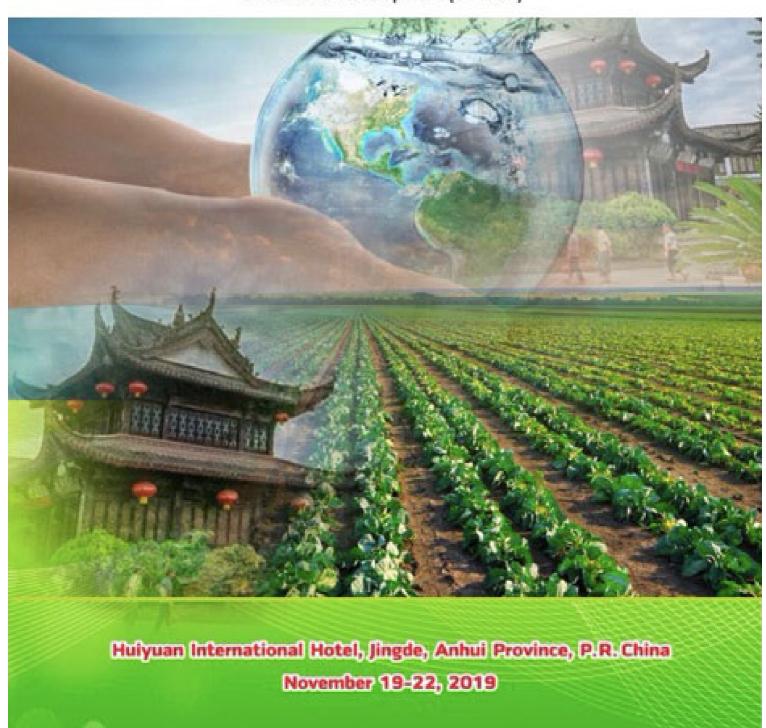


Proceeding of the 8th International Conference on Integration of Science and Technology for Sustainable Development (8th ICIST)



#### **FOREWORD**

Association of Agricultural Technology in Southeast Asia (AATSEA) has been organized International Conference on Integration of Science and Technology for Sustainable Development (ICIST) since 2011 which is started from Thailand to Laos, Vietnam, Myanmar, Philippines, Indonesia and this time is being in China. The 8<sup>th</sup> ICIST 2019 Organizing Committee wish to welcome all participants to the 8<sup>th</sup> International Conference on Integration of Science and Technology for Sustainable Development 2019 (8<sup>th</sup> ICIST 2019) held at Huiyuan International Hotel, Jingde, Anhui Province, P.R. China during November 19-22, 2019. AATSEA will provide the opportunity to discuss the recent advances and progress development in science and technology with the theme "Water Conservation, Biological Diversity, Food Safety and Agriculture". The theme of the conference is aptly chosen to address the current needs for academic, research and farm demands for further development and improvisation.

The integration of various topics in science and technology in agriculture is needed to build up the sustainable development of human being. As being a resource of knowledge, AATSEA realizes it is our responsibility to serve the community by providing education, research and development in science and technology, particularly in the multi-disciplinary aspects. Accordingly, this conference is targeted to initiate an international network among academic members, researchers, scientists and interesting peoples in science and technology. It is aimed to a venue for knowledge exchange and discussion among those seeking for new vision and insight in all topics related. For the technical point of view, the conference will explore various topics as following oral sessions: Organic Agriculture and Biological Products, Plant Biotechnology, Biodiversity, Taxonomy, Biological Activity, Crop Protection and Related Technologies, Water & Soil Management, Environmental Science, Animal Biotechnology and General Science and Technology. Poster Sessions will be demonstrated as follows:- Plant Science and Soil Management, Animal & Fishery Sciences Biotechnology and Food Science.

This year, there will be 3 keynote speakers, 101 oral and 64 poster presentations and attendants all together is about 250 participants who coming from 20 countries. AATSEA wish to acknowledge to all coorganized, CAS Asian Agriculture Bio Engineering (China), BioAgritech, Co. Ltd, Hanoi (Vietnam), CGC Organic Coffee, Champasack (Laos), Mahasarakham University (Thailand), Rajamangala University of Technology Tawanok (Thailand), Rambhai Barni Rajabhat University (Thailand), Shwe Kant Kaw Manufacturing (Myanmar), Society of Applied Biotechnology (India) and University of Bengkulu (Indonesia) for supporting our conference. Academicians, researchers, policy makers as well as extension experts contributed their expertise, experiences and research results to this conference. May the book of abstracts of this conference provide useful information and serve as references for those who are interested in the specific discipline. The book of abstracts and conference proceedings will be online at website: www.ijat-aatsea.com.

The selected full texts will be peer reviewed by Editorial board of International Journal of Agricultural Technology (IJAT) and external reviewers which will be online published in regular issue of the scopus indexed journal (IJAT) through website: www.ijat-aatsea.com.

I would like to express my sincerely thanks to all and hope to meet you again at the  $9^{th}$  ICIST 2020 in India.

**Editors** 

# 8<sup>th</sup> ICIST 2019

# Organized by

Asociation of Agriculture Technology in Southeast Asia (AATSEA); CAS Asian Agriculture Bio Engineering, China; King Mongkut's Institute of Technology Ladkrabang, Thailand; Rambhai Barni Rajabhat University, Thailand; Rajamangala University of Technology Tawan-ok, Thailand; Mahasarakham University, Thailand; Society of Applied Biotechnology, India; CGC Organic Coffee, Champasack, Laos; Shwe Kant Kaw Manufacturing, Ltd., Myanmar; BioAgritech, Co. Ltd, Hanoi, Vietnam University of Bengkulu, Indonesia.

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Ms. Rungrat Vareeket

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Ms. Rungrat Vareeket

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Mr. Suchart Chayhard, Mr. Akarat Jantub, Mr. Sarayos Puangkrasaer

## Proceedings and souvenirs:

Ms. Rujira Tongon

#### Audiovisual aid and Sub-session Conveners

Mr. Sarayos Puangkrasaer, Mr. Pheaktra Phal, Mr. Sommart Yoosukyingsataporn

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Ms. Rujira Tongon

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Chair and Co-chair in each session will be scored and made decision.

#### **Best Poster Presentation Awards Management:**

Chair and Co-chair in each session will be scored and made decision.

#### **AATSEA Awards Management:**

Ms. Jiaojiao Song

## **Session Chairpersons**

Keynote Session Prof. Dr. Fucheng Lin (China), Prof. Dr. Nanik Setyowati (Indonesia)

Session 1:	Prof. Dr. Zainal Muktamar (Indonesia), Dr. Younes Danesh (Iran), Prof. Dr. Danilo Josue (Philippines)
Session 2:	Dr. Bernadette Colle Mendoza (Philippines), Dr. Nanik Setyowati (Indonesia), Prof. Dr. Somdej Kanokmedhakul (Thailand), Dr. Bancha Wiangsamut (Thailand)
Session 3:	Prof. Dr. Kwanjai Kanokmedathakul (Thailand), Dr. Sundaram Lalitha (India), Dr. Samantha C. Karunarathna (Sri Lanka)
Session 4:	Prof. Dr. Raphael Okigbo (Nigeria), Prof. Dr. TSSK Patro (India), Dr. Hoang Pham (Vietnam)
Session 5:	Prof. Dr. Timo Korpela (Finland), Dr. Moammar Dayoub (Syria), Dr. Phrattraporn Soytong (Thailand)
Session 6:	Prof. Dr. John Moreki (Botswana), Prof. Dr. Hiroyuki Konuma (Japan), Ville Myllynpaa (Finland)
Session 7:	Prof. Dr. Nagia Farag Ali Mohamed (Egypt), Asst. Prof. Dr. Preeyanan Sittijinda (Thailand), Asoc. Prof. Dr. Pakkapong Poungsuk (Thailand)

# **Best Poster Award Committees**

Session 1:	Dr. Bernadette Colle Mendoza (Philippines), Dr. Fahrurrozi Fahrurrozi (Indonesia), Dr. Mohammad Chozin (Indonesia), Asst. Prof. Dr. Ajchara Bunroj (Thailand)
Session 2:	Dr. Dwatmadji (Indonesia), Dr. Kittichon U-taynapun (Thailand), Dr. Anurug Poeaim (Thailand)
Session 3:	Dr. Quyen BT HO (Vietnam), Dr. Widodo Widodo (Indonesia), Dr. Supattra Poeaim (Thailand), Dr. Naruemon Mongkontanawat (Thailand), Dr. Sararat Monkhung (Thailand)



# The 8<sup>th</sup> International Conference on Integration of Science and Technology for Sustainable Development (8th ICIST 2019)

# Huiyuan International Hotel, Jingde, Anhui Province, P.R. China November 19-22, 2019

**PROGRAM** 

19 November 2019 - registration and poster display

20 November 2019 – conference day

21 November 2019 – study tour in Jingde

22 November 2019 – study tour to Huangshan mountain

## 19 November 2019

14:30 Registration and poster display

# 20 November 2019

7:00 Registration

8:30 Parade of National Flags and Representatives on Stage

Guest representatives (20 countries):

AATSEA (Dr. Kasem Soytong)

Bangladesh (Dr. MD Asaduzzaman Sarker)

Botswana (Dr. John Moreki) Cambodia (Mr. Pheaktra Phal) China (Dr. Fucheng Lin) Egypt (Dr. Nagia F. Ali)

Finland (Dr. Timo Korpela) India (Dr. Sundaram Lalitha) Indonesia (Dr. Nanik Setyowati)

Iran (Dr. Younes Danesh) Japan (Dr. Hiroyuki Konuma) Korea (Mr. Young Ah Choi) Laos (Bouthsakone Inthalangsee) Myanmmar (Mr. Thet Lwin Htay) Nigeria (Dr. Raphel Okigbo)

Philippines (Dr. Teodoro Mendoza)

Russia (Dr. Elvira Khalikova)

Sri Lanka (Dr. Samantha C. Karunarathna)

Syria (Dr. Moammar Dayoub)

Thailand (Dr. Kampon Sriwatanakul)

Vietnam (Mrs. Hoang Hai)

Welcome address:

Prof. Dr. Timo Korpela (Chairman of International Organizing Committee)

CAS representative (Organizing Committee)

Dr. Kasem Soytong (AATSEA President) – opening with the theme song "IMAGINE"

## 8:45-9:00 AATSEA Awarding Ceremony:

9:00-9:10

AATSEA awardees will be given Award Plaques and Souvenirs by Dr. Kasem Soytong

#### **AATSEA Outstanding Leadership Award**

Beichun Chen (China), Agriculture Development

Yiming Zhu (China), Agriculture Development

**Professor Dr. Firma C. Viray (Philippines),** AATSEA Outstanding Leadership in Education and Research

**Kyaw Swar Soe (Myanmar),** AATSEA Outstanding Leadership in Agriculture Extension **Dr. Samantha C. Karunarathna (Sri Lanka),** AATSEA Outstanding Leadership in Research and Development

Dr. Sundaram Lalitha (India), AATSEA Outstanding Leadership in Research and Development

## AATSEA recognition awards to organizers

- 1. CAS Asian Agriculture Bio Engineering, China (Ms. Yi Zhao)
- 2. BioAgritech, Co. Ltd, Hanoi, Vietnam (Mrs. Hoang Hai)
- 3. CGC Organic Coffee, Champasack, Laos (Mr. Young Ah Choi)
- 4. Mahasarakham University (Dr. Wannasakpijitr Boonserm)
- 5. Rajamangala University of Technology Tawan-ok, Thailand (Dr. Naruemon Mongkontanawat)
- 6. Rambhai Barni Rajabhat University, Thailand (Asst. Prof. Dr. Preeyanan Sittijinda)

**GROUP PHOTO** 

- 7. Shwe Kant Kaw Manufacturing, Myanmar (Mr. Thet Lwin Htay)
- 8. Society of Applied Biotechnology, India (Prof. Dr. T. S. S. K. Patro)
- 9. University of Bengkulu, Indonesia (Dr. Zainal Muktamar)

Keynote Session	Chair: Prof. Dr. Fucheng Lin (China), Prof. Dr. Nanik Setyowati (Indonesia)
9:10-9:40	Prof. Dr. Hiroyuki Konuma (Japan): Global trend of food security and future outlook
9:40-10:10	<b>Prof. Dr. Teodoro Mendoza (Philippines):</b> The $4^{th}$ industrial revolution and the need for sustainable strategies of addressing the large-scale challenges
10:10-10:40	Prof. Dr. Fucheng Lin (China): Control of rice blast disease
10:40-11:00	COFFEE BREAK

# **Special Session**

# Meeting on CAS Biological products and Organic Agriculture Green Technology (Chinese Language)

13:00-15:20	Chairman: Wenrong Feng
13:00-13:20	Beichun Chen (China): Experience in green technological agriculture
13:20-13:40	Yiming Zhu (China): Experience in green technological agriculture
13:40-14:40	<b>Dr. Kasem Soytong (Thailand):</b> Experience Research in Biological products and Organic Agriculture
14:40-15:20	The success of CAS biological products distributional demonstration in China
15:20-15:40	COFFEE BREAK

# RESEARCH FORUM

# 11:00 -18:20 ORAL PRESENTATIONS

18:30

Session 1	ORGANIC AGRICULTURE AND BIOLOGICAL PRODUCTS
Room 1	Chairs: Prof. Dr. Zainal Muktamar (Indonesia), Prof. Dr. Danilo Josue (Philippines)
11:00-11:20	Invited Speaker: Prof. Dr. Kampon Sriwatanakul (Thailand): Nutritional values and health benefits of durian, the King of Fruits
11:20-11:40	<b>Invited Speaker: Dr. Nanik Setyowati (Indonesia):</b> Shifting and dominance of weed species following solarization treatment under organic farming system in tropical highland
11:40-12:00	Invited Speaker: Dr. Bernadette Colle Mendoza (Philippines): Detection of coliforms and salmonella strains in salad vegetables from selected organic farms in Laguna and Quezon, Philippines
12:00-13:00	LUNCH BREAK
13:00-13:20	<b>Invited Speaker: Dr. Fahrurrozi Fahrurrozi (Indonesia):</b> Yield performances of potato ( <i>Solanum tuberosum</i> L.) as amended with liquid organic fertilizer and vermicompost
13:20-13:40	<b>Invited Speaker: Dr. Mohammad Chozin (Indonesia):</b> Progress in sweet corn hybrid breeding program for organic agriculture in the tropics
13:40-14:00	Invited Speaker: Dr. Thet Lwin Htay (Myanmar): Organic agriculture movement in Myanmar
14:00-14:20	Invited Speaker: Dr. Teodoro Mendoza (Philippines): Obtaining heterosis in hybrid rice: The innovation pathway for increasing rice yield
14:20-14:40	<b>Karinporn Yodsenee (Thailand):</b> Encapsulation of <i>Pediococcus pentasaceus</i> RSU-Nh1 into pectin-sodium alginate and chitosan coating
14:40-15:00	<b>Patcharin Surin (Thailand):</b> Residual effect of different organic fertilizer types and application time on rice growth and yield
15:00-15:20	<b>Pakkapong Pongsuk (Thailand):</b> Organic farm learning and practical networks: Facilitation for learning and innovation for sustainable agriculture
15:20-15:40	COFFEE BREAK
15:40-16:00	Invited Speaker: Dr. Younes Danesh (Iran): Optimizing techniques on in vitro propagation of arbuscular mycorrhizal fungi
16:00-16:20	<b>Invited Speaker: Prof. Dr. Zainal Muktamar (Indonesia):</b> CO <sub>2</sub> emission and accumulation of soil organic matter under sweet corn stand in the long term organically managed land
16:20-16:40	Invited Speaker: Prof. Dr. M.A. Sarker (Bangladesh): Role of botanical pesticide in sustainable crop protection
16:40-17:00	<b>Dwi Wahyuni Ganefianti (Indonesia):</b> Superiority test of mixed-cropping models for chili pepper hybrid varieties through participatory plant breeding
17:00-17:20	Kasem Soytong (Thailand): Organic agriculture model
17:20-17:40	<b>Chanitnan Suriyawittayawate (Thailand):</b> Growing organic herbs to promote the local enterprises for sustainability under plant genetic conservation project, Suratthani, Thailand
17:40-18:00	<b>Puji Harsono (Indonesia):</b> Weed inhibition and sorghum yield as affected by organic mulch in tropical coastal environment
18:00-18:20	<b>Suneeporn Suwanmaneepong (Thailand):</b> Farmers' adoption and attitude towards organic rice production in Chachoengsao province, Thailand

**CLOSING CEREMONY** 

Session 2	PLANT BIOTECHNOLOGY
Room 2	Chairs: Dr. Bernadette Colle Mendoza (Philippines), Dr. Nanik Setyowati (Indonesia), Prof. Dr. Somdej Kanokmedhakul (Thailand), Dr. Bancha Wiangsamut (Thailand)
11:00-11:20	<b>Invited Speaker: Prof. Dr. Somdej Kanokmedhakul (Thailand):</b> Bioactive secondary metabolites from <i>Chaetomium</i> spp.
11:20-11:40	Invited speaker: Dr. Bancha Wiangsamut (Thailand): Yield and growth of pak choi and green oak vegetables grown in substrate plots and hydroponic systems with different plant spacing
11:40-12:00	Pannipa Youryon (Thailand): Efficiency of preharvest calcium chloride, potassium oxide or salicylic acid treatment on internal browning incidence of Queen pineapple during cold storage
12:00-13:00	LUNCH BREAK
13:00-13:20	Suriyan Supapvanich (Thailand): Effects of preprocess elicitors immersion on physicochemical
	quality of fresh-cut papaya cv. 'Holland' fruits during cold storage
13:20-13:40	Kannikar Charoensuk (Thailand): Sugars, nutritional and organic acids content in granulated
	Rambutan (Nephelium lappaceum Linn.) sugar
13:40-14:00	Lin Li (China): MoFap7, a ribosome assembly factor, is required for fungal development and
	plant colonization of Magnaporthe oryzae
14:00-14:20	Widodo Widodo (Indonesia): Kinds of retardants inducing potato (Solanum Tuberosum L.)
	microtuber production
14:20-14:40	Sutisa Chaikul (Thailand): Effects of compost and chelated ca on early growth of Para rubber
	(Hevea brasiliensis)
14:40-15:00	Wasinee Chanchum (Thailand): Influence of harvesting day and potassium chloride on
	sweetness of melon grown in plastic house
15:00-15:20	Pimpisuth Boonsopin (Thailand): Effect of growing media and 20-20-20 fertilizer rate on yield
	performance of melon grown in plastic house
15:20-15:40	COFFEE BREAK
15:40-16:00	Patinya Virulchatapan (Thailand): Effect of harvesting periods on physicochemical properties
	and in vitro digestibility of banana flour
16:00-16:20	Kannika Kunyanee (Thailand): The effects of dual modification with ultrasound and annealing
	treatments on properties and glycemic index in Thai glutinous rice (RD6)
16:20-16:40	Atra Romeida (Indonesia): Plant growth regulator formulation for propagating red chili
46.40.47.00	(Capsicum annum L.) UNIB CH23 hybrid stem cuttings
16:40-17:00	Xue-Ming Zhu (China): The crosstalk between autophagy and endocytosis in <i>Pyricularia</i>
17.00.17.00	oryzae
17:00-17:20	Pramote Pornsuriya (Thailand): Augmented analysis for yield and pod characters of yardlong
17-20 17-40	bean (Vigna unguiculata (L.) Walp. ssp. sesquipedalis Verdc.) lines
17:20-17:40	<b>Bunyarit Chumthong (Thailand):</b> Influence of ethephon spraying on growth and yield of Stevia ( <i>Stevia rebaudiana</i> Bertoni.)
17:40-18:00	Athiwat Joedsak (Thailand): Knowledge, attitude and practices towards good agricultural
17.40-16.00	practice of rice farmers under large agricultural plot scheme (LAPS) in
	Khlongudomchonlajorn, Chachoengsao province, Thailand
18:00-18:20	Sommart Yoosukyingsataporn (Thailand): Influences of trinexapac-ethyl on development and
10.00-10.20	sugar content of sorghum bicolor
18:20-18:40	Isagani Angeles (Philippines): Phytochemical constituents, Proximate composition and
10.20-10.40	Antimicrobial property of <i>Padina sp.</i> ( <i>Lap lapayag</i> ) collected along Gonzaga, Cagayan
	Philippines
18:30	CLOSING CEREMONY
20.00	CLOSING CENEMONI

Session 3	BIODIVERSITY, TAXONOMY, BIOLOGICAL ACTIVITY
Room 3	Chairs: Prof. Dr. Kwanjai Kanokmedathakul (Thailand), Dr. Sundaram Lalitha (India), Dr. Samantha C. Karunarathna (Sri Lanka)
11:00-11:20	Invited Speaker: Prof. Dr. Kwanjai Kanokmedathakul (Thailand): Compounds isolated from the fungus Neosartorya pseudofischeri
11:20-11:40	Invited Speaker: Dr. Samantha C. Karunarathna (Sri Lanka): New edible fungi from Southeast Asia: discovery to production
11:40-12:00	<b>Nion Chirapongsatonkul (Thailand):</b> The impact of the mixture of biofloc fermentation medium and vinasse on attractability, palatability and antibacterial property against multiantibiotics resistant <i>Aeromonas veronii</i>
12:00-13:00	LUNCH BREAK
13:00-13:20	<b>Invited speaker: Dr. Quyen BT HO (Vietnam):</b> Biodiversity and cultivation characteristic of oyster cultivation in Southern Vietnam
13:20-13:40	<b>Kittichon U-taynapun (Thailand):</b> Species diversity and virulence gene existence in clinical <i>Aeromonas</i> spp. causing motile <i>Aeromonas septicamia</i> (MAS) Isolated from cultured nile tilapia ( <i>Oreochromis niloticus</i> )
13:40-14:00	<b>Teeka Yotapakdee (Thailand):</b> Evaluation of the effectiveness of community development on naturally mychorrhizal mushroom cultivation technology for forest restoration and community food bank at Northern, Thailand
14:00-14:20	<b>Supattra Poeaim (Thailand):</b> Effective of <i>Neosartorya</i> use to control <i>Colletotrichum</i> causing anthracnose disease on papaya
14:20-14:40	<b>Apinya Khamson (Thailand):</b> Optimization of microbial collagenolytic enzyme production by <i>Bacillus subtilis</i> subsp. <i>Subtilis</i> S13 using Plackett-Burman and response surface methodology
14:40-15:00	<b>Kittiya Showpanish (Thailand):</b> Pectin extraction from fruit wastes on growth of <i>Pediococcus pentosaceus</i> RSU-Nh1 and <i>Lactobacillus plantarum</i> RSU-SO2
15:00-15:20	<b>Jiaojiao Song (China):</b> Fine particles of fungal active metabolites constructed from <i>Emericella</i> sp to control rice blast disease
15:20-15:40	COFFEE BREAK
15:40-16:00	<b>Invited Speaker: Dr. Sundaram Lalitha (India):</b> Effect of PGPR on the phytoremediation potential of <i>Vigna radiata</i> . L in contaminated soil
16:00-16:20	<b>Jatuporn Anuchai (Thailand):</b> Effect of BA and NAA on shoot induction from callus of <i>Dendrobium</i> spp.
16:20-16:40	<b>Kritsada Phongkaranyaphat (Thailand):</b> Species diversity and utilization of herbal plant in community forest, Phrae Province, Northern Thailand
16:40-17:00	<b>Pansaporn Plengsaengsri (Thailand):</b> Effect of lactic acid bacteria on the physicochemical and microbiology properties of drinking yogurt from rice milk
17:00-17:20	<b>Pradipha Pradapphai (Thailand):</b> Classification and diversity assessment of <i>Pyricularia oryzae</i> according leaf and neck blast pathogenisis on Khao Dawk Mali 105
17:20-17:40	<b>Tatik Suteky (Indonesia):</b> Silver nanoparticles from leaves extract of melastoma malabatricum and its nematocidal effects
17:40-18:00	<b>Racha Tepsorn (Thailand):</b> Antimicrobial potential of vapour phase propionic acid against <i>Salmonella</i> typhimurium contaminated on cherry tomato ( <i>Solanum lycopersicum</i> var. cerasiforme)
18:00-18:20	<b>Wiwat Samritphol (Thailand):</b> Hydrolytic properties of crude protease from <i>Bacillus subtilis</i> subsp. Subtilis M13
18:30	CLOSING CEREMONY

Session 4	CROP PROTECTION AND RELATED TECHNOLOGIES
Room 4	Chairs: Prof. Dr. T. S. S. K. Patro (India), Dr. Hoang Pham (Vietnam)
11:00-11:20	Invited Speaker: Prof. Dr. Raphael Okigbo (Nigeria): The impact of biopesticides on agricultural yields and food security in Africa
11:20-11:40	Chaisit Preecha (Thailand): Study on fruit spot caused by <i>Fusarium</i> sp. of pummelo ( <i>Citrus maxima</i> (Burm.) cultivar Tabtimsiam and screening fungicides and antagonist for alternate control <i>in vitro</i>
11:40-12:00	<b>Reny Herawat (Indonesia):</b> Analysis of polyethylene glycol (PEG) and proline to evaluate drought stress of double haploid new type upland rice lines
12:00-13:00	LUNCH BREAK
13:00-13:20	<b>Invited Speaker: Prof. Dr. T. S. S. K. Patro (India):</b> Importance of bioagents in mitigating the major diseases of nutri cereals
13:20-13:40	<b>Charida Pakasap (Thailand):</b> Effect of larval-stage mealworm ( <i>Tenebrio molitor</i> ) powder on qualities of bread
13:40-14:00	<b>Thawatchai Masiriyanan (Thailand):</b> Evaluation and selection of chili germplasms resistant to pepper yellow leaf curl Thailand virus (PepYLCVTH)
14:00-14:20	<b>Waree Laophemsuk (Thailand):</b> Identification of morphology and pathogenicity of <i>Pyricularia</i> sp. causing blast disease in grass
14:20-14:40	<b>Rosemarie Del Rosario Josue (Philippines</b> ): Biological control using parasitoids <i>Comperiella calauanica</i> hastens the recovery of the coconut trees from coconut scale insect (CSI) infestation in Basilan, Philippines
14:40-15:00	<b>Nattawut Suanphrom (Thailand):</b> Comparison of growth and yield of maize ( <i>Zea mays</i> L.) grown after using intercropping and monocropping systems
15:00-15:20	<b>Hathairat Kingkampang (Thailand):</b> Phenols and peroxidase activity in pepper yellow leaf curl virus resistant and susceptible chili ( <i>Capsicum annuum</i> L.) genotype
15:20-15:40	COFFEE BREAK
15:40-16:00	Invited speaker: Dr. Hoang Pham (Vietnam): The Rotting fungi of street plant in Ho Chi Minh City
16:00-16:20	<b>Watchareeporn Suksiri (Thailand):</b> Resistance genes investigation in a broad-spectrum resistance indigenous rice Yang Mawng variety
16:00 -16:40	<b>Rungrat Vareeket (Thailand):</b> Natural products of fine particles derived from <i>Neosartorya hiratsukae</i> against brown spot of rice cause by <i>Drechslera oryzae</i>
16:40 -17:00	Jeremias Lacsamana Ordonio (Philippines): Identification of quantitative trait loci for seedling stage salinity tolerance using NSIC Rc222 X Jumbo Jet BC1F2 population
17:00-17:20	<b>Suphattra Janthasri (Thailand):</b> Genetic inheritance of gene controlling leaf blast disease resistance in Dawk Pa-yawm Rai variety (GS23774)
17:20-17:40	<b>Laxmi Rawat (India):</b> Enhancement of growth, yield & yield contributing traits and alleviation of sheath blight disease caused by <i>Rhizoctonia solani</i> in barnyard millet ( <i>Echinocloa frumentacea</i> L.) with a particular reference by using <i>Trichoderma</i> isolates through seed biopriming and pre colonized FYM
17:40-18:00	<b>Danilo Sarcon Josue (Philippines):</b> Promotion of improved rice farming technology for resilient and profitable enterprise
18:00 -18:20	<b>Teerawat Sarutayophat (Thailand):</b> Drought-tolerant characters, yield and its component of an elite landrace upland rice cultivars in Thailand

18:30

**CLOSING CEREMONY** 

Session 5	WATER & SOIL MANAGEMENT, ENVIRONMENTAL SCIENCE,
Room 5	Chairs: Prof. Dr. Timo Korpela (Finland), Dr. Phrattraporn Soytong (Thailand)
11:00-11:20	Invited Speaker: Prof. Dr. Timo Korpela (Finland): Purification and disinfection of waste water with electrical current
11:20-11:40	Invited Speaker: Dr. Moammar Dayoub (Syria): Trends of precipitation and temperature in morogoro region in Tanzania
11:40-12:00	Artemio Jr Asuncion Martin (Philippines): Production potential of marginal lands to selected biofuel feedstocks in Cagayan Valley, Philippines
12:00-13:00	LUNCH BREAK
13:00-13:20	Invited Speaker: Dr. Phattraporn Soytong (Thailand): Analysis of water resources and water potentials under conditions of climate change and agriculture-industrial - urban development change in the Eastern region
13:20-13:40	<b>Patticha Kulsuwan (Thailand):</b> The carbon footprint assessment from electricity of undergraduate students Mahidol university Amnatcharoen campus Thailand for Eco university
13:40-14:00	<b>Suchart Chayhard (Thailand):</b> Application of aerial photography with Visible Atmospherically Resistant Index by using Unmanned Aerial Vehicles for seagrass bed classification in Kung Krabaen Bay, Thailand
14:00-14:20	Ha Thi Thu Hue (Vietnam): Social Aspects to Fish catch in Songkhla Lagoon, Thailand
14:20-14:40	Tamonwan Thepphakhun (Thailand): Allelopathic potential of praxelis and its use in soil application
14:40-15:00	<b>Duangkamon Chudaeng (Thailand):</b> Effects of soil moisture contents and arbuscular mycorrhizal fungi on phosphorus fractions in soil
15:00-15:20	<b>Muchammad Farid (Indonesia):</b> Field assessment on altitude variation to correlate soil conductivity and porosity for validation of archi formulation
15:20-15:40	COFFEE BREAK
15:40-16:00	<b>Kingkan Puansurin (Thailand):</b> A study of the relationship between urban heat island phenomena and land use: A case study of Chiang Rai Municipality, Thailand
16:00-16:20	<b>Elderico Tabal (Philippines):</b> Carbon stocks of selected agroforestry systems in Zamboanga city, Philippines
16:20-16:40	Likhit Noichaisin (Thailand): Drought risk area assessment in Sa Kaeo province by using GIS
16:40-17:00	<b>Pichitporn Pholgerddee (Thailand):</b> The application of Geo-informatics technology to study the vegetation index and humidity: A case study analysis of drought in special economic eastern region, Thailand
17:00 -17:20	<b>Paphawan Onkum (Thailand):</b> Soil microbial activities in Alfisol with different green manures application
17:20-17:40	Margie Daylusan Fiesta (Philippines): Farmer's climate change cognition and adaptation in agroecological zones of Mindanao, Philippines
17:40-18:00	<b>Charanya Khaeksawad (Thailand):</b> The effect of water deficit at different growth stage on yield and quality of sweet corn ( <i>Zea mays saccharata</i> Sturt.)
18:00-18:20	<b>Pimolwan Katepan (Thailand):</b> Effect of number of plowing on okra productivity in Suphan Buri Province, Thailand
18:30	CLOSING CEREMONY

Session 6	ANIMAL BIOTECHNOLOGY
Room 6	Chairs: Prof. Dr. John Moreki (Botswana), Prof. Dr. Hiroyuki Konuma (Japan)
11:00-11:20	<b>Invited Speaker: Prof. Dr. John Moreki (Botswana):</b> Cattle, identification and traceability system in Botswana and its challenges
11:20-11:40	<b>Thaworn Chimliang (Thailand):</b> Effects of Garcinia cowa leaf powder supplementation in diets on productive performance of broilers
11:40-12:00	<b>Poramech Moomak (Thailand):</b> The effect of sex, age, and final live weight on carcass traits of fattening dairy cattle
12:00-13:00	LUNCH BREAK
13:00-13:20	Invited Speaker: Dr. Dwatmadji D (Indonesia): Intake and digestibility of mix-herbal supplements block for bali cattle fed agricultural by-product in Bengkulu, Indonesia
13:20-13:40	<b>Jirapon Aungtikun (Thailand):</b> Essential oils from <i>Zingiber mekongense</i> Gagnep, <i>Myristica fragrans</i> Houtt and <i>Curcuma zedoaria</i> Roscoe as larvicidal agents against <i>Aedes albopictus</i> (Skuse)
13:40-14:00	<b>Ajaree Karakad (Thailand):</b> Effects of sodium chloride replacement with potassium chloride and flavour enhancers on the physicochemical and sensory characteristics of low-fat chicken frankfurter sausage
14:00-14:20	<b>Kamonthip Kongsrirat (Thailand):</b> Sous-vided restructured goat meat: Changes in meat qualities during refrigerated storage
14:20-14:40	Pattraphorn Patthararangsarith (Thailand): Effects of heart fatty acid binding protein (H-FABP) gene, housing system, and sex on carcass and meat quality of commercial crossbred pigs
14:40-15:00	Rapeepat Thawornwat (Thailand): Biological, farm, and slaughter month factors affecting dairy carcass traits and the relationship between the traits, thoracic cavity depth, and carcass length
15:00-15:20	<b>Peeradon Phonkhokkong (Thailand):</b> Influence of sex, age, slaughter weight, and farm on carcass composition of fattening dairy cattle
15:20-15:40	COFFEE BREAK
15:40-16:00	Sampan Promhom (Thailand): Population biology of mantis shrimp in Satun province
16:00-16:20	<b>Dede Hartono (Indonesia):</b> Study on tropical eels aquaculture in Bengkulu province of Indonesia
16:20-16:40	<b>Kunya Tuntivisoottikul (Thailand):</b> Paternity testing of crossbred beef cattle with 15 microsatellite markers
16:40-17:00	<b>Pinatthinee Jitkham (Thailand):</b> Prevalence of trematode metacercariae in cyprinoid fish from Chiang Rai province, Thailand
17:00-17:20	<b>Nella Saida Marjukka Sihvonen (Finland):</b> Ways to decrease greenhouse gas emissions from cattle
17:20-17:40	<b>Kasemsak Tongsuk (Thailand):</b> Influence of Duroc sire lines on carcass and meat quality traits of commercial crossbred pigs
17:40 -18:00	Jirapon Aungtikun (Thailand): Mosquito-repellent activity of <i>Illicium verum</i> Hook.F., <i>Amomum villosum</i> Lour. and <i>Amomum krervanh</i> Pierre. essential oils against <i>Aedes albopictus</i> (Skuse)

# **CLOSING CEREMONY**

18:30

Session 7	GENERAL SCIENCE AND TECHNOLOGY
Room 7	Chairs: Prof. Dr. Nagia Farag Ali Mohamed (Egypt), Asst. Prof. Dr. Preeyanan Sittijinda (Thailand), Asoc. Prof. Dr. Pakkapong Poungsuk (Thailand)
11:00-11:20	<b>Invited Speaker: Dr. Nagia Farag Ali Mohamed (Egypt):</b> Antimicrobial characteristics of wool fibers treated with chitosan and propolis nano composite and dyed with natural dye extracted from red prickly Pear
11:20-11:40	Amorn Kritsanaphan (Thailand): Intermediaries and local actors in agricultural technology and management in peri-urban Bangkok
11:40-12:00	Naruemon Mongkontanawat (Thailand): Product development of mushroom drink with high β-glucan content from local mushrooms
12:00-13:00	LUNCH BREAK
13:00-13:20	Naphatrapi Luangsakul (Thailand): The effects of the reduced sugar on macaron quality
13:20-13:40	Rungarun Poonsin (Thailand): Investigation of rice blast resistant genes in Thai elite rice varieties ( <i>Oryza sativa</i> L.) for improvement of broad-spectrum blast disease resistance variety
13:40-14:00	<b>Krittiya Khuenpet (Thailand):</b> Inulin extraction from Jerusalem artichoke ( <i>Helianthus tuberosus</i> L.) tuber powder and its application to yoghurt snack
14:00-14:20	<b>Bertoka Fajar Surya Perwira Negara (Indonesia):</b> The utilization of seaweeds as an inexpensive source of new drugs agent
14:20-14:40	<b>Wanwipa Kheawrod (Thailand):</b> The influence of fattening and ageing periods on meat quality of Charolais crossbred steers
14:40-15:00	<b>Sirima Takeungwongtrakul (Thailand):</b> Development of strawberry gummy jelly with reduced sugar content from strawberry syrup
15:00-15:20	<b>Surassawadee Promyou (Thailand):</b> Physicochemical quality improvement of ready to cook baby corns using calcium propionate immersion
15:20-15:40	COFFEE BREAK
15:40-16:00	<b>Rujira Tongon (Thailand):</b> Natural products of fine particles derived from <i>Chaetomium</i> spp. to inhibit fusarium wilt of tomato
16:00-16:20	<b>Rawiporn Polpued (Thailand):</b> Formulation of sweetened condensed rice-milk fortified with cereals in retortable pouch
16:20-16:40	Kodchawan Chaturattanachaiporn (Thailand): Development of sterilized durian cake roll
16:40-17:00	<b>Pheaktra Phal (Cambodia):</b> Application of agricultural inputs for organic asparagus production in the field
17:00-17:20	<b>Nitipat Chotikulapat (Thailand):</b> Antifungal activity of <i>Chaetomium</i> isolate CNC2 against <i>Alternaria</i> spp. causing leaf blight of kales
17:20-17:40	Fredisminda Matias Dolojan (Philippines): Product development and training centre (PDTC): Food product development-hub of the province Quirino, Philippines
17:40-18:00	<b>Kanittha Chepoo (Thailand):</b> Shortage of sugarcane workers, Nong Na Kham district, Khon Kaen province of Thailand
18:00-18:30	Chirathip Yimcharoen (Thailand): Bioactive test of <i>Chaetomium</i> spp. to control <i>Pestalotia</i> spp. causing leaf spot of orchid
18:30	CLOSING CEREMONY

# **List of Posters**

PLANT SCIENCE AND SOIL MANAGEMENT
Chairs: Dr. Rosemarie Del Rosario Josue, Dr. Fahrurrozi Fahrurrozi, Dr. Mohammad Chozin, Dr. Sutisa Chaikul
Alisa Kongjaimun Yoshida (Thailand): Genetic analysis of seed dormancy QTL in yardlong bean [Vigna unguiculata (L.) Walp. ssp. unguiculata cvgr. sesquipedalis]
<b>Anurug Poeaim (Thailand):</b> The influence of plant growth reglurators for plant regeneration of Chinese chaste ( <i>Vitex negundo</i> L.)
Apisara Pholjad (Thailand): In vitro propagation from nodal segments of Arachis glabrata cultivar Florigraze
<b>Bernadette Colle Mendoza (Philippines):</b> Heterotrophic and coliform loads, and <i>E. coli</i> and <i>Salmonella</i> detection in farm soils and organic fertilizers from selected organic farms in Southern Luzon, Philippines
Chaowanee Laosutthipong (Thailand): Lignin biosynthesis genes ( <i>OsPAL</i> and <i>Os4CL3</i> ) sequencing of native upland rice varieties from Pala U village, Thailand
Isagani Angeles (Philippines): Phytochemical constituents and proximate composition of spiny
amaranth Amaranthus spinosus Linn. Leave Isagani Angeles (Philippines): Production of azolla and duckweed with or without application of urea
or ammonium phosphate in tank based system
Kanittha Chepoo (Thailand): Production behavior, factors affecting the sale of ripe mango and SWOT analysis of Nam Dok Mai mango collaborative farming, Bang Phli district, Samut Prakan province, Thailand
<b>Krittayaporn Meesook (Thailand):</b> Times for callus propagation of indica rice ( <i>Oryza sativa</i> L.) cultivar Sangyod in suspension culture
Natchada Asoko (Thailand): Improvement of <i>Dendranthemum grandiflora</i> cv. canter with Colchicine <i>in vitro</i> .
Nattaya Montri (Thailand): The effects of teak (Tectona grandis L.f.) leaves crude extract on
controlling anthracnose disease in "Kluai Hom thong" (Musa AAA group)  Nonglek Parinthawang (Theiland). Dath aganisity of Divisularia angles and alite rise cultivars and
Nonglak Parinthawong (Thailand): Pathogenicity of <i>Pyricularia oryzae</i> on elite rice cultivars and geographical distribution of avirulence genes causing blast disease in Thailand
<b>Panida Duangkaew (Thailand):</b> Effect of $\beta$ -mannanase on nutrient composition of Palm Kernel Meal (PKM) mixed with yeast cake
Patcharaporn Suwor (Thailand): The effect of composting manures on growth of green oak, frillice
Iceberg and green cos ( <i>Lectuca sativa</i> L.) <b>Pimchanok Tongsad (Thailand):</b> The effects of abscisic acid and sorbitol on the slow growth in vitro
preservation of Mahesak ( <i>Tectona grandis</i> L.)
Sakulrat Hansuek (Thailand): Effects of plant growth regulators and type of media on shoot proliferation of neck orange ( <i>Citrus reticulate</i> Blanco)
Sarayut Phonpho (Thailand): Selection of appropriate of ornamental plant species for outdoor vertical garden
Somporn Na Nakorn (Thailand): Fruit growth and development of neck orange at the optimal time
for harvesting under the difference period climate of the year
<b>Somsak Kramchote (Thailand):</b> Effect of foliar application of chitosan on growth and fruit quality of 'Densuke' and 'Kinnaree' watermelons
Somsak Rayan (Thailand): Spatio-temporal variation of the distribution of aquatic plants in Nong Han
wetland, Thailand  Thausan Thangina (Thailand): Comparative officers of plant extracts, netroleum eil and insecticides
<b>Tipawan Thongjua (Thailand):</b> Comparative efficacy of plant extracta, petroleum oil and insecticides to control citrus Leaf-miner ( <i>Phyllocnistis citrella</i> Stainton) in Neck Orange ( <i>Citrus reticulate</i> Blanco)
Usana Prangthong (Thailand): Callus and hairy root induction of Melaleuca cajuputi Powell
Wanrawee Kunwanlop (Thailand): Effects of 6-benzylaminopurine and meta-topoline on
micropropagation of <i>Dendrobium chrysanthum</i> Lindl <b>Wattana Na Nakhon (Thailand):</b> Testing of growth and development of seashore ground cover plants on different salty level for development as ornamental plants

#### Poster **ANIMAL & FISHERY SCIENCES** Session 2 Chairs: Prof. Dr. Zainal Muktamar (Indonesia), Dr. Dwatmadji (Indonesia), Dr. Kittichon U-taynapun (Thailand), Dr. Anurug Poeaim (Thailand) 1 Bancherd Sornsupharp (Thailand): The study of density on growth and yield of African catfish (Clarias garriepinus) in Turbidity Water Pond 2 Bhutharit Vittayaphattananurak Raksasiri (Thailand): Effect of the aging period on meat quality and consumption properties of spent laying hens 3 Duangkamon Namee (Thailand): Effectiveness of long pepper (Piper retrofractum Vahl) extracts against adult of cowpea weevil (Callosobruchus maculatus Fabricius) and southern cowpea weevil (Callosobruchus chinensis Linnaeus) Fakeetah Bupphadong (Thailand): Concentrated pineapple extract, Na-Cid®, facilitates the digestion 4 of soybean meal and shrimp diet Isagani Angeles (Philippines): Growth performance, immune response and disease resistance of Nile 5 tilapia Oreochromis niloticus fed diet with different levels of Amaranthus spinosus leaf meal following Aeromonas hydrophila infection Isagani Angeles (Philippines): Rearing performance and disease resistance of Freshwater eel Anguilla 6 sp. fed diet with different levels of Water fern Azolla filiculoides extract following Aeromonas hydrophila infection 7 Kaewalee Viboonkit (Thailand): Morphometric and meristic parameters of short mackerel (Rastrelliger brachysoma) in the Gulf of Thailand 8 Kanokrat Srikijkasemwat (Thailand): Prevalence and genetic diversity of Trypanosoma evansi infections causing abortions among cattles and buffaloes in eastern border area of Thailand-Cambodia 9 Patinya Yeamsamai (Thailand): The effect of supplementary food on the adult growth of Zophobas mario Fabricius 10 Piyada Tavitchasri (Thailand): Stock identification of short mackerel (Rastrelliger brachysoma) from the Gulf of Thailand using morphometric and meristic parameters 11 Potchaman Lomwong (Thailand): The study of appropriate feed formulation for rearing super worms (Zophobas mario Fabricius) 12 Prasit Deewatthanawong (Thailand): Molecular and morphological identification of banana thrips in Chantaburi province, Thailand 13 Ronachai Sitthigripong (Thailand): The effect of okara in diets on productive performance of broiler 14 Thiamphop Kanloung (Thailand): The reproductive performances of Murrah and swamp buffaloes in **Thailand**

#### Poster **BIOTECHNOLOGY AND FOOD SCIENCE** Session 3 Chairs: Dr. Quyen BT HO (Vietnam), Dr. Widodo Widodo (Indonesia), Dr. Supattra Poeaim (Thailand), Dr. Naruemon Mongkontanawat (Thailand), Dr. Sararat Monkhung (Thailand) 1 Angsana Akarapisan (Thailand): Bacillus velezensis SD03 - potential biocontrol agent for diseases of 2 Bootsrapa Leelawat (Thailand): Development of grass jelly processing using modified starches and higher efficient extraction method 3 Chadchasa Tep-Ubon (Thailand): Antagonistic Bacillus sp. AAHMRU15 and yeast extract product, Beta-Sac Plus®, inhibit the growth of AHPND causing Vibrio parahaemolyticus (VPAHPND) 4 Chaiwut Kumueang (Thailand): Effect of sterility value on qualities of Chinese braised culled steer beef in retort pouch 5 Elvira Khalikova (Russia): Isolation and identification of cyclic lipopeptides from Paenibacillus ehimensis 6 Hang Thi Thuy Le (Vietnam): A survey on acid hydrolysis in analyzing the monosaccharide composition of exopolysaccharide from *Ophiocordyceps sinensis* 7 Hoang Tai Nguyen (Vietnam): Optimization of culture parameters of Selenium-enriched Ophiocordyceps sinensis by response surface methodology 8 Ky Khac Lam (Vietnam): In vitro antioxidant and anti-inflammatory activities of Cordyceps neovolkiana DL0004 extracts isolated in Vietnam 9 Nidchaporn Nabhadalung (Thailand): Study on arbuscular mycorrhizal fungi related to soil P, soil OM and soil pH in cassava field from Thailand 10 Ninh Thi My Pham (Vietnam): The cytotoxic activity of extracts from the biomass and fruit bodies of mushroom Isaria tenuipes on MCF-7 cancer cell line 11 Pattrarat Teamkao (Thailand): Floods increase soil microbial activity in paddy soil: A case study in Sakon Nakhon province, Thailand 12 Peamsuk Suvarnakuta (Thailand): Antioxidants and product development of bread crust 13 Pitukpol Pornanek (Thailand): Dietary supplementation of Beta Glucan enriched molasses yeast powder on immune response to swine fever virus and hematology of starter grower pigs 14 Pongpan Pragthong (Thailand): Temperature dependent expression of virulence genes in Vibrio parahaemolyticus AHPND strain (VPAHPND) Prapasri Theprugsa (Thailand): Effect of tomato powder on quality of Chinese sausage 15 16 Rachsawan Mongkol (Thailand): Antifungal and antibacterial activities of essential oil from Som Keaw (Citrus nobilis) in Thailand 17 Rujira Deewatthanawong (Thailand): GC-MS analysis and biopesticide properties of different crude extracts of Annona squamosa and Annona muricata 18 Sairung Sornsupharp (Thailand): Preliminary study on chlorophyll a concentration and phytoplankton diversity in Huai Jorakhemak reservoirs, Buriram province, Thailand 19 Saowapar Khianngam (Thailand): Isolation of cellulase producing bacteria as using sunflower meal as substrate 20 Sararat Monkhung (Thailand): Evaluation of the antifungal activity of various agricultural residue extracts to control seed borne pathogens of maize in Thailand 21 Supaluk Sorapukdee (Thailand): Quality characteristics and storage stability of reduced-fat Northeastern Thai fermented sausage (Sai Krok E-san) with konjac gel during chilled storage 22 Suteera Vatthanakul (Thailand): Development of banana flavor carrageenan jelly drink fortified with 23 Trairat Kaewsaad (Thailand): The usage of rice bran flour and pea protein in pork patties and pork

meatballs

## **CLOSING CEREMONY**

18:30 BEST PAPER AWARDS for Oral and Posters

Conclusion Remarks:

Prof. Dr. Timo Korpela (Chairman of International Organizing Committee)

Annoucement and welcoming to India for the 9<sup>th</sup> ICIST 2020 by local organizers:

Prof. Dr. T. S. S. K. Patro (India): ANGR Agricultural University,

Vizianagaram 535 001, AndhraPradesh, India

Dr. Sundaram Lalitha (India): Department of Botany, Periyar University,

Periya Palakalainagar, Salem-636011, Tamil

Nudu, India

Closing Address and Future Remark: Dr. Kasem Soytong (President of AATSEA) Closing with the ICIST Theme Song "IMAGINE"

19:00 FAREWELL DINNER

21 November 2019--Jingde city tour (AATSEA offer)

22 November 2019 – Huangshan one day tour (618 RMB, Optional)

23 November 2019 – leave from Jingde

## **AATSEA Outstanding Leadership in Agriculture Development**



Mr. Beichun Chen (China)

Mr. Beichun Chen, He was birth in 1978, graduated Bachelor degree in Heilongjiang Institute of Technology. He is currently the chairman of the Gray Vegetable Professional Cooperative of Jude County and the General Secretary of the Industrial Poverty Alleviation Alliance of Jingde County. In 2010, the Gray Vegetable Professional Cooperative was established in Jingde County to promote contract farming. Cooperatives have a stable return through the unified seedling, training in planting techniques, cultivation plans, and using agricultural inputs.

Mr. Beichun Chen focuses on innovation, research and development, brand and quality. The cooperative is registered the trademark, declared the pollution-free certification of horseradish, and obtained the patent for the invention of chrysanthemum. It has successively formulated the local standards of Anhui Province, "Technical Regulations for Horseradish Cultivation", "Technical Regulations for Cultivation of Royal Chrysanthemum", and "Technical Regulations for Processing of Royal Chrysanthemum".

In 2012, He was awarded "2010-2011 Entrepreneur Leadership" in Anhui Province. In 2016, the Gray Cooperative was awarded him the Anhui Provincial Demonstration Cooperative. In 2017, he was elected as member of the 10th CPPCC National Committee of Jingde and was named the "Star of Entrepreneurship" in Xuancheng City. In 2018, the preparatory coalition poverty alleviation alliance has prepared which leading 20 agricultural enterprises to participate in poverty alleviation. In the same year, he also won the first batch of "four belts" local agricultural celebrities in Xuancheng City, Xuancheng "Top Ten Youth Entrepreneurship Stars", the "Outstanding CPPCC members" in Jingde County, and the "Entrepreneur Star" in the led collective economy of Jingde County. In 2019, the Gray Cooperative was selected him to be a "National Cooperative Model" by the Ministry of Agriculture.

## **AATSEA Outstanding Leadership in Agriculture Development**



Yiming Zhu (China)

His honor deserved as in 2015-2017 to be "Wuxi City Model Workers", 2017 "The most beautiful young farmers in Wuxi", in in 2017 Xishan Mengchuang Lecture Hall "Innovation Pioneer", 2018 "Wuxi Excellent Agricultural Entrepreneur"In The 4th Jiangsu Province Star of Honesty Wuxi Youth Farmers Entrepreneurship Star. He is working experiences in agricultural enterprises in Wuxi. He graduated Bachelor degree of Marketing with Honours from University of Liverpool. He is the chief executive of Wuxi Doushan Baichayuan Tea Culture Co., Ltd.

He has appointed the professional skills as follows:-National secondary tea assessor, National Senior Dietitian, Soil selenium-enriched technology, Tea introduction and cultivation techniques, Product marketing and branding, Tea Derivatives Development.

He has done to adhere to high quality of agriculture, actively improve the soil condition of enterprise's tea bases, apply ecological bio-fertilizers, adhere to sustainable development of agriculture. He is appraised by the Agricultural Products Quality and Safety Center of the Ministry of Agriculture and granted a Certificate of "Certification for the Production of Pollution-free Agricultural Products" along with the title of "No pollution agricultural products".

He has contributed to build a corporate brands actively, cooperate with experts from China Tea Society to conduct special training for enterprise's production teams. The "Xibei Brand" trademark held by Wuxi Doushan Baichayuan Tea Culture Co., Ltd. was awarded "Jiangsu Famous Trademark" and "Wuxi's well-known trademark", the enterprise product "Taihu Cuizhu Tea" won the "Wuxi City's most popular and high quality agricultural products in 2018"; While he was developing the traditional Doushan tea industry, to be the first enterprise that has innovated and introduced the selenium-enriched golden leaf tea, overcome the problems of soil improvement technology, agricultural internet of things technology, and selenium-enriched fertilizer application technology. The project was funded by R&D of Wuxi Agricultural Science and Technology Support Project. The company became the first company in Wuxi to develop selenium-enriched tea.

He is also presided over the establishment of enterprise product traceability mechanism, tea field management and production management. Products sold were made to be transparent traceable. Wuxi white tea, Taihu Cuizhu, Biluochun tea that are produced by Wuxi Doushan Baichayuan Tea Culture Co., Ltd. products won the certificate of "China's Green food".

He is actively protected the intellectual property rights of the enterprise; applied for a number of design patents and utility model patents for the company's tea packaging and a invention patent for the production of black tea made from golden tea leaves, hosted the establishment of an online sales platform for agricultural products and formulated supporting sales plans.

He has carried out special knowledge lectures, providing agricultural professional technical support for surrounding farmers. That leads the surrounding farmers to increase income and improve corporate social benefits. Actively build the public welfare image of the enterprise. The company was awarded the title of "Jiangsu Science Popularization Service Station".

He has improved the field management mechanism; introduced agricultural Internet of Things equipments; replaced with advanced tea production equipments; set up a high-quality service team; and increased the scale of the enterprise. The enterprise has won the honor of "Wuxi Municipal Agricultural Industrialization Leading Enterprise".

He insisted that innovation is the foundation to maintain the vitality of company. For that reason, the company innovated and developed mulberry wine and tea wine. "The innovation of tea and wine and industrial integration development" project won the first "China Ocean Cup" National Rural Entrepreneurship Innovation Competition third prize in Jiangsu Province and participated in the national competition on behalf of Jiangsu Province.

He has contributed to the establishment of Baichayuan Entrepreneurship Alliance and set up an expert advisory team which provides free technical consultation for new agricultural entrepreneurs. Therefore, has been awarded one of the "The most beautiful young farmers in Wuxi" in Wuxi in 2017 and one of the "Innovation Pioneer" in 2017; as long as "2015-2017 Wuxi City Model Workers", "2018 Wuxi Excellent Agricultural Entrepreneur", "The 4th Jiangsu Province Honest Star", "Wuxi Young Farmers Entrepreneurship Star", etc. In 2018, the company was awarded to be one of 100 the theme creative farmland of Jiangsu Province, the "Wuxi Youth Farmers Entrepreneurship Demonstration Base" and the "Wuxi City initiative to promote agricultural industry revitalization demonstration points" and other titles.

#### **AATSEA Outstanding Leadership in Education and Research**

Firma Caccam - Viray (Philippines)

Dr. Viray is a retired full Professor and had been awarded the highest rank as University Professor of the Central Luzon State University (CLSU), Nueva Ecija. She has excellent performance in various dynamic fields of education, related sciences of agriculture and had served as Faculty Regent of the Central Luzon State University Board of Regent for two years. In recognition to her academic and managerial capabilities, she was elected as of President of Core Gateway College, Inc., San Jose City, Nueva Ecija, Philippines for three years after her retirement Dr. Viray earned a Ph.D in Home Economics Education from the University of the Philippines (UP) at Diliman, Quezon City in 1995, M.S. in Home Economics Education, UP in 1987, and B.S. in Home Economics (1978) at CLSU. An avid community and extension worker whose goal is elevate the quality of life of farm workers and the empowerment of women in the different barangays of Central Luzon. Her multi-discipline as home economist, cut across on family quality of life, under privilege women and children, women empowerment, farming systems/management being a farmer herself. As an advocate and practitioner, she encourages families to buy and eat/use organic/sustainable agriculture products for health reasons, healthy lifestyle, and to help protect the environment, as well as the farm workers. Dr. Viray is one of the leading advocate of small scale organic agriculture in Nueva Ecija, Philippines through her lectures as extension worker and hands on farmer. This is no surprise as she was born to a farming family in La Union which motivated her to do actual farming. She does not simply preach but "walk the talk", she applied it to her everyday lifestyle.

She is a member of various honorific and professional societies like the Pi Lambda Theta, International Honor Society and Professional Association in Education (by invitation), regular member to National Research Council of the Philippines, as President of the Philippine Colleges and Universities China Studies Association, Inc. (PCUCSAI) and as active member and officer to more than ten academic, research civic organizations

As an academic, she is a prolific writer and researcher, she has authored seven textbooks in the National Service Training Program, 12 technical papers (Internationally refereed journals), presented more than 15 technical papers in regional, national and international conferences; and several power point presentations in conferences, trainings, seminars and symposiums in China, Indonesia, Thailand, Malaysia and Japan.

An identified academic and R/D leader in her field, Dr. Viray is also well recognized as she is frequently tapped as technical adviser and consultants in several local, regional, national and international schools, colleges/universities, agencies and organization besides being invited as motivational speaker to civic organizations. She is regularly tapped/selected as an external examiner and lecturer of graduate students as far as Bugema University in Uganda, Africa; University of Swaziland, Africa; Hunan Agricultural University, China; and Maejo University, Chiang Mai, Thailand and University of Gadjah Mada, Yogyakarta, Indonesia.

In 2014, She is recipient of Golden Grain Award, the highest recognition regarded to an alumnus of CLSU as dean, director, administrator, professor, and speaker to different local and international for a. Awarded in recognition to "committed services and valuable contribution to the academe" by her college and the Institute of Graduate Studies, at CLSU. Other recognitions she received include: Plague of Recognition dedication to duty as director and

coordinator of the National Service Training Program and Civic Welfare Training Service Respectively. Meritorious citations and recognitions given her local and abroad as motivational invited speaker.

Dr. Viray is also selected by the Philippine Sino-Center for Agriculture Technology (PhilSCAT), first China project to the Philippines as the international coordinator and facilitator of trainings between CLSU and other colleges in the Philippines mostly on technology and innovations in agriculture. Till now, she still find time to do researches in collaboration with the academic research members and graduate students with the objective to help them write their thesis into journal form with high quality and for publication in refereed journals.

## **AATSEA Outstanding Leadership in Agriculture Extension**

**Kyaw Swar Soe (Myanmar)** 

U Kyaw Swar Soe was born in Yangon on 19th May of 1965. His parents are U Hla Soe and Daw Saw Sein. He studied at Myit Kyee Nar Township and Lwikaw Township for Primary and Middle School. He attended High School in B.E.S.H No.1 Thingangyun, Yangon. He passed 10th Standard in and joined Hlaing University in Yangon as a Psychology major student but due to having strike and instability in Myanmar at that time, all university sites and schools gone close. So, he went to Bangkok (Thailand) and then to Tokyo (Japan). When he was in Japan, he found the different status and life style of Myanmar and Japanese farmers. He was so sorry and felt that there is a need to help and develop Myanmar farmers to improve their status and life style. In 1996, he went back to Myanmar and made commitment not to do any more politics.

In 1998 he went to Australia and studied in Sydney College. In 2001 he came back from Australia and lived in Salin Township, Agway Division (The native land of his father). For his living he set up a Saw Mill and grew paddy in 95 acres. As his grandparents were landowners, he also settled down in that field. He realized very well about the very difficult life of farmers.

In Myanmar there are about 60 billion people, 70% of those people are farmers and they are so poor. The country practiced the Land Law either as private landlordism (1953), absolute landlordism (1963) or both (after 1988). All systems are not helpful for the farmers, their lives are more difficult, poorer than poor and getting a lot of debts. So some farmers left their jobs and joined to different sectors.

Then U Kyaw Swar Soe tired to attempt the election in 2010 as well as 2012. As he failed for many reasons, he decided not try again. But many farmers from up country came to him and requested to stand in front of them as a leader. They expressed their difficulties and problems. They also mentioned that without having a good leader, they won't be over come from such a very bad situation.

So his dream for feeling of willingness to help for the development of Myanmar farmers' status and life style came true. He established the "Myanmar Farmers Development Party". At the same time he planned to use the flag and logo as the same as former Farmers' leader Sayar San. He got the permission from the government for the establishment of the "Myanmar Farmers Development Party". Farmers are very much delighted for existing this kind of party and very much proud of their flag and logo. The local leaders from Yangon Mandalay Division started to recruit right people under the supervision of much proud of their flag and logo. The local leaders from Yangon Mandalay Division started to recruit right people under the supervision of U Kyaw Swar Soe

In 2012, the Land Laws are printed and distributed to farmers and explained them to study thoroughly for protection themselves. U Kyaw Swar Soe brought good quality paddy seeds from Japan. He tried to get lands from the government to distribute farmers who do not have land. Now, thousands of acres of lands were distributed. There are at least 40 entrepreneurs supporting the "Myanmar Farmers' Development Public Company" and are trying to bring lighting systems in many villages.

To gain international knowledge, children of the farmers are given a chance to attend training from some international NGO organizations. There are 30 branch offices in townships to solve the problems of farmers actively and attentively.

The Japanese Government and the Prime Minister invited U Kyaw Swar Soe for a visit they agreed to help for the development of Myanmar farmers and their villages as quickly as possible. Toshiba (Japan) Company also agreed to provide lighting system to villages. The Japan Agriculture Development pledged to cooperate and develop Myanmar farmers to world level.

The main object of U Kyaw Swar Soe is to scarifies for the farmers who never come out from the basic level that will be able to have to change participate in Hluttaw for their lives after the 2015 election. He has also decided to try to succeed in his main objective to develop the country as well as Myanmar' farmers. For 100 years ago, the famers had no party, now he established party for farmers and they are very happy. He is doing his best to make the party appear and grow.

#### AATSEA Outstanding Leadership in Research and Development

Dr. Samantha Chandranath Karunarathna (Sri Lanka)

He is specialized in Mycology eg taxonomy and phylogeny of Lentinus, Pleurotus giganteus, Clarkeinda and selected species of Agaricus in northern Thailand. He has joined at Soil Biology Group, Kunming Institute of Botany, Chinese Academy of Science, 132 Lanhei Rd, Heilongtan, Kunming. He used to be visiting researcher in Botanic Garden Meise, Belgium, French National Institute for Agricultural Research, Mycologie et Sécurité des Aliments (MYCSA), France, and World Agroforestry Centre, East and Central Asia office, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

Research grants involved research assistant of the National Center for Genetic Engineering & Biotechnology grant (grant number: BRN049/2553) "Value added products from basidiomycetes: Putting Thailand's biodiversity to use" from June 2009 – July 2012. Research assistant of the National Research Council of Thailand (NRCT) grant (grant number: NRCT/55201020007) "Taxonomy, phylogeny and cultivation of Lentinus in northern Thailand" from November 2011 to December 2014. Research assistant of the Mae Fah Luang University grant (grant number: MFU/54101020048) "Taxonomy, phylogeny and cultivation of Lentinus in northern Thailand" from January 2012 to February 2013. Research assistant of the Thailand Research Fund grant (grant number: BRG 5580009) "Taxonomy, Phylogeny and biochemistry of Thai Basidiomycetes" from June 2012 to July 2015.

He has a journal editor of Scientific Editor of the International Journal Studies in Fungi from March 2017 to date, Associate book review editor of Fungal Diversity, an International Journal of Mycology from January 2012 to date, Handling editor (Basidiomycetes) of the International Journal "Phytotaxa" from August 2014 to date, Editor of the International Journals Fungal Diversity, Mycosphere and Current Research in Environmental and Applied Mycology (CREAM) from June 2014 to date, Senior editor of the International Journal "Journal of Fungal Diversity" from January 2018 to date.

He has contributed his advanced research mushroom through publications and conferences for examples diversity, taxonomy and phylogeny of Lentinus (Basidiomcetes) from northern Thailand. Current status of knowledge of Sri Lankan mycota. New edible mushrooms: from discovery to production, Education system in Sri Lanka, Lentinus (BASIDIOMYCOTA)- exiting findings from Thailand, Biodiversity of mushrooms in northern Thailand forests, economics of sustainable forestry practices, New edible fungi from Southeast Asia: discovery to production, New edible fungi from Southeast Asia: discovery to production, New edible fungi from Southeast Asia: discovery to production, Forest-Fungi Systems: From Yunnan to the Greater Mekong, 2018 Greater Mekong Forum on Water, Food and Energy", New edible fungi from Southeast Asia: discovery to production, Organic mushroom growing: Improving human health and livelihoods. He has internationally publication over 69 scientific papers.

## **AATSEA Outstanding Leadership in Research and Development**



Dr. Sundaram Lalitha (India)

Dr. Lalitha has demonstrated the ability to work well as an integral team member and independently. She is to be adept in planning, execution of experiments on her own and in reporting research results. She is very good at leading a team and managing people at laboratory. She has excellent ability of preparing scientific publications and culminated research results to a series of papers. She is good at using modern technology for teaching and presents materials clearly for diverse audience.

Dr. Lalitha has a good command over her subject area and her publication record that is a testimony of her perseverance and ingenuity. One thing that sets her apart is her dynamic personality which clearly reflects in her interpersonal, oratory and management skills

She showed an excellent visual presentation, very informative, effective facial expressions – hand gestures – body language, good display of confidence, excellent use of audience interaction, and voice modulations were positive aspects of her talk. She was the youngest invited researcher.

She showed interest to learn and does not hesitate to clear her doubts with her seniors or counterparts. She has a good command over her subject area and her publication record is a testimony of her perseverance and ingenuity. One thing that sets her apart is her dynamic personality which clearly reflects in her inter-personal, oratory and management skills. She is to be highly competent, enthusiastic, articulate and caring individual that interfaces well with faculty, staff and students alike. She is adept at planning, executing and reporting her research objectives well within the time frame. She executes work assigned to her with great precision and devotion.

Dr. Lalitha demonstrated the ability to work well as an integral team member and independently. She has excellent ability of preparing scientific publications and culminated research results to a series of papers. She is good at using modern technology for teaching and presents materials clearly for diverse audience. Dr. Lalitha will significantly contribute in meaningful manner to the research and teaching missions at her University and to the humankind.

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# Abstrac

# **Keynote Session**

Global trends on food security and future outlook

Konuma, H.

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4th Industrial revolution and the sustainable strategies of addressing the large-scale challenges

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The  $4^{th}$  Industrial Revolution ( $4^{th}$  IR) is still at infant stage but it is perceived already as "double edge sword". On one side, it can magnify the technology – led divides on "water, digital, food, economic, and socio-political standing" On the other hand, technologies of  $4^{th}$  IR can also address the pitfalls of the  $1^{st}$ ,  $2^{nd}$  and  $3^{rd}$  industrial revolutions that led to the current environmental problems i.e climate change, soil, water, air pollution, depletion of fishing stocks, waste on land and ocean, loss of biodiversity and deforestation. The most compelling is how to address climate change i.e. the urgency of sequestering back 1.2 TGt of  $CO_2$  eq to mitigate the harsh effects of global warming/ climate change. The food systems is the major source of of greenhouse gases (GHGe) calculated to emit up to 57% GHGe. This could be intensified or lessened in the  $4^{th}$  IR. Initiatives on lessening emissions are being done by many groups and institutions. Listed and briefly discussed in the paper are some  $4^{th}$  IR technologies and systems could be utilized to addressed the challenges.

Including Sustainability Sciences, there are 2 complementary disciplines (Food systems energy accounting and food thermodynamics) must be institutionalized in the academe to help assess  $4^{th}$  IR technologies in terms of their cash, energy,  $CO_2$  eq emissions as measures of their sustainability in addition to their inclusiveness and their risks. Likewise, earlier and on-going discussions on de-industrialization, post materialism and limits to growth are necessary.

Keywords: food systems, food energetics, food thermodynamics, technology-led divides, global warming/climate change

Control of rice blast disease

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#### Session 1 ORGANIC AGRICULTURE AND BIOLOGICAL PRODUCTS

Shifting and dominance of weed species following solarization treatment under organic farming system in tropical highland

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Weed control is essential to improve plant productivity under organic farming system. This practice often leads to shifting of weed species. The objective of this experiment were to determine weed shifting following solarization treatments in long term tropical highland organic farming system and to find out weed dominance. The experiment was conducted in CAPS Research Station situated in Air Duku Village, Bengkulu, Indonesia at 1054 m above sea level, employing Completely Randomized Block Design with three replications. The treatments were five different color plastic mulches, i.e., silver-black, black, clear, and red as well as control (without mulch). Plastic mulch was laid on 1m wide x 2 m long raised soil bed for four and eight weeks for solarization treatment. After solarization treatment, soil sample was collected and weed seed germination was tested in greenhouse. Species and number of weeds were observed after four weeks. It was observed that a month solarization treatment brought about alteration of weed species but the number was not significantly different. Eleusine indica was predominant in control whereas Bidens pilosa and Ageratum conyzoides were prevailing in solarization treatment. After two months of solarization, there was also a shift in weed composition. Eleusine indica was still dominant in control but Sinedrella nodiflora was prevailing in black and clear plastic mulch. Solarization treatment significantly decreased weed dry weight where a month of solarization reduced weed dry weight by 51.5, 48.17,41.58 and 36.10% in treatments of black, silver-black, red, and clear plastic mulch, respectively as compared to that control. In addition, two months of solarization decreased weed dry weight by 45.84, 13.08, and 57.09% in treatments of black, red, and clear plastic mulch, respectively in comparison to that control. After two month of solarization, clear plastic mulch is the most effective to reduce weed dry weight.

Keywords: weed shifting, weed dominance, organic farming, solarization, mulch

Detection of coliforms and Salmonella strains in salad vegetables from selected organic farms in laguna and Quezon, Philippines

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Increased consumption of raw vegetables as part of a healthier lifestyle has, ironically, also led to an increased risk of foodborne diseases due partly to bacterial pathogens. For enhanced awareness of both the farmer-producers and the consumers, this study was conducted to assess the extent of contamination with coliforms and *Salmonella* for probable food safety risks of salad vegetables grown in selected organic farms in Laguna and Quezon, Philippines. Samples of salad vegetables obtained at different time periods from four "practicing organic" farms were microbiologically analyzed for heterotrophic count, and for the presence of coliforms (total and putative *E. coli* counts) and *Salmonella*. Generally, the heterotrophic counts obtained varied by vegetable type within a farm, and by farm. Coliforms, commonly used as indicator organisms to detect the hygienic status of foods, water and the environment, were detected in all of the vegetable samples analyzed from all four farms. *E. coli*, a fecal coliform, and *Salmonella*, a known foodborne pathogen, were also detected putatively in all of the salad vegetables analyzed. Purified isolates are being phenotypically characterized and will be molecularly identified using 16S rRNA gene sequencing analysis.

Keywords: coliforms, E. coli, Salmonella, salad vegetables, organic farms, Quezon and Laguna, Philippines, food safety risks

Yield performances of potato (Solanum tuberosum L.) as amended with liquid organic fertilizer and vermicompost

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Closed Agriculture Production System (CAPS) Research Center, Faculty of Agriculture, Universitas Bengkulu has developed liquid organic fertilizer since 2014 by using locally available natural resources for vegetable production under closed agriculture production system. The effectiveness of liquid organic fertilizer has been evaluated on sweet corn, carrot, caisim, cauliflower, long bean, ground nut and broccoli. This experiment aimed to evaluate the yield performance of potato to liquid organic fertilizer under various vermicompost dosages. A field experiment was established using split-plot design with three replicates where vermicompost dosages (5, 10, 15, 20 and 25 ton ha<sup>-1</sup>) as main plot and liquid organic fertilizer (no liquid organic fertilizer versus liquid organic fertilizer) as sub-plot. Yield responses of potato were expressed as tuber diameter, total number of tuber per plant, total weight of tuber per plant, tuber weight per plot and number of marketable tubers. Results indicated that the use of liquid organic fertilizer increased total number of tuber per plant, total weight of tuber per plant, tuber weight per plot, but not tuber diameter and marketable tubers. The use of vermicompost increased tuber diameter, total weight of tuber per plant, tuber weight per plot and number of marketable tubers, but not total weight of tuber per plant. No interaction effects were recorded to all observed variables. Both vermicompost and liquid organic fertilizer could independently increase potato yields. Under closed production system, the used of 10 ton ha<sup>-1</sup> vermicompost was as effective as of 15, 20 and 25 ton ha<sup>-1</sup>. Still, research must be done to determine the optimum concentration of liquid organic fertilizer for potato production.

Keywords: liquid organic fertilizer, vermicompost, potato yields

Progress in sweetcorn hybrid breeding program for organic agriculture in the tropics

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The scarcity of varieties best suited to organic agriculture has been a problem faced by organic growers in the tropics for a long time. So far, most of the growers inevitably have to use varieties that were bred for the conventional production and such varieties often do not perform well under organic crop management systems. The current organic sweet corn breeding program is implemented to develop hybrid varieties best suited for organic crop management as demanded by the organic sweet corn growers. The program was initiated by evaluating 20 out of 25 sweet corn varieties based on their general performances under organic crop management. A polycross mating scheme was then performed on these 20 varieties and the resulting hybrids were subjected to three cycles of selfing and test cross for the determination of general combining ability for the traits of interest. A further series of selfing was performed to the eight inbred lines showing good combining ability in the test cross, named Caps 1, Caps 3, Caps 15, Caps 17A, Caps 17B, Caps 22, and Caps 23. At seventh generation of selfing, these lines were crossed in a half-diallel mating scheme to evaluate the genetic properties of the crosses. These included the general combining ability of the inbreds, the specific combining abilities of the resulting hybrids, and the heterosis effects. Results to date are 12 hybrids showing their potential for organic production. Lesson learned and direction of the breeding program are described and discussed.

Keywords: combining ability, hybrids, inbred lines, organic breeding, selection

Obtaining heterosis in hybrid rice: the innovation pathway for increasing rice yield

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As relatively new innovation, we tried to find agronomic ways to obtain heterosis in growing hybrid rice under low external input(organic) or fossil fuel energy based- inputs free production systems and compare it to agrochemical intensive production systems. Hence, series of studies were conducted as follows: In study 1, we evaluated 20 rice cultivars (17 inbreds, 3 hybrids, planted in 20x20cm) and they were grown under organic production systems. Of the 20 cultivars, highest grain yields were obtained in 20x20cm, and they were grown under organic production systems. Of the 20 cultivars, highest grain yields were obtained in 20x20cm, and they were grown under organic production systems. Of the 20 cultivars, highest grain yields were obtained in 20x20cm, and they were grown under organic production systems. Of the 20 cultivars, highest grain yields were obtained in 20x20cm, and they were grown under organic production systems. Of the 20 cultivars, highest grain yields were obtained in 20x20cm, and they were grown under organic production systems. Of the 20 cultivars, highest grain yields were obtained in 20x20cm, and they were grown under organic production systems. The arrows the contraction of the 20 cultivars are the 20 cultamong the inbreds. Best inbred (PSB RcI8, 8.5 t ha1) outyielded the hybrids when grown in double row planting pattern. In study 2, we evaluated hybrids and inbreds grown in 3 spatial arrangements and under organic and agrochemical intensive production systems. Under organic, Bigante plus (a hybrid) yielded the highest (5.7 t ha-1) when grown in 30cm x 30cm spacing. The energy return over input was 16 (EROI=16) and energy foot print was only 80 kg CO<sub>2</sub>e/ton. NSIC 222(inbred) yielded 5.3 t ha-1 had the same energy footprint with Bigante plus. Fossil fuel energy based- inputs intensive grown SL8 (hybrid) yielded the highest under double row spacing at 8.6 t ha-1. The energy foot print was 425 kg CO2e ton-1 of unmilled grain which was 5.3 times higher than organically grown rice. In study 3, fertilizer applied at 50% of recommended rates and planted the hybrids (SL8, M20) and inbreds (NSIC 222, PSB82) in 3 spacings (20x20cm,30x30cm, double row (2x25) x50cm). When grown and fertilized at 50% of recommended rates, SL-8, a hybrid yielded 9.2 t ha<sup>-1</sup> in double rows and 9.07 t ha<sup>-1</sup> when planted in 30cm x 30cm spacing. When planted in 20cmx20cm, yield was 21% lower at 7.2 t ha<sup>-1</sup>. As early as seedling stage, manifeststion of heterosis was noted when sparsely seeded @ 1,500 seed per square meter. Fifteen day-old transplanted hybrid seedlings had already 5 to 7 young tillers and tillering was sustained that produced more panicle bearing tillers with more grains per panicle, thus, higher grain yields. Hybrid seeds are 70 times more cash expensive than inbreds. But only 4 kg seeds/ha are required when transplanted at 30cm x30cm spacing. Application of fossil fuel energy based-inputs gave higher yields but higher energy footprint. Yields of 6.0 t ha<sup>-1</sup> using hybrids grown under organic method using 4 kg seeds/ha had the lowest energy footprint.

Keywords: hybrids, inbreds organic, fossil fuel based energy inputs, energy footprints

Encapsulation of Pediococcus pentasaceus RSU-Nh1 into pectin-sodium alginate and chitosan coating

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A number of lactic acid-producing bacteria have been used as probiotics. They are beneficial to the human intestine which inhibit the growth of pathogenic microorganisms and also used for lactic acid fermentation. However, lactic acid bacteria (LAB) could be partially digested when passing through the digestive system resulting in to decrased survival rate. Encapsulation might improve the survival of probiotic bacteria by protecting them from harsh conditions in digestive system of gastrointestinal tract. This research aimed to investigate the survival of *Pediococcus pentasaceus* RSU-Nh1, which was isolated from Thai fermented pork or "Nham", in different treatments which are pectin-sodium alginate, chitosan-sodium alginate and pectin-sodium alginate coated by chitosan at 0, 60, 120 and 240 minutes under simulated gastrointestinal conditions. In this study, the initial cell number of *Ped. pentasaceus* RSU-Nh1 of each treatment was approximately 10 CFU/ml. At the final time, the results displayed that the free cell had survived about 7.68 CFU/ml, whereas encapsulation by using sodium alginate combined with various pectin extracted from pomelo and passion fruit peel exhibited the survival cell approximately 7.35 and 8.12 CFU/ml, respectively. In addition to encapsulation with chitosan-sodium alginate, combining chitosan from shrimp shell and fish scale, showed survival cell approximately 8.59 and 8.08 CFU/ml, respectively. However, encapsulation with pectin from passion fruit and coated with chitosan

from shrimp shell showed the highest number of *Ped. pentasaceus* RSU-Nh1 about 9.24 CFU/ml in the gastrointestinal simulation. Our results revealed that encapsulation improved the survival of *Ped. pentasaceus* RSU-Nh1 in simulated gastrointestinal conditions.

Keywords: encapsulation, lactic acid bacteria, pectin, chitosan, simulation

#### Residual effect of different organic fertilizer types and application time on rice growth and yield

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Application rates of organic fertilizer depend on rice nitrogen requirement. Continuous applying of organic fertilizer can cause some elements to accumulate in the soil which may differ by organic fertilizer types and application time in the previous crop. This study was conducted to determine the residual effects of different organic fertilizer types and application time on the growth of rice RD 43 cultivar and nutrient uptake. The experiment was conducted in a greenhouse with a randomized complete block design (RCBD) with three replications. The treatment was seven treatments consisted of 1) soil without organic fertilizer application (control), 2) compost residue without split application (CP1), 3) compost residue with two split application (CM2), 6) compost and cow manure residue without split application (CM2), 6) compost and cow manure residue (CP1+CM1) and 7) compost and cow manure residue with two split application (CP1+CM2). The results showed that the residue cow manure with and without split application gave significantly highest tillering, grain fresh and dry weight as compared to other treatments. In addition, nitrogen uptake in grain and potassium uptake in straw from the cow manure residue was significantly different from the residual of compost. But, phosphorus uptake in straw and grain yield of all treatments was not significantly different.

Keywords: nutrient uptake, residual effect, compost, cow manure

Organic farm learning and practical networks: facilitation for learning and innovation for sustainable agriculture

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This paper examines the learning and practical networks and role of organic farmer networks in facilitating farmer innovations in complex agro-ecological farming systems through a study of a sample of organic farmers in the case of Praibueng district, Thailand. Forty-five representative of 145 farmers were sampled. Building on a knowledge systems and social learning perspective related learning and practicing program in school together with their child, illustrates how learning is triggered and agricultural innovations diffused among them using sustainable producers through an interactive, participatory style of problem-solving. Finally, the research links farmers' social learning processes thought integrated learning and practical networks to critical reflection on the potential niches in organic farmer management for extension practice together among farmer family and young generation.

Keywords: Organic farm, farmer participation, learning and practical networks, sustainable agriculture

CO<sub>2</sub> emission and accumulation of soil organic matter under sweet corn stand in the long term organically managed land

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It has been widely recognized that agriculture practice emits a significant amount of carbon dioxide. Long-term practice of organic agriculture accumulates organic matter in the soil; however, at the same time releases carbon dioxide. The study aimed to evaluate carbon release and soil organic matter accumulation under sweet corn stand in the long-term organically managed land. The experiment was conducted at Closed Agricultural Production System (CAPS) Research Station located in Air Duku Village at 1054 m above sea level, employing Randomized Completely Block Design (RCBD) with five treatments, i.e., 5, 10, 15, 20, and 25 Mg ha-1 of vermicompost. The treatment was replicated three times. Carbon dioxide release was measured at a day before and tillage, 1, 2, 3, 4, 10, 17, 28, 35, 41, 49, 56, 63, 70, and 77 days after tillage using modified Anderson Method (1982). At the same time, the soil temperature was recorded at 15 cm depth. At sweet corn harvesting, a soil sample was collected at 0-10 and 10-20 cm depths and analyzed for Total Soil Organic Carbon (TSOC), exchangeable Al and soil pH. The study indicates that carbon flux from the organically managed sweet corn stand fluctuates during the growing period. However, the increasing rate of vermicompost does not influence carbon flux unless soil disturbance has occurred (tillage and heaping). When soil disturbed, high rate of vermicompost emits carbon dioxide higher than the lower one. In addition, soil organic matter at 0-10 cm depth for 3-year accumulation increases at a higher rate of vermicompost but not at a depth of 10-20 cm. Soil organic matter accumulation led to reduction of exchangeable Al and increased soil pH. Carbon release from organically managed land is highly dependent on soil disturbance and crop growth stage.

**Keywords:** carbon emission, carbon accumulation, sweet corn, organic farming.

Superiority test of mixed-cropping models for chili pepper hybrid varieties through participatory plant breeding

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Mixed cropping system aims to increase chili pepper productivity by suppressing insect and pathogen developments. Participatory

plant breeding might produce desired variety and combination of hybrid varieties. This experiment aimed to obtain superior model for mixed cropping for chili pepper variety, insect and disease resistances as preferred by consumers. Experiment was conducted using randomized completely block design with three replicates. Treatments consisted of four chili pepper hybrids, i.e. K01=mono cropping of UNIB CH13, (2) K02=mono cropping of Dwiguna UNIB, (3) K03= mono cropping of UNIB CH63, (4) K04=mono cropping of UNIB CH65, (5) K05=mixed cropping of UNIB CH13 and Dwiguna UNIB, (6) K6=mixed cropping of UNIB CH13 and UNIB CH63, (7) K07=mixed cropping of UNIB CH13 and UNIB CH65, (8) K08= mixed cropping of Dwiguna UNIB and UNIB CH63, (9) K09=mixed cropping of Dwiguna UNIB and UNIB CH65, (10) K10=mixed cropping of UNIB CH63 and UNIB CH65. Results showed that the best mixed cropping for chili pepper hybrids was UNIB CH13 and Dwiguna UNIB. This combination produced total fruit weight of 300.12 g per-plant, longest fruit length of 13.66 cm, fruit stalk of 5.43 cm, canopy area of 3172.53 cm<sup>2</sup>, biggest stem diameter of 11.79 mm and highest fruit weight of 5117.22 g per-plot. Mixed cropping of UNIB CH13 and Dwiguna UNIB had the lowest Cercospora sp, though pathogen attacks of Anthracnose, Fusarium blight and yellow leaf curl Begomovirus were not significantly different.

Although consumers prefer to select mono cropping of UNIB CH13 and Dwiguna UNIB, consumers chose the combination of UNIB

Keywords: mixed cropping, chili pepper hybrid, participatory plant breeding.

CH13 and Dwiguna UNIB for fruit shape and homogeneity.

#### Organic agriculture model

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Research and development of bio-products as agricultural inputs for organic agriculture has been conducted since 1989. The bioproducts are released and contributed in many countries eg. Thailand, Laos, Myanmar, Cambodia, Vietnam and China eg. Super Ketomium as biofungicide, bio-decomposer, bioinsecticide, bionutrients and biofertilizers etc. Organic agriculture model has been demonstrated e.g KMITL and AATSEA Organic Models in Thailand, Organic Coffee in Laos, Organic coconut and other plants in Myanmar etc., to contribute to the research findings of biological products as agricultural inputs to be used for promoting organic agriculture to the farmers. The organic model is divided into six parts as follows:- production, agricultural inputs, organic certification, marketing, extension and training as well as research and development. The model is a process from production to marketing including research findings which necessary to serve the model. The organic model also serve for environmental protection, maintain agro-ecosystem and sustainable development, and Torganic products free from toxic agrochemicals. Association of Agricultural Technology in Southeast Asia (AATSEA) is non-profitable organization which established in 9 March 2011 and officially approved on 17 April 2012. AATSEA is active in a variety of training programs for agricultural technology, especially organic agriculture. It has also done organic certification in Thailand, Vietnam, Cambodia, Myanmar and Laos since 6 august 2018. Marketing is very important factor for cultivation thereafter production. The organic model has committed to produce organic vegetables and animal for delivery to the markets. Organic production planning would depend on production order. Which kinds of vegetables would be cultivated including the quality, quantity and price should be known earlier. Organic shop and home delivery had been started for marketing promotion. The training program for organic agriculture has been organized for organic farmers. A pilot project of transfer technology for organic agriculture model to expand learning network in the areas and to train interested farmers to gain knowledge for commercial scale organic agriculture had been initiated. Doing researches supportive to organic agriculture eg. agricultural inputs for organic agriculture, organic seeds, organic animal feeds, organic food processing etc.; are indeed necessary.

Keywords: Organic Agriculture Model, Agricultural inputs, bio-products

Growing organic herbs to promote the local enterprises for sustainability under plant genetic conservation project, suratthani, thailand

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This study was mainly focused on growing organic herbs to promote local enterprises for 34 members under plant genetic conservation project. The research design was both qualitative and quantitative. Data were collected by in-depth interview, participatory focus group discussion. The data received were analyzed by frequency, percentage, mean, standard deviation, and content analysis. The results found that 23 (67.65%) members were continually growing organic herbs with 5 organic herb types and processing for local enterprises. All of them gain knowledge from the program in terms of planting, management, processing, and marketing. Most of them applied their own organic fertilizer for planting (87.50%). In terms of herb types, customers order were followed that increased their income. We out found that members had improved cooperation together with a local organization to develop activities contributing to improving quality of life for the community members, health care, mental health, social and environmental relationships. Moreover, the members and community involvement approach of the related local organization was encouraged by promoting the awareness of the public, creating a cultural awareness in the community of using organic products for more sustainable living. Processing techniques, production tools, standards and product quality certificate were the need to improve further the project.

Keywords: Growing organic herbs, local enterprises, sustainability

#### Weed inhibition and sorghum yield as affected by organic mulch in tropical coastal environment

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Weed control is a common practice in sorghum (Sorghum bicolor, L.) cultivation to prevent yield loss. Organic mulch is believed to inhibit the growth of weed. The study was undertaken to determine the influence of selected organic mulch on the weed growth and yield of sorghum in the tropical coastal environment. The experiment was assigned in Randomized Complete Block Design (RCBD) with two factors. The first factor was organic mulches consisting of rice husk, rice straw, and control (no mulch) and the second factor was sorghum varieties, i.e., Keller, Super-2, and Samurai. The results showed that the most inhibited weed was observed in the treatment of rice straw mulch at 5 weeks after planting date (WAP) and at harvesting with a dry biomass weight of 13.49 g and 9.61 g, respectively. Nonetheless, the organic mulch had no prominent effect on sorghum yield. Keller variety had the highest biomass, but its yield was lower than the other two varieties. This result indicated that Keller variety is more suitable for animal feed while Samurai and Super-2 varieties are prospective for human consumption. In addition, all sorghum varieties tested in this study are potential to be cultivated in the tropical coastal environment.

**Keywords:** coastal, sorghum, rice straw, rice husk, weed control

#### Farmers' adoption and attitude towards organic rice production in chachengsao province, thailand

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This study aimed to describe the socio-economic characteristics of rice farmers, investigate the factors affecting the adoption of organic rice production by farmers, and explain organic farmers attitude towards organic farming. Primary data were collected through structured questionnaires from 108 farmers - 50 organic and 58 chemical rice farms in Chachengsao Province, Thailand. The samples were selected through purposive random sampling technique. The data analysis was carried out using descriptive analysis to describe the socio-economic characteristics of rice farmers. Logistic regression was also employed to investigate the factors influencing organic rice adoption. Results showed that organic rice farmers had an average age of 51 years old, gained 8 years of education, had 2 household members, had approximately 33 years of rice farming experience, and farming 16 rai of rice area. Regarding chemical rice farmers, on average, they were 56 years old, gained 5 years of education, had 2 household members who gained approximately 33 years of rice farming experience, and occupied 23 rai of rice production area. Logistic regression results revealed that education (positive), farming experience (positive) and farm size (negative) had highly significant (p < 0.01) influence on organic farming adoption. These results implied that as more farmers are educated the more farmers adopt to organic farming, similar to those who have more experience. This finding further indicated their willingness to take reasonable risks and new farming practices with the knowledge that they acquire. However, farm size showed negative significance of organic farming adoption which revealed that farmers owning smaller farm sizes are more likely to adopt organic farming because of less intensive labors and lower capital requirements, among others.

Keywords: organic rice, organic adoption, socio-economic, organic rice farming, adoption factors

#### SESSION 2 PLANT BIOTECHNOLOGY

Bioactive secondary metabolites from *Chaetomium* spp.

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Investigation on fungi isolated from Thai soil genus *Chaetomuim* spp. resulted in the isolation of numerous types of compounds for example *cytochalasans*, *azaphilones*, *xanthones*, *xanthoquinodins*, depsidones. The isolated compounds were evaluated for their bioactivities such as antimalarial activity against Plasmodium falciparum, antimycobacterial activity against Mycobacterium tuberculosis and antibacterial, as well as cytotoxicity against KB, BC1, NCI-H187 and cholangiocarcinoma cell lines. In addition, some of the isolated compounds were tested for a bio-control agent against plant diseases. This presentation will highlight our work on chemical and biological aspects of these isolated compounds.

Keywords: Chaetomuim, cytochalasan, azaphilone, depcidone, antimalarial, anti-TB, cytotoxic, antibacterial, plant diseases

Yield and Growth of Pak Choi and Green Oak Vegetables Grown in Substrate Plots and Hydroponic Systems with Different Plant Spacing

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The yields of Pak Choi and Green Oak vegetables grown in deep flow technique hydroponic system with narrow plant spacing of 10 cm x 12 cm (HydroDFT10x12) were significantly higher than those vegetables grown in other planting systems with different plant spacings. The yield of Pak Choi grown in HydroDFT10x12 was the highest due to having the highest plant density that compensated for the low fresh weight per plant, narrow canopy diameter, and low number of leaves per plant. Green Oak grown in HydroDFT10x12 had the second highest yield due to having high plant density, high fresh weight per plant, highest canopy diameter, high number of leaves per plant, and tallest plant height. Yields of Green Oak grown in Sub20x25 (substrate plot with wide plant spacing of 20 cm x 25 cm) and HydroNFT20x25 (nutrient film technique hydroponic system with wide plant spacing of 20 cm x 25 cm) were lower than in other treatments caused by the lowest plant density. Pak Choi had significantly higher yield than Green Oak, as Pak Choi grown with higher plant density compensated for its lower canopy diameter, lighter fresh weight per plant, and lesser number of leaves per plant. Plant density was the main factor for the higher yield of Pak Choi. HydroDFT10x12 gave the significantly highest yield, higher than Sub20x25, Sub10x12 (substrate plot with narrow plant spacing of 10 cm x 12 cm), HydroNFT20x25, and HydroDFT20x25 (deep flow technique hydroponic system with wide plant spacing of 20 cm x 25 cm), which was due to the high plant density, widest canopy diameter, tallest plant height, and high fresh weight per plant as indicated by

positive values of the correlation coefficients. Therefore, the HydroDFT10x12 is the most suitable planting system for producing Pak Choi and Green Oak vegetables.

Keywords: substrate culture, deep flow technique, plant spacing, Pak Choi, Green Oak

Effects of preprocess elicitors immersion on physicochemical quality of fresh-cut papaya cv. 'Holland' fruits during cold storage

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Postharvest treatment of elicitors such as salicylic acid (SA) and methyl jasmonate (MeJA) improve postharvest quality of fruits and vegetables. To investigate the preprocess immersions of SA or MeJA on physicochemical quality changes of fresh-cut papaya cv. 'Holland' fruits during cold storage, this study was conducted. The fruits were immersed in 2 mM SA or  $10^{-4}$  M MeJA for 1 hour and then stored at room temperature ( $26 \pm 1$  °C) for 24 hours before fresh-cut processed. The biochemical parameters such as firmness, total soluble solids (TSS), colour attributes, antioxidant activities and bioactive compounds of fresh-cut papaya fruit during storage at 4 °C for 8 days were monitored. Both SA and MeJA treatments effectively maintained firmness but had no influence on the changes in TSS and colour attributes over storage. Antioxidant capacity of the fresh-cut papaya was enhanced by SA or MeJA immersion while the free radical scavenging activity was only induced by SA immersion. The amount of total phenols and flavonoids of SA treated fresh-cut fruit were higher than those of control. MeJA immersion induced flavonoids content but did not induce total phenols. In conclusion, preprocess immersion of SA and MeJA could maintain texture and improve nutritional quality of fresh-cut papaya fruit during storage.

Keywords: elicitors, fresh-cut, papaya, physicochemical quality

Sugars, nutrition and organic acids content of granulated rambutan (Nephelium lappaceum Linn.) sugar

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Rambutan (*Nephelium lappaceum* Linn.) of the Sapindaceae family, originated from the Malayan archipelago, which includes Indonesia, Malaysia and Southern Thailand. The fruit is relatively rich in sugar, vitamins and mineral contents, and organinic acids. A 100 g sample of rambutan fresh is composed of 82.1% water, 0.9% protein, 0.3% fat, 0.3% ash, 2.8 g glucose, 3.0 g fructose, 9.9 g sucrose, no starch, 2.8 g dietary fiber, 0.05% malic acid, 0.31% citric acid, 0.5 mg of niacin, 15 mg of calcium, 0.1 to 2.5 mg of iron, 70 mg of vitamin C, 0.01 mg of thiamine, 0.07 mg of riboflavin, 140 mg of potassium, 2 mg sodium and 10 mg of magnesium (van Welzen *et al.*, 1988). The granulated rambutan sugar production process was published (Charoensuk *et al*, 2015). In this study we reported on the content of sugar, mineral and organic acids in the granulated rambutan sugar. The dominant sugars were sucrose that ranged from 85-87%w/w, and followed by glucose and fructose of 6.6 and 3.7%w/w, respectively. The granulated rambutan sugar was shown to be rich in calcium (28 mg), Sodium (16 mg) and ferous (0.3 mg) in 100 g. Citric and malic acid were 120 and 40 mg in 100 g, respectively. Granulated rambutan sugar as the sweet source for incorporation into diets is an option.

Keywords: sugars, mineral, organic acids, granulated rambutan sugar

#### MoFap7, a ribosome assembly factor, is required for fungal development and plant colonization of Magnaporthe oryzae

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Fap7, an important ribosome assembly factor, plays a vital role in pre-40S small ribosomal subunit synthesis in Saccharomyces cerevisiae via its ATPase activity. Currently, the biological functions of its homologs in filamentous fungi remain elusive. Here, MoFap7, a homologous protein of ScFap7, was identified in the rice blast fungus *Magnaporthe oryzae*, which is a devastating fungal pathogen in rice and threatens food security worldwide. Δ*Mofap7* mutants exhibited defects in growth anddevelopment, conidial morphology, appressorium formation and infection, and were sensitive to oxidative stress. In addition, site-directed mutagenesis analysis confirmed that the conserved Walker A motif and Walker B motif in MoFap7 are essential for the biological functions of *M. oryzae*. We further analyzed the regulation mechanism of MoFap7 in pathogenicity. MoFap7 was found to interact with MoMst50, a regulator functioning in the MAPK Pmk1 signaling pathway, that participates in modulating plant penetration and cell-to-cell invasion by regulating the phosphorylation of MoPmk1. Moreover, MoFap7 interacted with the GTPases MoCdc42 and MoRac1 to control growth and conidiogenesis. Taken together, the results of this study provide novel insights into MoFap7-mediated orchestration of the development and pathogenesis of filamentous fungi.

#### Kinds of retardants inducing potato (Solanum Tuberosum L.) microtuber production

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Tuber dormancy is believed to be one of the most important constraining factors in potato growing. This study was conducted to determine the effect of retardant on potato microtuber induction. The experiment was carried out at Bengkulu University from February 2017 to August 2017. The experiment used three microtuber retardants and microtubers were induced in vitro using MS medium supplemented with 125 ppm Paclobutrazol, 300 ppm CCC, and 75 Coumarine. The results showed that all of the media were able to induce potato microtuber *in vitro*. However, the best media was MS + 125 ppm Paclobutrazol.

Keywords: retardants, potatoes tubers

#### Fects of compost and chelated ca on early growth of para rubber (Hevea brasiliensis)

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Chanthaburi province is the major province for Para rubber (*Hevea brasiliensis*) production in the east of Thailand. A field trial was undertaken in the second rotation to determine any benefit of applying commercial compost together with NPK fertilizer for early growth. As Para rubber may also benefit from additional soil Ca in parts of Thailand, chelated Ca was included as a factor in the trial. A 4 x 4 factorial (4 rates of compost: 0, 1,500, 3,000 and 4,500 g tree<sup>-1</sup>; and 4 rates of chelated Ca: 0, 200, 400 and 600 g tree<sup>-1</sup>) RCBD field experiment was conducted with 4 replications on Para rubber "RRIM 600". Data for stem diameter, height, leaf and internode length were collected 1 year after planting. Application of commercial compost at 3,000 g tree<sup>-1</sup> promoted stem diameter, leaf length and internode length of Para rubber. Further research is required to identify any requirements for the addition of Ca and organic material for Para rubber growth during the later latex production stage.

Keywords: chelated Ca, compost, Para rubber

#### Influence of harvesting day and potassium chloride on sweetness of melon grown in plastic house

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This study determined the relationship between harvesting date and potassium chloride on melon fruit quality grown in plastic house. Melon cultivar used is "#20" orange flesh grown in the plastic bag 14-inch diameter. The growing media were soil and manure rate 3:1 mixed well under natural day light. The treatmeats were as follows: harvested on fruit date after fruit set on 45, 50, 30day+KCL 3g/pot 45day, 36day+KCL 3g/pot 50day, 30day+KCL 5g/pot 45day and 36day+KCL 5g/pot 50day. The statistical model was completely randomized design composed of 6 treatments and 3 replications. The results showed that melon increased in height according to increasing of planting date. On harvesting day therefore significantly difference among treatment in TSS and TA content of juice. The most TSS content of juice extract with the mean of 16.33 "brix received from those melon fruit treated with KCL 5 gram + 50 day harvested and showed significantly difference among treatmant. The second and the lowest TSS content got from those treated with 36day+KCL 3g/pot 50days and 45 days harverted with the mean of 16.20 and 15.07 "brix respectipely. The fruit weight had a range 2293.30 to 2405.30 gram while there is less impact on the fruit volume, fruit circumstance flesh and peel thickness. The harvesting date 50 days shown more pronounce effect on TSS content of fruit juice than 45 days harvested. On the other hand, harvesting date also had influence of L \*and a \*on flesh melon grown in plastic house.

Keywords: melon, harvesting, potassium chloride, plastic house

#### Effect of growing media and 20-20-20 fertilizer rate on pield performance of melon grown in plastic house

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This study aimed to find out the type of growing media and the rate of 20-20-20 per pot on quality of melon cultivar used as #204. The statistical model was 4x4 compossed factorial in completely randomized design composed of two factors as factor A growing media coir, coir dust, rice husk and rain tree leaves while factor B has 4 rates of 20-20-20 per pot as 3 5 7 and 9 grams per pot. The plastic pot size as 15 inch in diameter were used with plastic house under natural day light. The result indicated that melon which grown in rain tree leaves: coir: manure (2:1:1) plus 20-20-20 7 gram per pot gave the significantly highest fruit weight and the most TSS content with the mean of 1345.90 gram and 12.78 ° brix Melon grown in coir plus 20-20-20 9 gram per pot gave the lowest fruit weight with the mean of 779.70 gram. The melon received from those grown in coir only gave the most palatability with the mean of 27.00 score. This study indicated that growing media and 20-20-20 fertilization rate had positive effects on quality and quantity of melon grown in plastic house.

Keywords: growing media, fertilizer, melon, yield components, yield quality

## Effects of harvesting periods on physicochemical properties and in vitro digestibility of banana flour

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Banana are processed into banana flour to increasing their value. Banana flour is used as raw materials in various products such as bakeries and beverages with the properties of glutein-free, low glycemic index (GI) and high resistant starch content (RS). In the banana farms, unripe bananas (60-80 days of the harvesting periods after blooming) are frequently damaged from windys. In addition, in some seasons, the oversupply of banana happened. This study is focused on determining the effects of unripe banana

in 3 different harvesting periods: 60-, 70- and 80-days after blooming on the physicochemical properties, *in vitro* digestibility and the yield of banana flour. The results showed that the peel color of banana was changed from green to light green with increasing harvesting days. The diameter and pulp weight of fruit at 80-days of the harvesting had significantly the largest diameter (35.82  $\pm$  2.74 mm) and highest weight (116.69  $\pm$  1.64 g) (p <0.05). The ratio of pulp was not significantly different (p $\geq$ 0.05) with values in the range of 57.25 to 69.89%. The yield of banana flour was ranged between 19.36-25.12%. The pH of banana flours at the harvesting periods of 70-days (5.19  $\pm$  0.09) and 80-days (5.30  $\pm$  0.09) was significantly higher than that of banana flour at 60-days. The total soluble solids of all samples were not significantly different (p $\geq$ 0.05). For the swelling power and solubility of flour at 80-days had the significantly the highest but the lowest, respectively. The gelatinization temperature of all banana flour was in the range of 80.98 to 83.14 °C. The thermal properties of all three harvesting periods flour showed no significant values (p $\geq$ 0.05). Banana flour at 80-days had RS content (77.72 $\pm$ 3.22%) and estimated glycemic index (43.43  $\pm$  0.33) which was significantly higher than 60-days (p<0.05).

Keywords: harvesting period, banana flour, physicochemical property, in vitro digestibility

The effects of dual modification with ultrasound and annealing treatments on properties and glycemic index of thai glutinous rice (RD6)

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As source of carbohydrate, glutinous rice is popular for consumption in the North and North-Eastern area in Thailand. However, the consumption of glutinous rice is unhealthful for type II diabetics due to the glycemic index (GI) as GI=75-92. Thus, the reduction of the GI of glutinous rice is important for diabetics. Ultrasound technique, which is mechanical waves with a frequency above 16 kHz, had been reported to modify starch. Annealing treatment (ANN) is the hydrothermal method for starch modification. ANN had been reported to reduce starch hydrolysis and improve its relative crystallinity, but affected its thermal properties. To reduce the GI of Thai glutinous rice (RD6), the combination of ultrasound and annealing treatment was applied in this study. The RD6 treated with ultrasound of 100% amplitude for 15 min following with 4 °C for 24 h storage (U) and finally treated with annealing at 45, 50, and 55 °C (U-ANN45, U-ANN50 and U-ANN55, respectively) were studied. The study was elaborated on their effects on pasting properties (pasting temperature, pasting viscosity, breakdown, final viscosity and setback), gelatinization properties, starch hydrolysis, and glycemic index. All the pasting properties of RD6 treated with U and U-ANN increased significantly (p<0.05) from its native. U and U-ANN significantly increased onset temperature ( $T_0$ ) and enthalpy of gelatinization ( $T_0$ ) of thermal properties, particularly the treatment of the higher annealing temperature ( $T_0$ ) and enthalpy of gelatinization U reatment decreased the starch hydrolysis and promoted a decrease in their glycemic index (GI) compared with its native and U treatment.

Keywords: glutinous rice, ultrasound treatment, annealing, glycemic index

Plant growth regulator formulation for propagating red chili (Capsicum annum L.) UNIB CH23 hybrid stem cuttings

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Vegetative propagation may serve as a sound practice to propagate hybrid chili with suitable formulations of auxin. An experiment was conducted to evaluate the type and concentration of auxin growth regulator for stimulating root growth of stem cuttings of a hybrid cultivar "UNIB CH23". Treatments included 35 plant growth regulator formulas which were single or combination of auxin type and concentration, added to a basic formulation. These treatments were arranged in a completely randomized design with five replications. The type of auxin was 2,4-Dichlorophenoxyacetic acid (2,4-D), Indole-3-butyric acid (IBA) and 1-Naphthaleneacetic acid (NAA), used alone or in combination at a concentration of 1, 5 or 10 ppm. Stem cuttings were taken from Chili hybrid cultivar "UNIB CH23" plants growing five dichotome branches. The base of cuttings was immersed in a 20 ml root growth promoting formula for 60 minutes. Theresults demonstrated that 5 out of 35 formulas promoted root growth of chili stem cuttings of the

hybrid red chili. These were Formula 20 (1 ppm IBA + 10 ppm NAA) Formula 21 (10 ppm IBA + 1 ppm NAA), Formula 22 (5 ppm IBA + 10 ppm NAA), Formula 25 (1 ppm 2,4-D + 1 ppm NAA + 1 ppm IBA) and Formula 26 (1 ppm 2,4-D + 1 ppm NAA + 5 ppm IBA). Formula 22 showed as the best auxin combination formula for root growth enhancement for chili hybrid cultivar "UNIB CH23", resulted in the greatest root number (8 roots per cuttings), the longest root (8.8 cm), the greatest percentage of rooting (80%) and the greatest number of leaves (8 leaves per cutting) at 5 weeks after planting.

Keywords: plant growth regulator, red chili hybrid, vegetative propagation, auxin, stem Cutting

The crosstalk between autophagy and endocytosis in Pyricularia oryzae

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Pyricularia oryzae is an excellent model fungus, causing the rice blast disease worldwide. Autophagy was shown to play important roles in P. oryzae development and plant infection. The P. oryzae endosomal system is highly dynamic and has been shown to be associated with conidiogenesis and pathogenicity as well. So far, the crosstalk between autophagy and endocytosis has not been explored in P. oryzae. Here, we identified three P. oryzae VPS9 domain-containing prkoteins, PoVps9, PoMuk1, and PoVrl1. We found that PoVps9 and PoMuk1 localized to vacuolar membranes and co-localized with PoVps21, which was recognized as a marker of early endosomes. Deletion of PoVPS9 resulted in severe defects in endocytosis and autophagosome degradation and impaired the localization of PoVps21 to endosomes. In addition, deleting the PoMUK1 gene in the  $\Delta Povps9$  mutant background, the  $\Delta Povps9\Delta Pomuk1$  mutant exhibited more severe defects in development, autophagy and endocytosis compared with the  $\Delta Povps9$  mutant, indicated that PoMuk1 play a certain role in autophagy and endocytosis. Pull-down assay showed that PoVps9 interacted with PoVps21, PoRab11, and PoRab1, which have been verified to participate in endocytosis. Furthermore, the yeast two-hybrid and co-immunoprecipitation assay confirmed that PoVps9 interacted with the GDP form of PoVps21 (PoVps21 $^{525N}$ ) directly. Thus, our study depicts that PoVps9 is a pivotal protein involved in autophagy and endocytosis, which are two branches of the vesicle\vacuele vacuele digestion system.

Keywords: VPS9 domain-containing proteins, endocytosis, autophagy, pathogenicity, Pyricularia oryzae

Augmented Analysis for Yield and Pod Characters of Yardlong Bean (Vigna unguiculata (L.) Walp. ssp. sesquipedalis Verdc.) Lines

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Selected 40 yardlong bean lines in the sixth generation ( $F_6$ ) of the cross between 2 parental cultivars were compared with 4 check cultivars viz. Bangpra Purple, Bangpra2 (parental cultivars), Lamnamchee and Tarnthong (commercial cultivars) in augmented design. The results showed that the 40 lines were significantly different in pod length, number of seeds per pod (P<0.01) and yield per hectare (P<0.05). Means for pod length and yield of the lines were significantly higher than those of the checks. Eight lines had pod length ranking from 70.35 to 75.85 cm that longer than the higher parent (69.50 cm of Bangpra2) but not significantly different (P>0.05). Twenty-one lines possessed pod length from 61.41 to 75.85 cm that significantly different (P>0.05) from the higher commercial cultivar (52.52 cm). Bangpra2 gave the significantly highest yield (19.16 t/ha) among the checks. There were 4 promissing lines possessing yield ranking from 20.60 to 22.80 t/ha tended to give more value than Bangpra2. Most of the selected  $F_6$ -lines were proved to be better performance than the commercial cultivars, and some were better than their parents. Thus, these promising lines would be selected for yield trial in the next generation.

**Keywords:** asparagus bean, cross, generation, performance

#### Influence of ethephon spraying on growth and yield of Stevia (Stevia rebaudiana Bertoni.)

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A pot experiment was done to determine the effects of different ethephon spraying concentrations and times of spraying on growth and yield of Stevia (*Stevia rebaudiana* Bertoni.) at Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok. The treatments consisted of three different times of ethephon spraying (such as 1) one time of spraying at 30 days after transplanting (DAT), 2) two times of spraying at 30 and 60 DAT and 3) three times of spraying at 30, 60 and 90 DAT) and seven doses of ethephon (0, 50, 100, 150, 200, 250 and 300 ppm.). A split plot in randomized complete block design with three replications was employed whereas variations of times of spraying and seven doses of ethephon were as main plot and sub plot, respectively. The results indicated that spraying three times with ethephon, gave the highest growth (such as stem, leaf, root dry weight and total dry weight) and leaf dry weight yield followed by two times and one time of spraying, respectively. At seven doses of ethephon spraying, Stevia with ethephon 200 ppm gave the highest of total dry weight and leaf dry weight yield compare to the other doses and control. Application of ethephon at 200 ppm and three times of spraying at 30, 60 and 90 DAT gave the highest vegetative growth and yield for stevia.

Keywords: ethephon, growth, yield, Stevia.

Knowledge, attitude and practices towards good agricultural practice of rice farmers under large agricultural plot scheme (LAPS) in Khlongudomchonlajorn, Chachoengsao province Thailand

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This study investigated the knowledge, attitude and level of practice regarding good agricultural practices (GAP) of rice farmers under Large Agricultural Plot Scheme (LAPS) Khlongudomchonlajorn, Chachoengsao Province, Thailand. Data were collected by using questionnaires and 38 farmers interviewed from March to July 2019. Data were analyzed using frequency, percentage, mean, and standard deviation. The results showed that most of the farmers were male (57.9%) with an average age of 56.7 years (65.8%), completed primary education (73.7%), had marital status and had an average number of household members 4.1 people. In term of farmer's knowledge on GAP for rice, the results had shown that the knowledge levels of the respondents were medium level (65.8%), high (26.3%) and low (7.9%). As for the attitudes towards GAP for rice, it was found that most of farmers had a positive attitude (that is, strongly agreed 55.3%, and agreed 44.7%) towards GAP for rice. Regarding the level of farmer's practice toward GAP for rice, it was found that farmers produced rice according to GAP standards at a high level (52.6%), highest level (39.5%) and moderate level (7.9%). The level of GAP compliance that the farmers performed at a low level were inspect water quality and harmful contamination ( $\vec{X}$ =2.16) and record plantation area codes and information. ( $\vec{X}$ =2.55). Relevant agencies should provide training program on knowledge of GAP standards for rice in every item for farmers under LAPS, especially water and plantation area should be inspected and recorded.

Keywords: knowledge, attitude, practices, Good Agricultural Practice, GAP rice, Large Agricultural Plot Scheme (LAPS)

#### Influences of trinexapac-ethyl on development and sugar content of Sorghum bicolor

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The aim of this study was to investigate the influences of a plant growth regulator called Trinexapac-ethyl at different spraying doses and different pre-harvest weeks on the development and sugar content of two sweet sorghum cultivars. The experimental design was 2x4x3 Factorial and randomized complete block with three replications. The experiment was from July to December 2017. Factor A was two sweet sorghum cultivars (KKU 40 and Cowley). Factor B was four Trinexapac-ethyl doses (0, 0.05, 0.10 and 0.20 ppm), while three periods of pre-harvest weeks were Factor C (1, 3 and 5 pre-harvest weeks). No-interactions were found among the cultivars. However, the different trinexapac-ethyl doses and different periods of pre-harvest weeks provided significantly different sweet sorghum growth and yield at the p < 0.05. The control provided the highest growth but low sugar yield. The trinexapac-ethyl ripener affected the highest growth and sugar yield when it was applied at 0.05 ppm and 1 pre-harvest week. On the other hand, trinexapac-ethyl (0.20 ppm) level inhibited the growth of sweet sorghum when applied at 5 pre-harvest weeks. At 0.05 ppm dose and 1 pre-harvest week, the chemical ripener provided the tallest plant height, biggest stalk diameter, and largest biological, juice extract, and sugar yields. Based on these findings, we recommend using trinexapac-ethyl at 0.05 ppm dose and spraying it 1 week before harvest. The recommended sweet sorghum varieties are the KKU 40 cultivar.

Keywords: sweet sorghum, trinexapac-ethyl, yield, growth

#### SESSION 3 BIODIVERSITY, TAXONOMY, BIOLOGICAL ACTIVITY

#### Bioactive compounds isolated from the fungus Neosartorya pseudofischeri

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The genus Neosartorya belongs to the family (Trichomaceae). Many of its members have been reported as source of bioactive secondary metabolites. These compounds show cytotoxicity against several cancer cell lines *in vitro*. In our search for bioactive compounds from soil fungi isolated in Thailand, the fungus *Neosartorya pseudofischeri* was our focus. Its asexual (conidial) state appear morphologically very similar to *Aspergillus fumigatus*. Our *N. pseudofischeri* sample was collected and identified by M. Soytong. The dried fungal biomass sample was investigated by chromatographic separation and identification of isolated compounds as well as evaluation of their biological activities. Several of isolated compounds exhibited potent cancer cell lines tested and showed antibacterial activity. The result of this finding will be presented.

Keywords: Neosartorya pseudofischeri, Trichomaceae, bioactive compounds, cytotoxic, antibacterial activity

## New edible fungi from Southeast Asia: Discovery to production

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The forests of Southeast Asia is a rich source of cultivatable edible fungi. Although significant amounts of research on the taxonomy and phylogeny of edible mushrooms have been carried out, fewer studies have been focused on the domestication of wild fungi. Today, the most commonly cultivated strains are temperate species, but tropical and subtropical mushrooms are both abundant and highly diverse, with many species having long histories of human consumption. In addition, many new species have recently been introduced to science, including numerous species of high nutritional and medicinal value. The domestication and cultivation of tropical mushrooms therefore provides an enormous opportunity for Southeast Asian countries. Due to the difficulties of cultivating mycorrhizal species, we have concentrated on saprobic species. Most tropical and subtropical mushrooms, if provided with appropriate conditions, grow and produce fruiting bodies more quickly than temperate species. Tropical and subtropical mushrooms can be produced using cheap, readily available waste products such as sawdust, corn cobs, rice straw, sugarcane bagasse, and other forest and agricultural residues, making them an ideal crop for smallholder farmers. We have collected and isolated numerous strains of wild mushroom species from Southeast Asian forests, and have published some initial results documenting our progress in domesticating these species. Using a variety of steps including sample collection, isolation, spawn production and fruiting body production in sawdust and compost media, we showed for the first time that it is possible to domesticate the following fungi: Pleurotus giganteus; a new Thai-French hybrid strain of Agaricus subrufescens; A. flocculosipes; A. subtilipes; Auricularia thailandica; A. cornea (white); Panus roseus; Macrolepiota dolichaula; Ganoderma australe; G. resinaceum; G. gibbosum and G. leucocontextum. These discoveries may create new opportunities for the mushroom growing industry and for smallholder farmers in Southeast Asia in particular.

Keywords: compost media, edible fungi, sugarcane bagasse, temperate species

The effects of the mixture of biofloc fermentation medium and vinasse on attractability, palatability and antibacterial property against multi-antibiotics resistant *Aeromonas veronii* 

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One of the important problems in aquaculture are diseases especially those caused by bacteria. Regarding the food safety concept, diverse natural products have been developed to enhance aquatic animal immunities and control the pathogens replacing antibiotics. However, in-feed application of these products noticeably affects the intake in aquatic animals which may be associated with their attractability and palatability. This work therefore aimed to develop the natural substance which has antibacterial activity and increase the attractability and palatability of the feedstuff. The mixture of biofloc fermentation medium after harvesting the biomass and vinasse, residues from bioethanol production, (PBFCM) was produced and characterized. The antibacterial potency of this product was determined against a multi-antibiotics resistant *Aeromonas veronii*, a pathogenic bacterium causing losses in freshwater aquaculture. Moreover, its ability as feeding attractant was tested, in terms of attractability and palatability, in juvenile Nile tilapia (*Oreochromis niloticus*). The attractability was verified using soybean meal, a plant protein source which typically used to replace fishmeal. The palatability was determined using the herbivorous fish diet (25% protein) that normally shows low intake levels. The results showed that our developed product was capable of inhibiting the growth of the multi-antibiotics resistant *A. veronii* and improving the attractability and palatability of the test materials.

Keywords: attractability, Biofloc fermentation medium, multi-antibiotics resistant Aeromonas veronii, palatability, vinasse

#### Biodiversity and Cultivation Characteristics of Oyster Cultivates in Southern Vietnam

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In Vietnam, although many *Pleurotus* species are popularly cultivated, but most of them are imported. These strains are easily degenerated when they are cultivated in large scale, because they hardly adapt to the environment of Vietnam. This research aims to obtain the collection of *Pleurotus* spp. with details of taxonomy, genetic diversity, cultivating traits. The monokaryotic mating types focused on Pleurotus strains with the high yield, genetic stable and adaptability to Vietnam environment. *Pleurotus* strains were collected from some provinces in South Vietnam. By morphology and phylogenetic analysis based on ITS sequences, these strains were identified as *P. citrinopileatus*, *P. ostreatus*, *P. cystidiosus*, *P. pulmonarius*, *P. cornucopiae*. PL4, PL8 and PL1 strains adapted well to Vietnam environment. PL2, PL5, PL6 and PL9 are cold strains and formed fruiting bodies slowly. The biological efficiencies of most strains are over 50 %. Monokaryotic isolates of PL1, PL2 and PL8 are collected and determined the mating type.

Keywords: edible mushroom, monokaryon, mushroom cultivation, Pleurotus

Species diversity and virulence gene existence in clinical *Aeromonas* spp causing Motile *Aeromonas* Septicamia (MAS) isolated from cultured Nile tilapia (*Oreochromis niloticus*)

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Motile Aeromonas septicemia (MAS) caused by virulent clonal isolates of Aeromonas spp. including A. hydrophila, A. veronii, A. veronii ver sobia, and A. jandaei, is emerging as a major disease in Nile tilapia (Oreochromis niloticus) aquaculture in Thailand. It is an opportunistic pathogen causing disease in fish cultured under stress conditions. The bacterium produces a number of pathogenic factors, and the most important among them are lipase, elastase, enolase, aerolysin (aerA) and heatlablie cytotonic enterotoxin (alt) provoking the disease. However, MAS in Nile tilapia was widely believed that A. hydrophila while intrinsic bacterial pathogen species were neglected. In this study, we investigated the species diversity and virulence genes existence of Aeromonas spp. by analyzing 5 MAS incidences (10 Aeromonas isolates from each case). According to the partial 16S rDNA sequences and phylogenetic analysis, 50 test isolates were assigned to 4 different species with A. veronii as a dominant species (39 isolates, 78 %), followed by A. hydrophila (6 isolates, 12%), A. veronii ver sobia (3 isolates, 6%) and A. jandaei (2 isolates, 4%), respectively. Among 39 confirmed A. veronii isolates, the most prevalent general virulence genes were elastase and enolase (100%) followed by alt (32 isolates, 82.05%), aerA (24 isolates, 61.54%) and lipase (18 isolates, 46.15%). This study indicated that there are currently 4 major pathogenic species of Aeromonas in Nile tilapia cultured of Thailand. Moreover, it revealed that A. veronii is a major pathogen that causes MAS and is widely distribution in this area.

Keywords: Aeromonas veronii, Motile Aeromonas Septicamia, Nile tilapia, species diversity, virulence genes

Evaluation of the effectiveness of community development on naturally mychorrhizal mushroom cultivation technology for forest restoration and community food bank at Northern, Thailand

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Forests in Northern Thailand had been changed to intensive farming grown to high yield crops, using fertilizers and pesticides These farming practices and inputs have harmful effects to the environment and food supply for the households. The aim of this study was to evaluate the effectiveness community development on naturally mycorrhiza mushroom cultivation technology for forest restoration and community food bank. Knowledge transfer workshop about naturally mycorrhiza mushroom cultivation technology on seedling was done. People that registered to get the knowledge were around 355 and did workshop to about 253 samples (71.27%) from 10 communities at Phrae province. Most of them of the farmers that participated had an average of 56.23 years old. Top five trees were Citrus reticulata Blanco., Hopea odorata Roxb., Gymnema inodorum (Lour.) Decne, Spondias pinnata (L.f.) Kurz, and Melientha suavis Pierre, respectively. Naturally mycorrhiza mushroom cultivation technology on seedling were Astraeus hygrometricus 159 samples (62.8%) and Thaeogyroporus porentosus 152 samples (60.1%) was a food supply at community. Farmers want to plant seedling average 9 trees/person at home garden because they want to pick mushroom around home 131 samples (51.8%) and easy take care trees at home 129 samples (51%). Some farmers want to plant seedling average 22 trees/person at garden because they want to start mushroom business (126 samples ,49.8%) and they want to develop the garden for fruit business also 118 samples (46.6%). On the other hand, farmers who want to plant seedling average 34 trees/person at community forest  $because they want food security and forest restoration for future. \ Forest restoration and food security in Northern, Thailand to the following the follo$ are co-benefits and that food supply and forest restoration in the community can be achieved at the same time.

Keywords: food security, seedling, Astraeus hygrometricus, Thaeogyroporus porentosus

Effectivity of Neosartorya use in controlling Colletotrichum causing anthracnose disease on papaya

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Isolation of *Colletotrichum* causing papaya anthracnose disease that were collected from markets of Bangkok, Chonburi and Suphanburi provinces was done. The isolates were identified based on morphological characteristics and DNA sequencing of the internal transcribed spacer (ITS) region. Two species of *Colletotrichum gloeosporioides* and *C. truncatum* were identified. For fungal pathogenicity test, *C. gloeosporioides* CBI1 was most pathogenesis. In vitro test by dual culture technique, *C. gloeosporioides* CBI1 was co-cultured with three isolates of antagonist fungi (*Neosartorya hiratsukae* EU06, *N. pseudofischeri* EU13 and *N. fenneliae* CHA03-A11). The result showed the best antagonistic was *N. pseudofischeri* EU13 which inhibited *C. gloeosporioides* CBI1 as 70.16%. The hexane, ethyl acetate and methanol crude extracts of *N. pseudofischeri* EU13 were assessed for antifungal activity against *C. gloeosporioides* CBI1 by poison plate method. The ethyl acetate crude extracts of *N. pseudofischeri* EU13 showed the highest antagonistic activity. Effect of ethyl acetate on inhibition of mycelial growth and spore germination of *C. gloeosporioides* CBI1, the effective dose at 50% (ED50) was 45.63 ppm.

**Keywords:** Neosartorya, anthracnose, Colletotrichum sp.

Optimization of microbial collagenolytic enzyme production by *Bacillus subtilis* subsp. *Subtilis* S13 using Plackett-Burman and response surface methodology

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Bacillus subtilis subsp. Subtilis S13 had been found t produce collagenase. An optimized production for this enzyme was carried out with two statistical approaches. The Plackett-Burman (PB) design was used for screening of 11 parameters including medium composition (gelatin, glucose, glycerol, sodium chloride, magnesium sulfate, calcium chloride, and yeast extract) and culture condition (initial pH, inoculation size, speed, and incubation time). The results showed that gelatin concentration, initial pH, and incubation time were accounted for significant factors from PB design and then applied for a central composite design (CCD) under response surface methodology (RSM) for optimization of significant factors were performed. The optimum parameters for the enhancing gelatinase production through CCD and response surface methodology were 19 g/L of pork gelatin, initial pH 6.16 for culture medium, and 59 h of incubation time, which provided the predicted maximum collatinolytic activity of 65.36 U/mL. This condition allowed approximately 4-fold increase as compared to un-optimized condition (15 U/mL). The obtained optimal activity of this enzyme collagenase showed several applications including meat tenderizing.

Keywords: protease production, collagenolytic protease, central composite design

Pectin extraction from fruit wastes on growth of Pediococcus pentosaceus RSU-Nh1 and Lactobacillus plantarum RSU-SO2

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Pectin is a group of polysaccharides found in plants and fruit which are used widely in the food industry. This aimed to compare different pectins extracted from pomelo, durian, passion fruit and salacca fruit wastes, and to determine the growth enhancement of two lactic acid bacteria consisted of *Pediococcus pentosaceus* RSU-Nh1 and *Lactobacillus plantarum* RSU-SO2, which was isolated from Thai fermented pork (Nham) and fermented spring onion (Ton-Hom-Dong), respectively. Four pectin extracts were analyzed for degree of esterification (DE) and galacturonic acid content. The range of extract pectin was approximately from 80.48 to 86.68% and 52.36 to 64.23%, respectively. Moreover, the tested lactic acid bacteria produced the antimicrobial substance (AMS) against three Gram-positive and eleven Gram-negative bacteria. These AMSs were degraded by various proteolytic enzymes, indicating that they are bacteriocin. However, the addition of pectin each can increase a number of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2, especially pectin from the peels of pomelo and passion fruit. They showed the highest number about 9.94 CFU/ml and 10.47 CFU/ml at 48 hr, respectively. Moreover, the activity of bacteriocin was also increased when these pectins were added in *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 culture. Our results indicate that these pectin extracts could be used as prebiotics to promote the growth of lactic acid bacteria.

Keywords: pectin, fruit waste, lactic acid bacteria, bacteriocin

#### Fine particles of fungal active metabolites constructed from Emericella sp to control rice blast disease

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The biological control of rice blast was investigated by using the fine particles of active metabolites constructed from *Emericella* sp. The metabolite fine particles were inhibited *Magnaporthe oryzae* causing rice blast disease by using poisonous food method. Fine particles-EN inhibited the blast pathogen of 62 % in 12 days. *In vivo* biological activity of fine particles from active metabolites were proved to control blast disease of rice var. Co39 in tested tube and pot experiments compared with chemical fungicide, tebuconazole. Test tube experiment resulted that fine particles -EN reduced the blast disease incidence of 49 % which higher than tebuconazole of 33 %n when compared to the inoculated with *M. oryzae*. Pot experiment showed that tebuconazole treatment reduced the blast incidence of 63 % and fine particles-EN reduced the blast incidence of 44 % when compared to the inoculated control. The further research findings are being investigated for rice immunity to blast disease.

Keywords: rice blast, Magnaporthe oryzae, Emericella sp., fine particles

#### Effect of PGPR on the phytoremediation potential of Vigna radiata. L in contaminated Soil

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Heavy metal contamination due to natural and anthropogenic sources is as a global environmental concern. The remediation of heavy metal-contaminated soil has become a critical issue due to toxic effects of these metals on living organisms. A green-house experiment was conducted to investigate the efficiency of two heavy metal-tolerant plant growth promoting bacterial strains (PGPR) *Pseudomonas fluorescens (MK478897)*, *Bacillus subtilis (MK483262*) to alleviate the heavy metal's toxic effects on Green gram plant (*Vigna radiata*, L.) in addition to their ability to enhance plant growth. The growth characteristics photosynthetic pigments, heavy metals uptake was estimated in green gram cultivated in soil contaminated with heavy metals under green-house conditions. Results showed that the application of these PGPR strains as biofertilizer of green gram help plant to ignore the toxic effects of heavy metals enhance growth characteristics.

Keywords: heavy metal-tolerant bacteria, greengram, PGPR, biofertilizer

#### Effect of growth regulator on shoot induction from callus of *Dendrobium* spp

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This study aimed to identify suitable formulations for the development oF shoot derived calluses of *Dendrobium* in aseptic conditions cultured on MS formula supplemented with different growth regulator. Nine treatments of different concentration of NAA (0, 0.1 and 0.5 mg/l) and BA (0, 2 and 3 mg/l) were used. Each treatment consisted of 9 treatments, with 5 replications, each replication contained 5 calluses. After 8 weeks of cultured, treatment which supplemented with NAA 0 and BA 2 mg/l is the formula that is suitable for use as medium in tissue culture of *Dendrobium*. The results showed that the highest number of shoot (19.6 shoots), shoot length (1.30 cm), fresh weight (1.82 g), chlorophyll A and chlorophyll B (0.19 and 0.11 mg/gFW, respectively) in treatment which was supplemented with NAA 0 mg/l and BA 2 mg/l. For total non-structural carbohydrate (TNC), the result showed that the highest value was in treatment which was supplemented with NAA 0.5 and BA 0 mg/l (5.54 mg/FW).

Keywords: tissue culture, plant growth regulator, dendrobium, orchid

Species diversity and utilization of herbal plant in community forest, phrae province, northern thailand

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The objectives of this research were to study species diversity and utilization of herbal plant in community forest in Phrae Province, northern Thailand. The data were collected in June, 2018 by surveying geographical features that included mountain slope, mid-hill mountain, and mountain ridge. Three blocks of mountains were constructed through mountain slope sampling study area (1 kilometer). The data gathered from every block were analysed to determine the density, frequency, relative density, relative frequency and importance value index. The results showed that 83 herb species in 77 genera and 70 families, had the highest density of 1885 plants/ha, the highest 5 Importance Value Index of significance were *Chromolaena odorata, Centotheca lappacea, Harrisonia perforate, Curcuma sessilis, and Amorphophallus paeoniifolius of* 7.52, 6.99, 5.40, 4.87, and 4.87 percent, respectively. The herbs found were 37 species in 35 genera and 21 families in a hillside, middle of the hill are 26 species in 26 genera and 19 families and the ridges found are 20 species in 20 genera and 13 families. The top 10 most widely used herb species in the Maesai community are *Tacca chantrieri, Thunbergia laurifolia, Acalypha spiciflora, Walsura trichostemon, Ventilago denticulate, Boesenbergia rotunda, Tiliacora triandra, Butea superba, Croton persimilis, Zixiphus oenoplia.* 

Keywords: species diversity, utilization of herbal plant, community forest

Effect of lactic acid bacteria on the physicochemical and microbiology properties of drinking yogurt from rice milk

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Rice yogurt is an alternative to dairy products that are suitable for those who are lactose intolerant, protein allergies, hypercholesterolemia and are vegetarian. Commercial lactic acid bacteria was isolated from the plants that are suitable for other cereal yogurts such as soy or almond but not for rice. This work aimed to develop formulation in producing drinking yogurt from rice milk, then study physiochemical, microbiology of yoghurt during storage in order to select the commercial lactic acid bacteria suitable for rice drinking yogurt and acceptability of consumers. Drinking yogurt from rice milk made from gelatinized rice flour, rice protein concentrate, rice bran oil, glucose and use three types of commercial lactic acid bacteria including of SACCO® Lyoflora SYAB1, DANISCO® VEGE 033 LYO and YoFlex® YF-L01. Firs, we t study about factor effect to growth of LAB including content of rice flour gelatinized and glucose. The formulas were varied as 10,30,50 and 70 % of gelatinized rice flour with 5, 10, and 15 % of glucose. At 50% gelatinized rice flour and 10% glucose take the least time of incubation to ph 4.5-4.8 and had highest acceptability score therefore use to producing yogurt. LAB count and quality parameters like pH, titratable acidity, total solid content, colour and viscosity were analysed to infer how about effect of different commercial lactic acid bacteria would impact to yoghurt during storage. The different commercial lactic acid bacteria influenced pH, titratable acidity, colour and viscosity. Lactic acid bacteria populations were around 6.35 and 7.52 log(cfu/ml) follow by SACCO® Lyoflora SYAB1, DANISCO® VEGE 033 LYO, respectively then decrease from first day. Whereas YoFlex® YF-L01 were around 6.00 log(cfu/ml) throughout storage and had highest of consumer acceptability. These results could be applied in the production of drinking yogurt from rice milk as alternative products for lactose intolerance, milk protein allergies and vegetarian.

Keywords: rice milk, drinking yogurt, non-dairy product, cereal yogurt

Classification and diversity assessment of *Pyricularia oryzae* based on leaf and neck blast pathogenesis on Khao Dawk Mali 105

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Keywords: leaf blast, neck blast, Pyricularia oryzae, avirulence (Avr) genes

#### Silver nanoparticles from leaves extract of melastoma malabatricum and its nematocidal effects

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Silver nanoparticles have potential advantages in various range of biomedical dicipline. The aim of this research was to determine the potency of silver nanoparticles (AgNPs) synthesized using the leaves extract of Melastoma malabatricum as anti-nematode. Wormicidal activity in-vitro assay was conducted on mature Haemonchus contortus. Two diferent concentrations of silver nanoparticles and ethanol 96% extract of Melastoma malabatricum were tested. Albendazole was used as standard reference of nematocidal and saline water as negative control. The phytochemical study showed that ethanol extract of Melastoma malabatricum presence of tannin, flavanoid, terpenoid and steroid. The synthesized silver nanoparticles are identified by visual assessment as colour changes of the solution and using ultraviolet-visible nanophotometer (UV-Vis). Analyzed using UV -Vis nanophotometer and the spectrum showed a clear peak around 365 nm with the pH 5.9. Nematocidal assay on adult Haemonchus contortus showed that the paralyse time of worm in silver nanoparticle significantly (P<0.05) shorter than control positive. The death time of worms in all treatment was not more than 1 hour, and for the negative control, worm remained active up to 2 hours post exposure. The biochemical study also conducted to verify the efficacy of wormicidal activity.

Keywords: silver nanoparticles, Melastoma malabatricum, Haemonchus contortus

Antimicrobial potential of vapour phase propionic acid against *Salmonella* typhimurium contaminated on cherry tomato (*Solanum lycopersicum* var. *cerasiforme*)

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Salmonella sp. is increasingly recognized as significant cause of foodborne illness. Several decontamination procedures have been applied to reduce the number of this organism. This study examined the potential of Mechanically Vapourized Propionic acid solution (MVP) on the reduction of Salmonella Typhimurium contaminated on on Cherry tomato (Solanum lycopersicum var. cerasiforme). In vitro surface inhibitions at low population and high population were examined. MVP at the concentration of 70.0% demonstrated the completely inhibition within 15 min at 4°C. At the concentration of 70.0% the absolutely inactivated was observed within 5 min at 50°C. For the evaluation of antimicrobial activity of MVP over time, the results indicated that ca. 8.00 Log<sub>10</sub> CFU/ml reductions were observed within 5, 10, 15, 20, 25 and 30 min at the concentration at 5.0%, 10.0%, 30.0%, 60.0% and 70.0%, respectively at 25±2°C. The effectiveness of MVP increased when the temperature of MPV process increased. The reduction of S. Typhimurium contaminated on Cherry tomato using MPV was also determined. The effectiveness of MVP on the reduction of S. Typhimurium depended on the concentration of Propionic acid solution, the fumigation time and temperature. The studies on biological and physical changes of Cherry tomato during 15 days of storage at room temperature and 4°C after fumigated demonstrated that MPV at concentration of 70.0% for 5 min at 50°C, and 4°C vapourized of that at 70.0% for 15 min, indicated that the completely inhibition of S. Typhimurium contaminated Cherry tomato was accomplished. Moreover, the colour and physical appearance of fumigated Cherry tomato was not different from fresh and control those. This study can conclude that Propionic acid in vapour phase demonstrated the antimicrobial potential against S. Typhimurium at both after fumigation process and storage time.

Keywords: mechanically vapourized propionic acid, Salmonella Typhimurium, tomato, antimocrobial

#### Hydrolytic properties of crude protease from Bacillus subtilis subsp. Subtilis M13

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A new *Bacillus subtilis* subsp. *Subtilis* M13, isolated from meat, had been proven as the collagenase producing bacteria based on high degradation of denatured form of collagen or gelatin. This study was conducted to further estimate several hydrolytic activities against pH, temperature, and various substrates including collagen, elastin, and myofibrillar protein of crude protease from the culture supernatant. The molecular weight of the crude enzyme was approximate to be around 21 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Maximum collagenolytic activity based on gelatin as a substrate was attained at 50°C and pH 6.0 with citrate buffer. Furthermore, this enzyme was preferable to hydrolyze insoluble collagen, followed by elastin at 37°C and pH 6.0. Maximum hydrolysis for collagen was showed by the highest release of amino acid at 998  $\mu$ g/mL after incubation for 24 h, and for elastin at 656  $\mu$ g/mL after incubation for 12 h. However, the hydrolytic activity against myofibrillar protein extracted from meat was relatively lower than 3.3  $\mu$ g/mL at 6 h and there was no or slight hydrolytic effect after prolonged incubation. The results indicated the potential protease activity of collagenase produced from bacteria Bacillus subtilis subsp. Subtilis M13 for use as meat tenderizing enzyme with high degradation of collagen and elastin with low hydrolysis of meat myofibrillar protein.

Keywords: bacteria protease, collagenolytic protease, meat tenderizing enzyme

#### SESSION 4 CROP PROTECTION AND RELATED TECHNOLOGIES

Screening of fungicides and antagonist for alternate control in vitro for fruit spot caused by *Fusarium* sp. of pummelo (Citrus maxima (Burm.) cultivar Tabtim Siam

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Fruit kanker-spot caused by *Fusarium* sp., was the disease complex on canker symptom caused by *Xanthomonas axonopodis* pv. *citri*. It is the new serious yield damaging disease. It has not have been reported in this area. Twenty fungicides collected from the agrochemical stores at this area were brought to preliminary screen against *Fusarium* sp. *in vitro* using poison medium technique and antagonistic *Trichoderma harzianum*, *Bacillus amyloliquefaciens* KPS46 and *Paenibacillus pabuli* SW were parallel tested by dual culture technique. The results showed that prochloraz (0.19 mg/ml), thiabendazole (0.60 mg/ml) and thiophanate-methyl (0.7 mg/ml) inhibited mycelial growth 100% at recommendation dose and fallowed with propiconazole (0.25 mg/ml) and fluopyram (0.12 mg/ml) + trifloxstrobin (0.12 mg/ml) inhibited mycelial grow of 85 and 83.3% respectively. The high efficacy control fungicides in preliminary screen mention above were trialed for optimal dose. The optimal doses to control of prochloraz, thiabendazole and thiophanate-ethyl were 0.5 mg/ml with 100% inhibition and for propiconazole was 1 mg/ml. Only fluopyram+trifloxstrobin showed the highest inhibition only 95.3% at 0.5 mg/ml. For antagonistic test, we found that all antagonistic *Trichoderma harzianum*, *Bacillus amyloliquefaciens* KPS46 and *Paenibacillus pabuli* SW01/4 showed the control efficacy by inhibition mycelial growth of *Fusarium* sp. *in vitro* test.

Keywords: chemical control, biological control, disease complex

#### Analysis of polyethyleneglycol (PEG) and proline to evaluate drought stress of double haploid new type upland rice lines

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Evaluation and characterization, as well as a selection of rice that are tolerant to drought stress, is an essential stage in plant breeding. To make the process of selection of double haploid lines, especially those related to drought tolerance can be done by looking at the morphological features on the root system of each genotype. The treatment of PEG solution into the planting medium is expected to create conditions of stress because of the availability of water for plants is reduced. Molecular size and the concentration of PEG in the solution determining the osmotic potential that occurs. The mechanisms used by plants to defend on drought stress is through the accumulation of proline for adjustment osmotic, production and accumulation of free amino acids like proline in plant tissues during drought stress, an adaptation response in these conditions. In this research, PEG 6000 inhibited germination (33.9 percent), root length (60.8 percent), and shoot length (80 percent) of upland rice lines. Drought stress treatment (60 percent of field capacity) at the flowering period showed no significant reduction in the growth of doubled haploid upland rice but reduced the weight of grains per hill (52.11 percent). Drought stress decreased total chlorophyll (20.7  $\mu$ mol/cm) and increased proline content in leaves (30.3  $\mu$ mol/g). The content of proline in the leaves varies in inbreds due to drought stress. The tolerant genotype had high proline content based on PEG 6000 are P3-31, followed by P6-95 respectively 30.33, 20.82  $\mu$ mol/g, and genotype moderate line P6-291 at 20:42  $\mu$ mol/g. Drought stress led to a decrease in total chlorophyll, and increase the proline content in the leaves.

Keywords: drought stress, doubled haploid, upland rice, polyethyleneglycol, proline

# Importance of bioagents in mitigating the major diseases of nutri cereals

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Millets are group of small grained cereal food crops which are highly tolerant to drought and other extreme weather conditions and are grown with low chemical inputs such as fertilizers and pesticides. Most of millet crops are native of South East Asia and are popularly known as Nutri-cereals as they provide most of the nutrients required for normal functioning of human body. Millets are gluten free and non- allergenic. Millet consumption decreases triglycerides and C- reactive protein, thereby preventing cardiovascular disease. Millets are nutri cereals comprising of sorghum, pearl millet, finger millet (Major millets) foxtail, little, kodo, proso and barnyard millet (minor millets). Millets are prone to various diseases across the India. The major diseases occuring in millets are Blast, Banded sheath blight etc. Diseases are major cause for reducing the economic yield and losses upto 75%. Control of these pathogens is difficult because of its ecological behavior, Its extremely broad host range and the high survival rate of sclerotia under various environmental conditions. For management of these pathogens, various methods, that is, chemical control, biological control, resistant varieties, cultural control and physical control, are applied. Resistant varieties are the best and cheapest method for managing the diseases. However, resistant break down occur over the years. Resistance against some pathogen resistant are not available. Chemical management is the second-best option for managing the diseases, due to continuous and irrational use of the chemicals; pathogens have developed resistance against certain class of fungicides/bactericides. Moreover, these chemicals also assist in environmental pollution and toxicity in the produce. Bio-agents are naturally occurring living organisms, which are found in rhizosphere, phylloplane, etc. These bio-agents help in not only managing the diseases but also increasing the crop yield. Therefore, the use of bio-agents for biological management of millet crops is the focused research area worldwide. Also, biological control assumes special significance as it is ecologically conscious and cost-effective alternative strategy and presently has vital role in the present farming system. Bioagents, such as Trichoderma spp., Bacillus subtilis and Pseudomonas flouroscens are effective in controlling of the major diseases in millets. Seed treatment and foliar sprays with bioagents reduces

the incidence of the diseases in millets. If we promote the use of Bioagents for control of various diseases of millets in South East Asia we could be a global leader in Agri business and farmers could sustain their livelihood.

Keywords: millets, nutri cereals, blast, banded blight, bioagents, Trichoderma spp., Bacillus subtilis, Pseudomonas flouroscens

#### Effects of larval-stage mealworm (Tenebrio molitor) powder on qualities of bread

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Larval-stage mealworm (*Tenebrio molitor*) was prepared and dried to be powderized. The water activity ( $a_w$ ) of powder was 0.185 while its protein content of 41.22%. The fortification of larval-stage mealworm powder at 4 levels of 0, 5, 10 and 15% of wheat flour weight in bread formulas was studied. The results showed that the wheat flour without the addition of larval-stage mealworm powder (control; 0%) had the highest peak viscosity of 139.14 RVU. The addition of higher levels of larval-stage mealworm powder resulted in significantly decrease in peak viscosity of wheat flour (p<0.05). The pasting temperatures of all samples were between 87.20 and 89.50°C. The hardness of bread without the addition of larval-stage mealworm powder was 108.50  $g_{force}$  whereas the hardness of fortified bread samples raised approximately 4 times when adding larval-stage mealworm powder to 5-15% (425.83 to 487.67  $g_{force}$ ). It was found that an increase of larval-stage mealworm powder leading to lower specific volume and higher hardness in bread after baking. The fortification of larval-stage mealworm powder also caused the increase of color intensity due to the original brown color of larval-stage mealworm powder and the occurrance of maillard reaction during baking. The non-uniformity of porosity of the bread products was observed in fortified bread samples. The bread products added by larval-stage mealworm powder at 0, 5, 10 and 15% of wheat flour had protein contents of 9.63, 12.63, 13.21 and 13.73% respectively. The bread products fortified at 5% of wheat flour had the most comparable quality to the control bread sample.

**Keywords:** bread, insect, mealworm, protein fortification

## Evaluation and selection of chili genotypes resistant to Pepper yellow leaf curl Thailand virus (PepYLCVTH)

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Pepper yellow leaf curl virus (PepYLCV) belongs to Begomovirus causes leaf curl disease prevalent in chili production areas of subtropics and tropics. The objective of this study was to evaluate and select parental line of chili genotypes resistant to Pepper Yellow Leaf Curl Virus (Thailand isolate). For phenotypic evaluation, 21 chili genotypes consisting of Thai local lines (4), Khon Kaen University (10) and (7) varieties obtained from World Vegetable Center, Taiwan were inoculated with PepYLCV-TH isolate by grafting method at the seedling stage (45 day after sowing). The experiment was designed by Randomized completed block design (RCBD) with six replications and five plants per replication. The disease symptoms were scored at 19, 26, 33, 40, 47 and 54 days after inoculation (DAI). The virus verification was identified by universal primers PAL1v1978B and PAR1c715H. The chili genotypes were classified into 5 groups based on their responses to PepYLCV-TH isolate. The symptom occurred in the susceptible line at the 19 DAI, but not expressed in resistant genotypes. The first group consisted of a resistant check. The second group showing resistant disease reaction consisted of four chili genotypes. Nine and two genotypes were moderately resistant and susceptible, respectively. Ten chili resistant lines shown no virus infection and they could be used as source of resistance.

Keywords: against, breeding, capsicum, disease

#### Identification of morphology and pathogenicity of *Pyricularia* sp. causing blast disease in grass

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Fungal species from the *Pyricularia* genus are associated with blast disease in plants from the Poaceae family. The fungi cause losses in economically important crops such as rice, oat, rye, barley, wheat and triticale. Grass leaves expressed blast symptoms were collected from blast epidemic area in Thailand during the year 2018 and 27 isolates were obtained. Morphological characterization was studied on RFA (Rice flour agar) media and 8 colony types were grouped. The colony color of 27 isolates were greyish green. The conidia shapes were pyriform or pear shape with rounded base and narrow toward the tip which is pointed or blunt. Study of pathogenicity on 40 Thai elite rice varieties showed that only 3 out of 27 isolates could infect different Thai elite rice varieties. Spot lesion to eye shape symptoms were founded on rice leaves. The isolate LPG61102 infected the most numbers of Thai elite rice varieties with the virulence index of 0.15. While 24 isolates were unable to infect any of 40 tested rice varieties. This first report in Thailand on *Pyricularia* sp. causing blast disease in grass provided preliminary information between the two host species.

Keywords: blast disease, blast fungus, pathogenicity test

Biological control using parasitoids *Comperiella calauanica* hastens the recovery of the coconut trees from Coconut Scale Insect (CSI) infestation in Basilan, Philippines

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Rapid ground assessments (RGAs) of the presence of the parasitoids *Comperiella calauanica*, were done before and after their release in the Coconut Scale Insect (CSI) – infested farms in Basilan, Philippines from October 2017 to July 2018. Before the release of parasitoids, based on RGAs, all coconut trees sampled had the CSIs but no sightings of the parasitoids *Comperiella calauanica*. After the release of approximately 379,500 parasitoids as biological control agents, parasitizations on the CSI among the coconut trees were noted. After a month, parasitizations of about 12% were recorded in November 2017. In February 2018, 100% parasitizations were already observed. Continuing rapid ground assessments separately made by the partner agency found similar results. Actual observations and photo-documents taken by both partner agencies showed high recovery. The previously "severe" CSI–infested coconut trees, which were characterized by "yellowing to browning of almost all leaves of mature coconut trees" in November 2017 were noticeably restored to trees with "normally green leaves" in February and July 2018. As of July 2019, the coconut trees in all 23 sample farms are "all normally green" and the newly grown leaves showed "no infestation" with CSI. A significant number of the trees are now bearing fruits. These indicate that using the parasitoids *Comperiella calauanica* is an effective biological control of the CSI in a shorter period.

Keywords: Rapid ground assessment, Biological control, Parasitoids, Coconut Scale Insect, Infestation.

#### Comparison of growth and yield of maize (Zea mays L.) grown in intercropping and monocropping systems

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Maize is one of a few crops that the Thai government is trying to encourage farmers to grow instead of off-season rice because it needs less water. This study aimed to compare the growth and yield of maize in a low-moisture field after it was used for intercropping (maize, peanut and sweet potato) and monocropping (rice) systems in the previous season. The experiment was conducted in a farmer's field in Phichit province, Thailand. Two plots of the fields were previously used for intercropping system and a monocropping system. Maize was planted with spacings of 75 x 25 cm at a rate of 1-3 seeds per hill. The intercropping plot was divided into 560 grids of 2 x 2 m area and the monocropping field was divided into 200 grids of 6 x 6 m area before the data was sampled. Soil and plant data were sampled from randomly selected 14 and 20 grids of intercropping and monocropping fields, respectively, then the data were compared by a nonpooled t-test method. Soil sample was analyzed for its chemical properties. The soil of the intercropping plot had higher amounts of organic matter, available phosphorus, exchangeable potassium and magnesium extractable iron, manganese, zinc and copper than those in the monocropping plot, resulting in non-significant higher growth, yield and yield components at p < 0.05. However, the trend was clear. The total dry matter and yield provided by the intercropping system was 10.1 and 8.4 % higher than those provided by the monocropping system, respectively. These findings were only from one season. Data on the growth of maize grown in these plots after two or more seasons are needed to confirm these benefits.

Keywords: intercropping system, monocropping system, off-season, maize

Phenols and peroxidase activity of resistant and susceptible chili (Capsicum annuum L.) genotypes to Pepper yellow leaf curl virus

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Chilli (Capsicum annuum L.) is most commonly cultivated species of the genus Capsicum. Pepper yellow leaf curl virus (PepYLCV) causes serious leaf curl disease of chili. In order to understand the plant defence mechanism to PepYLCV disease, this study reports response of total phenols and peroxidase activity in two pepper genotypes (resistant and susceptible) against PepYLCV. The resistant (PBC148) and susceptible (KKU-P31118) genotypes were graft inoculated with Pepper yellow leaf curl virus (PepYLCV) at 45 and 60 days after sowing (DAS). The disease score, total phenolics and peroxidase activities were observed and estimated at 14 days after inoculation and after five weeks after inoculation. Genotype PBC148 was confirmed to be most resistant to PepYLCV. In contrast, genotype KKU-P31118 was highly susceptible. The total phenols were increased in the inoculated resistant genotype as compared to uninoculated condition. However, in case of susceptible genotype, the amount of total phenols decreased under artificial inoculated condition as compared to uninoculated condition. On the other hand, in both chili genotypes, the amount of peroxidase activity increased under inoculated condition. Hence, our finding suggests that the total phenols may act as defence mechanism in resistant genotype PBC148.

Keywords: disease response, PepYLCV, peroxidase, phenolic

#### The Rotting fungi of street plant in Ho Chi Minh City

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This study aimed to investigate wood decay fungi from plant cultivated along street in 8 central Districts of Ho Chi Minh City, Vietnam. There are twenty-one dominant species included three group: frequent split or collapsed branch (*Peltophorum pterocarpum*, *Albizia saman*, *Delonix regia*, *Dipterocarpus alatus*, *Hopea odorata*, *Khaya senegalensis*), large trees which have more implicit hazards (*Terminalia molineti*, *Lagerstroemia speciosa*, *Cassia fistula*, *Tectona grandis*, *Pterocarpus indicus*, *Cinnamomum camphora*, *Diospyros mollis*, *Tamarindus indica*, *Swietenia mahagoni*, *Berrya mollis*, *Mimusops elengi*) and potential trees (*Sindora cochinchinensis*, *Tabebuia rosea*, *Chukrasia velutina*, *Dracontomelon duperreanum*). In results, 4.22% of the sampled trees had decay fungi and the incidence was highest in *L. speciosa* (1.76%) and *D. alatus* (1.17%). Most of rotting fungi were confined in the older and larger diameter plant. Eight species of white rot fungi found in the fruiting body stage were *Marasmiellus palmivorus*, *Crinipellis setipes*, *Favolus aff. tenuiculus*, *Inonotus henanensis*, *Xylaria feejeensis*, *Fulvifomes sp., Physisporinus vitreus and Ganoderma multipileum*. The location of most of these species was often in woody roots, lower trunks or tree wounds caused by pruning or insect bites. The presence of fruiting structure of decaying fungi was positive indicators to assess of the hazard risk of street tree system in Ho Chi Minh City.

Keywords: street plant, white rot fungi, wood decay

Investigation of broad-spectrum blast resistance genes of indigenous rice, Yang Mawng variety

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Rice blast caused by *Pyricularia oryzae* is a major disease of rice. Thai indigenous rice Yang Mawng variety is highly resistant to the infection of *P. oryzae*. In this study, disease assessment was done using 27 isolates of *P. oryzae* derived from different blast disease epidemic regions of Thailand. Yang Mawng seedling was inoculated by each isolate of *P. oryzae* and disease severity was scored 7 days after inoculation. The results showed that Yang Mawng was highly resistant to 24 isolates and moderately resistant to 3 isolates of *P. oryzae*. The results confirmed the broad-spectrum resistance to blast disease of Yang Mawng variety. The pathogenicity test of 27 isolates of *P. oryzae* was conducted on 31 Near Isogenic Lines (NILs). The results showed that the virulence index (VI) ranged from 0 to 0.50 and the most pathogenic isolate on NILs was UBN2010 13515. The investigation for 8 rice blast disease resistance genes of Yang Mawng variety was done using blast resistance gene specific primers and PCR technique. The result showed that 5 blast resistance genes including *Pi9, Pigm(t), Pi54, Pib* and *Pita*, were detectable. The information of this research is preliminary data for the study of resistance genes of Yang Mawng variety.

Keywords: Pyricularia oryzae, rice blast disease, pathogenicity, Yang Mawng variety

#### Natural products of fine particles derived from Neosartorya hiratsukae against brown spot of rice cause by Drechslera oryzae

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Efficacy of *Neosartorya hiratsukae* was confirmed to control brown leaf spot of rice caused by *Drechslera oryzae*. In bi-culture antagonistic test, *Neosartorya hiratsukae* had highest significantly inhibited the colony growth and spore production which were 51.10% and 55.65% respectively. Crude methanol extract metabolites of *N. hiratsukae* gave significantly highest inhibition of colony growth and spore production of *D. oryzae* which ED50 was 168  $\mu$ g/ml. Testing fine particles constructed from metabolites of *N. hiratsukae* showed that fine particles from methanol crude extact of *N. hiratsukae* gave significantly highest inhibition of colony growth and spore production of *D. oryzae* which ED50 of 4.11  $\mu$ g/ml.

Keywords: Neosartorya hiratsukae, brown spot, rice disease

Blast resistance evaluation and genetic inheritance of gene controlling leaf blast resistance in Dawk Pa-yawm Rai variety (GS23774)

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Rice blast disease is an important disease and known to occur in most rice producing areas of Thailand. The disease caused by the fungus *Pyricularia oryzae*. The Thai indigenous variety, Dawk Pa-yawm Rai was selected from a previous screening for broad spectrum resistance to rice blast disease and therefore can be used as a good genetic resource in the breeding program. In this study, disease assessment to blast resistance of Dawk Pa-yawm Rai variety was conducted using 25 blast fungal isolates collected from different blast disease epidemic areas of Thailand. Each fungal isolate was inoculated on Dawk Pa-yawm Rai variety and 3 rice control cultivars (KDML105 (susceptible, S), Jao Hom Nin and IR64 (resistance, R)) and the disease reactions were scored 7 days after inoculation. The results showed that Dawk Pa-yawm Rai variety was highly resistant to 18 blast isolates, moderately resistant and susceptible to 5 and 2 blast isolates, respectively. Therefore, Dawk Pa-yawm Rai variety was broad spectrum resistance to many blast isolates and can be used as genetic resource in breeding program for blast disease resistance rice variety. The genetic inheritance of blast resistance in F<sub>2</sub> population was identified. Seven hundred and thirty F<sub>2</sub> plants from a cross between KDML105 (recipient) and Dawk Pa-yawm Rai (donor) were generated. The disease assessment revealed 524 F<sub>2</sub> plants were resistant and 206 F<sub>2</sub> plants were susceptible. The segregation of resistance and susceptible phenotypes showed a goodness of fit to the ratio 3:1 (R:S). The data found in this study is important and useful in finding and mapping of blast resistant gene in Dawk Pa-yawm Rai variety.

Keywords: rice blast disease, Pyricularia oryzae, genetic inheritance

Enhancement of growth, yield & yield contributing traits and alleviation of sheath blight disease caused by *Rhizoctonia solani* in barnyard millet (*Echinocloa frumentacea* L.) with a particular reference by using *Trichoderma* isolates through seed bio-priming and pre colonized FYM

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Trichoderma spp. were tested to determine if they promote growth, yield and induce resistance against biotic stresses for barnyard millet (Echinocloa frumentacea L.). Physiological and biochemical parameters were also monitored under greenhouse conditions to explore the mechanism underlying plant biotic stress resilience in response to Trichoderma inoculation. The isolates of Trichoderma spp. were used through seed bio-priming technique along with value added FYM. The treatment T3 (Seed bio-priming with Trichoderma isolate Th-14+ FYM pre-colonized by Th-14) was found significantly superior over all the treatments with respect to all the studied traits related to growth, yield and yield contributing attributes at the same time reducing sheath blight disease caused by Rhizoctonia solani followed by the treatment T5 (Seed bio-priming with Trichoderma isolate Th-21 + FYM pre-colonized by Th-21) and T4 (Seed bio-priming with Trichoderma isolate Th-19+ FYM pre-colonized by Th-19) which were statistically at par. The study also revealed that the bio-control strains showed significant potential when used along with FYM. However, the treatment T3 proved to be most effective in improving the growth and yield attributes and also helped in suppressing incidence of sheath blight disease in barnyard millet. In comparison with the untreated plants, characterization of Trichoderma treated plants confirmed that they had reinforced contents of proline along with relatively higher levels of chlorophyll content, SPAD value, total phenols, membrane stability index and SOD activity while lower accumulation of malondehyde content in comparison to untreated plants under the present materials and environmental conditions. The research merits attention and could additionally open the avenue for the use of putative bio-control agents in enhancing growth and yield parameters and suppressing the most destructive diseases of barnyard millet in the mid hill regions of Uttarakhand.

Keywords: barnyard millet, seed bio-priming, FYM, Trichoderma, Proline, phenols, SOD, malondealdehyde

Promotion of improved rice farming technology for resilient and profitable enterprise

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Rice Production Technology Demo-farms were established in 5 barangays of Datu Odin Sinsuat, Maguindanao, Philippines in 2 croppings. The improved rice production technology (*irptech*) was designed to increase rice productivity, lower farmer's production cost, provide appropriate production and management prerogatives and use of biodynamic principles for farmers that experience adverse conditions like drought and floodings in rice agroecosystem. The adoption of improved crop management options to include the proper choice of rice varieties, weed control, timing of planting, right amount and kind of fertilizers, weed, diseases and pest control measures, water management, pre- and post-harvest handling, time management and adherence to some principles of biodynamic farming enabled the farmers to improve productivity and profitability at the same time avoid and protect the farms from the adverse environmental conditions during the croppings. Compared with the conventional farming, the improved technology *irptech* reduced the gross margin on operating cost by 15.45%, lessened crop damages, improved crop performance, increased rice yield by 20%, obtained higher income by 20% and averted environmental rice stresses observed during those periods. The break-even production cost using the technology is Php 8.45 /kilogram which is lower by 26%, and break-even quantity is 1,482.16 kilograms/hectare which is lower by 15% when using the conventional farming. These show that farmers can operate profitably even with that level of production provided that management prerogatives and biodynamic principles of farming were strictly followed.

Keywords: rice technology, resilient, profitable enterprise, biodynamic

## Drought-tolerant characters, yield and its component of an elite landrace upland rice cultivars in Thailand

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In Thailand, approximately 10% of total 9.28 million ha of rainfed rice are grown in unbunded upland which are usually subject to drought stress due to eratic rainfall throughout growing season leading to low yield. Six elite landrace upland rice cultivars namely; 1) Niaw Dum Luem Phua, 2) Dawk Pa Yawm, 3) Phukaothong 2, 4) Sew Gliang, 5) Sam Deuan, and 6) Leb Nok were studied in 2 experiments, separately with IR1552, and Khao Dawk Mali 105, a moderate drought-tolerant check varities. The first experiment was carried out to study 4 drought-tolerant characters at maximum tillering including longest root, root dry wt, root/shoot dry wt ratio, and stomatal conductance. The germinated seeds were grown in 14-inch in diameter plastic pot, data were recorded for 10 hill/experimental unit. Completely Randomized Design with 3 replications was used. The second experiment was yield tested in an unbunded sandy-loam upland with 3 replications in Randomized Complete Block Design, ungerminated seeds were direct planted with space of 25x25 cm, yield and yield components were sampling recorded for 10 hill/experimental unit. All studied characters among cultivars; longest root, root/shoot dry wt ratio, stomatal conductance, yield and all yield components except root dry wt were highly significant different (p≤0.01). IR1552 had the highest yield of 72.52 g/hill. Among 6 landrace cultivars, Niaw dum luem phua showed the best drought tolerant-related characters, the best yield components, and highest rough yield of 59.77g/hill.

Keywords: upland rice, elite landrace cultivar, drought-tolerant character, yield and its component

#### SESSION 5 WATER & SOIL MANAGEMENT, ENVIRONMENTAL SCIENCE

#### Purification and disinfection of waste water with electrical current

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Humankind is encountering increasing problems of municipal and industrial waste waters which should be purified and reused. Physical, chemical, and biological purification processes have been developed. Electroflotation is an established purification method especially for industrial waste waters. Low voltage (5-24V) direct current is applied to achieve decomposition of water to hydrogen, oxygen and other gases depending on the inorganic ion in water. Electricity causes various secondary reactions in the solutions which cause solubility of colloidal particles to decrease. The particles form flocks which bind to generate gas bubbles and are lifted by their buoyance forces to the water surface and form relatively stable foam or froth which can be then removed. Purification and microbial disinfection of waste waters are interconnected tasks. Removal of organic load from waste water removes also microbes. When biologically purified water is released in nature, it still contains microbes which may interfere with the natural habitats. Pathogens may cause aberrations in the ecosystems although they will not directly infect humans. The possible harmful effects largely depend on the climatic and soil/drainage basin conditions of the waste discharge environment. However, even occasional infections may be harmful and therefore there is a need to secure that the discharge liquids are microbiologically safe. The disinfection of contaminated waters can be divided into chemical and physical techniques. The chemical ones include treatment with active chlorine compounds. Ozone is popular in disinfection of drinking water. Various methods to produce ozone and chlorine gas or compounds which release them are in use. Chemical methods have the drawback of yielding residues of unnatural chemicals and their reaction products. Low-voltage direct current has been deployed for disinfection of waste waters in various formats. Solar cells are especially attractive source for electricity. *In situ* production of oxidative chlorine compounds from concentrated NaCl solutions and chlorine gases from anode are introduced to water to be disinfected. The gases contain also other disinfecting compounds like reactive oxygen, ozone, together with generated alkali and acids. The electrodes may include semipermeable membranes or porous filters so that gases at anode and cathode do not mix. In situ production of disinfection gases has the advantage of production of poisonous chemicals without need of their storage and transport. The main disadvantage of the in situ processes is the use of semipermeable membranes which tend to clog and must be regularly cleaned and/or changed.

The electrochemical cathode reactions produce basic hydroxyl ions and molecular hydrogen. The hydrogen is generated about 0.4 liter (NTP) per Ah. The hydrogen formation is less useful for the disinfection while it is important for obtaining the flotation effect. In proper conditions the reduction power at cathode can be transformed to hydrogen peroxide formation which may be used as a disinfectant. Anodic corrosion is a serious problem in electroflotation, as well as, in the disinfection systems. It can be diminished by specific coatings of the electrodes. High current density increases the generation of electrochemical oxidants. Because the stability of the grainy nano-coating is dependent on the polarity of the electrode, the polarity cannot be changed to prevent the clogging of electrodes. Electrode corrosion (dissolving) is also exploited in waste water purification to produce metal ions, like Feand Al- salts, which precipitate biomolecules and phosphate. This process avoids the need to add them separately as salts. Especial hindrance for the adoption of electric technologies in waste water purification is the realization of convenient and long-life maintenance-free equipment. The primary problems arise from electrode passivation by deposition of materials on the electrodes. When considering the overall economy, usual low-corrosion anode materials, like titanium and stainless steel may be optimal allowing change of the polarity of the electrodes. Here is presented a new device construction, to work out electroflotation and microbial disinfection, which can be used to produce drinking water.

**Keywords:** waste water purification and sterilization, electroflotation, sanitation with solar energy.

Analysis of water resources and water potentials under conditions of land use-urban-industrial-agriculture change and climate change in the eastern region of Thailand

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Analysis of water resources and rainwater-runoff potentials under climate change conditions and land use development, urban, economic and industrial change in the eastern region, as well as analyzing models and evaluating water costs in the eastern watershed, namely the Eastern Coastal Basin, Bang Pakong River Basin, Prachinburi Basin, Tonle Lake Basin by using the SWAT model (Soil and Water Assessment Tool) and a Cellular Automata (CA)-Markov chain model to predict land use change related the amount of runoff demand for water in the year 2012-2018 has an average water demand of 10,316.44 - 13824.96 million cubic meters (consumption 158.40-212.27 million cubic meters, industry and tourism 369.36-494.98 million cubic meters, agriculture 9,338.18 - 12514.05 million cubic meters). The economic development of the Eastern Seaboard area has changed due to higher temperature, hot and drought during the summer season as high as 35 - 45 degrees Celsius, along with changes in land use of the eastern region from year 2006 to year 2016, increasing of urban areas, industries and buildings (mostly is a infrastructure), by 305.29 square kilometers, especially in Rayong, Chonburi, and Chachoengsao province. Agricultural area increased by 446.74 square kilometers which is growing by 10 - 15%, result of government policies, development of special economic zones, Coastal Economic Corridor, and water demand increased 10 - 20 % per year in each sector. The Water Supply of Eastern Thailand has 56 reservoirs with 6 large reservoirs with a combined capacity of 1467.75 MCM and 49 medium-sized reservoirs with a total capacity of 647 MCM. that a storage volume of 2115 MCM. Eastern region has 48 industrial estates and total factories have both located insite and outsite industrial estates about 10853 factories. Water demand of agriculture sector used about 85-90 % or more than 5,600 million cubic meters: MCM./Year of the total water supplies. Industial sectors used about 8-10 % or more than 570 MCM./Y. Urban, domestic and tourism used about 5-7% or more than 370 MCM./Year.

**Keywords:** SWAT: Soil and Water Assessment Tool, Eastern basin, CA-Markov Chain

Assessment of carbon footprint from electrical energy usage of students in the mahidol university amnatcharoen campus establishment project for eco university

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This research aimed to 1. Assess carbon footprint from electrical energy usage in habitation of students in the Mahidol University Amnatcharoen Campus Establishment Project; 2. Study electrical energy usage behavior of the students in the Mahidol University Amnatcharoen Campus Establishment Project for Eco University. The samples are 157 students with study in the Mahidol University Amnatcharoen Campus Establishment Project in 2nd semester, academic year 2017. The tool used in this research is assessment of carbon footprint from electrical energy usage of students in the Mahidol University Amnatcharoen Campus Establishment Project. To analyze data, the statistical techniques were applied, including descriptive statistics, percentage, mean, standard deviation and calculation to analyze carbon dioxide emissions (CO<sub>2</sub> Emission). The study revealed that in the assessment of carbon dioxide equivalent emissions per year class of study, it was assessed that the students had a total of carbon dioxide emissions at 1,784.32 ton CO<sub>2</sub>/capita, with an average of 1.64 ton CO<sub>2</sub> /Capital/person. Considering by year class of study, it was found that the year class with highest amount of carbon dioxide equivalent emissions was the 2nd year, at 767.52 ton CO<sub>2</sub>/capita (including 2 programs: Bachelor of Public Health Program, and; Bachelor of Science Program in Agriculture). In terms of knowledge on electrical energy usage of the students, the study showed that most of them were aware that "maintenance of electrical appliances helps to save energy" followed by the knowledge that "putting too many stuffs in the refrigerator will cause the refrigerator to consume more power than usual" and "electricity generation in Thailand does not use fuel". In the behavioral aspect, it was found that the electrical energy usage behavior of students in overall was at often level. The information obtained from this research is expected to be guide for crafting policies to conserve electrical energy and reduce carbon dioxide emissions of the Mahidol University Amnatcharoen Campus.

Keywords: carbon footprint, electrical energy usage behavior, greenhouse gas

Application of aerial photography with visible atmospherically resistant index by using unmanned aerial vehicles for seagrass bed classification in kung krabaen bay, Thailand

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The aim of this research was to study seagrass classification by using aerial photography with Visible Atmospherically Resistant Index (VARI) in the Kung Krabaen Bay, Chanthaburi, Thailand, which covers an area of 5.59 km2 and has an average depth of 2.5 m in the shallow zone. The classification based on VARI resulted in three classes, namely (i) long-leaved species (*E. acoroides*), (ii) short-leaved species (*H. pinifolia* and *H. uninervis*), and (iii) other objects. Results showed that aerial photographs could clearly differentiate seagrass species having different digital number value ranges with the VARI approach. The overall accuracy of visual interpretation was higher than that of supervised classification of 88.88% and 61.11%, respectively. This technique could be useful for seagrass species mapping in other areas. The results also showed that *H. pinifolia* and *H. uninervis* were distributed on sandy clay and seashell substrates while *E. acoroides* was distributed only on sandy areas.

Keywords: aerial photography, Visible Atmospherically Resistant Index, seagrass bed classification

## Effects of soil moisture contents and arbuscular mycorrhizal fungi on phosphorus fractions in soil

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Drought is an environmental stress that impacts on plant growth, crop productivity and nutrient availability. After nitrogen, phosphorus (P) is often limited as a nutrient for crops because of fixation by ferrous (Fe), aluminum (Al) and calcium (Ca) in the soil. Effects of soil moisture content and arbuscular mycorrhizal fungi (AMF) addition were investigated on seven inorganic P forms as P-water, P-loosely, P-Al, P-Fe, P-reductant, P-Ca and P-apatite in Ultisol. The soil incubation study was set as 4×2 factorial in completely randomized design (CRD). The first factor was soil moisture content at 0% (control), 25% (drought), 50% (moderate drought) and 75% (non-drought) soil water holding capacity (WHC). The second factor was AMF addition consisting of inoculation and non-inoculation. The experiment was run for 90 days. Results showed that soil moisture affected on P-water, P-loosely, P-Fe and P-apatite with statistical significance. On day 90 of the experiment, P-loosely and P-apatite contents were high at 0% WHC, while P-water and P-Fe contents were high at 75% WHC. AMF inoculation affected P-Al, P-Ca and P-apatite contents with statistically significant differences. AMF inoculation reduced amounts of P-Al, P-Ca and P-apatite in soil. Interaction between soil moisture and AMF affected P-loosely and P-apatite with statistically significant differences. During the study period, P-Fe, P-Ca and P-apatite increased while P-water, P-loosely and P-Al decreased. Findings suggested that drought stress reduced P-water content, while AMF inoculation assist P transformation from unavailable to available forms to promote plant growth.

Keywords: soil moisture, arbuscular mycorrhizal, phosphorus fractions

Field assessment of altitude variation correlate d soil conductivity and porosity for validating archi formulation

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Archi formula collated with soil conductivity and porosity has been recognized for about 30 years. The method implemented by Archi was not fully explained how the equation could be formulated. In addition, the elevation criterion was not also completely defined in the Archi formula. This study was undertaken to validate the Archi Formula for land at different altitudes. The locations selected for the field assessment were coastal area, agriculture field, and forest, representing low terrain (5 m above sea level), the low-medium terrain (20 m above sea level) and medium terrain (40 m above sea level), respectively. Data of conductivity were collected using Geoelectric and soil conductivity at each depth and calculated using Resis2inv software. Undisturbed soil sample for porosity measurement was collected using an auger to 6 m depth with 1 m fractions. Soil porosity of each fraction was measured at the Soil Science Laboratory, Faculty of Agriculture, University of Bengkulu. Both data were utilized to create a model which was compared to the Archi formula. The results showed that there were corrections for consideration to validate the Archi formula. Correction of sand and clay contents should be considered for low and low-medium altitudes, respectively, while soil hardness for medium terrain. These corrections will benefit to the application of the Archi formula for practical use.

Keywords: conductivity, porosity, Archi formula, elevation, and soil types

#### A study of the relationship between urban heat island phenomena and land use: a case study of chiang rai municipality, Thailand

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This study aims to identify the relationship between urban heat island phenomena and land covers in Chiang Rai municipality as the study area. The direct thermal measurement was used to measure the thermal difference between the heats at the fixed weather station and the heats at the moving points along the traverse route. The experimental data were analyzed in order to develop the isothermal map of the study area at 20:00. Consequently, the thermal difference between urban area and rural area was slightly high (5 °C). The least squares analysis was applied to identify the relationship between urban heat and land covers that were classified into two categories (man-made structure and natural area). As the result, the thermal differences (Urban Heat Island: UHI) varied directly with the man-made structures but they were inversely with the natural areas following  $Y = 0.0393X_1 - 0.7533$  ( $R^2 = 0.8996$ ) and  $Y = -0.0393X_2 + 3.1765$  ( $R^2 = 0.8996$ ) equations.

Keywords: Urban heat island, direct thermal measurement, mobile traverse, land cover surface, least squares analysis

## Quantifying the carbon stocks of selected agroforestrysystems in zamboanga city, Philippines

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The importance of agroforestry as a land-use system for carbon (C) sequestration had been recognized. This study was conducted to determine the various levels of C stocks expressed into  $CO_2e$  sequestered of major agroforestry systems (AFSs) across the 16 community-based forest management (CBFM) sites in Zamboanga City, Philippines. The major AFSs were compared to pure forest stand. Among the AFSs, the rubber-based had the highest C stocks at 68.93 tC ha<sup>-1</sup> compared to lanzones-based (60.33 tC ha<sup>-1</sup>), marang-based (60.23 tC ha<sup>-1</sup>), mango-based (60.01 tC ha<sup>-1</sup>), respectively. The rest had below 60.0 tC ha<sup>-1</sup> carbon stocks. In terms of net carbon dioxide equivalent (net  $CO_{2e}$ ) sequestered, the pure forest had the highest at 1,098.62 t $CO_{2e}$  ha<sup>-1</sup> compared to the top four AFSs with the highest net t $CO_{2e}$  sequestered such as the rubber + based, lanzones, marang and mango-based AFSs where each had about 248.66, 217.70, 218.17 and 217.41 t $CO_{2e}$  ha<sup>-1</sup>, respectively. The pure forest stand had 5-7 times higher  $CO_{2e}$  sequestered compared with the top four AFSs. Results of this study had provided data that none of the AFSs can replace the real pristine forest in terms of C sequestration (5.0 ha AFS is equal to 1.0 ha pristine forest) and its watershed role as they are net user of water. It was observed that no water is available in the 16 CBFM sites and community residents had to fetch water for their household use. Also, Zamboanga City, Philippines had insufficient water for domestic use during extended rainless or El Nino months.

Keywords: agroforestry systems, carbon sequestration, carbon yield on biomass, soil organic carbon, pure forest stand

Drought risk area assessment in sa kaeo province by using geographic information systems (GIS)

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This study aims to assess drought risk area in Sa Kaeo province by using Geographic Information System (GIS) based on potential influencing factors, namely: average annual rainfall, volume of groundwater, distance from water sources, distance from irrigation area, soil drainage, slope and land use. The weighting factors suggested by environmental experts in the field of environment and natural resource management were 7:5:5.2:5.5:5:4.5:4.3 for each factor, respectively. The results indicated that drought risk area in low, moderate, high, and very high level were  $103,394 \, \text{m}^2$  (1.44% of provincial area), 3,482,673.76  $\text{m}^2$  (48.40% of provincial area), 3,406,193.74  $\text{m}^2$  (47.34% of provincial area) and 203,174.31  $\text{m}^2$  (2.82% of provincial area), respectively. Comparing to real scenario, the overall accuracy of assessed results was 77.35%. The results of this study can be used as information for the local administration to alleviate the continuation of drought in Sa Kaeo province.

Keywords: drought risk, Geographic Information System, Sa Kaeo Province

The application of geo-informatics technology to study the vegetation index and humidity: a case study analysis of drought in special economic eastern region, Thailand

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The objective of this research was to evaluate the changes in vegetation as related to drought in Special Economic Eastern Region, Thailand. The data analyzed and measured were the changes detection in Normalized Difference Vegetation Index (NDVI), Difference Water Index (NDVI), and Difference Enhanced Vegetation Index (EVI). Secondly, the seasonal cycle of growth stages was studied. The establishment of relationships between rainfall assessments of change was determined by the thresholds of the mean indices and order of standard deviation from the mean of picture difference Index as determination size of changes. The result achieved describe the change of vegetation covering differently and it indicated the spatial-temporal pattern of drought. These represented each of the NDVI, NDWI, and EVI value of spatial-temporal pattern image covering over the NDVI and EVI which had higher value; namely, it referred to the differences among the areas of various vegetation covering. The NDVI, and EVI had greater degree of NDWI provided the difference in terms of a combination of vegetation covering and water content. Finally, the map of severity for classifying the drought in the spatial-temporal pattern was made. The spatial-temporal pattern of drought obtained from the NDVI NDWI and EVI could be used instead of the meteorological data in cases where there is inadequate coverage of climatic data.

**Keywords:** Geo-informatics Technology, Drought, Normalized Differential Vegetation Index, Normalized Difference Water Index, Enhanced Vegetation Index

## Soil microbial activities in alfisol with different green manure applications

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Reduction of soil fertility is a pressing global problem. Green manure is an alternative method to increase soil fertility and reduce disease accumulation in the fields. In Thailand, many varieties of legumes are used as green manure. This study focused on the activity of soil microorganisms after green manure application for 30 days in Alfisol. Completely Randomized Design (CRD) was used for the experiments with 8 treatments as 1) control, 2) sunn hemp, 3) jack bean, 4) cowpea, 5) mung bean, 6) soybean, 7) African sesbania and 8) earleaf acacia. Soil samples were collected every 10 days and analyzed for soil respiration, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and enzymatic activities (cellulase, protease, acid-phosphatase and urease). After 30 days, the highest soil respiration was found under African sesbania application with 0.5478 mgCO<sub>2</sub>/g soil, while lowest MBC was found under cowpea application with 29.68 mgCO<sub>2</sub>/g soil. MBN was found at the highest value under non-application of green manure (control) at 2.20 ng/g soil. Urease activity was highest under African sesbania at 12.58  $\mu$ g NH<sub>4</sub>-N/g dwt. However, activities of cellulase, protease and acid-phosphatase were not statistically different between treatments. Enzymatic activities increased during the study period with highest activities found on day 20 and 30 of the experiment. Soybean promoted the highest microbial biomass while jack bean promoted the highest soil respiration during the study period. Changes of enzymes followed the same trend, except for urease. Decomposition rate related to plant quality which control activity in soil.

**Keywords:** green manure, enzymatic activity, microbial activity

#### Farmer's climate change cognition and adaptation in agroecological zones of mindanao, Philippines

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The Farmer's Climate Change Cognition and Adaptation in Agroecological Zones Datu Odin Sinsuat of Mindanao, Philippines were analyzed. All 90 respondents in the 3 agroecological zones (lowland, upland and coastal areas) believed that there are changes in the last 30-40 years, there are less involvement in traditional farming but there is rampant use of chemicals, loss of large number of big dipterocarp trees, high charcoal making activities, less presence of wild trees and animals and high erosion rates. Mostly are knowledgeable and aware about climate change. The practices that contributed to the changes are cutting of trees and illegal logging, charcoal making, use of chemicals, burning of plastics and throwing of garbage in rivers, seas and everywhere. The changes are long dry spell, very hot even during rainy days, drying of rivers and long rainy seasons. The effects are large deforested areas, high incidence of insect pest, soil erosion, crop losses and death of livestocks, drought and destroyed roads. These result to low productivity, income and poverty. To lessen the effects, most of them rely on prayers. Other options are through planting and replanting, avoid cutting of trees, stop illegal logging, encourage cooperativism, follow laws on conservation, maintain cleanliness and proper waste disposal, not use of chemicals and observed timing of planting.

Keywords: climate change, cognition, adaptation, agroecological zones

#### The effect of water deficit at different growth stage on yield and quality of sweet corn (Zea mays saccharata sturt.)

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The effect of drought stress on growth, yield and quality of crop plants especially, sweet corn are usually affected due to the severity of drought and growth stages at which drought stress occurred. Finding out for the critical growth stage to drought stress may provide suitable planting date in each rainfall pathern or for higher water management efficiency. This field experiment was conducted to examine the effect of water deficit severity at different growth stage of sweet corn var. ATS8 in dry season (Jan-Mar), 2017. Randomized Complete Block Design (RCBD) with 4 replications was used, sweet corn was planted in space of 75x25 cm with 21 plants/row, 6 rows/plot, data were recorded from 2-middle rows, and space between plot was 2 m. The experiment was conducted in a silty-clay soil (Pak-chong series) at the National Corn and Sorghum Research Center (Suwan Farm), central Thailand. Seven water applications comprised of 2-water deficit severity; 1) moderately deficit (MD; withholding water until soil moisture at 25 cm from surface was less than 5% for 3 days), and 2) severely deficit (SD; withholding water until soil moisture at 25 cm from surface was less than 5% for 7 days) at 3 growth stages; 1) 2-wk before silking  $(V_{13})$ , 2) silking  $(R_1)$ , and 3) 2-wk after silking  $(R_2)$  and full irrigation (FI; after emergence 2-wk, sprinkle irrigation was applied weekly) was control treatment. Water application treatments showed highly significant effects (p≤0.01) on ear yield, ear size (ear length, diameter), and number kernels/ear. Full irrigation, MDV<sub>13</sub>, SDV<sub>13</sub>, and MDR<sub>2</sub> had a non-significant green yield of 16.03, 16.31, 16.06 and 15.98 t/ha, respectively, while these 4 treatments had significantly higher yield than  $SDR_2$ ,  $MDR_1$  and  $SDR_1$  with green yields of 8.43, 7.89 and 12.78 t/ha, respectively. The effects of water deficit at 3 different growth stages on kernel sweetness (% brix) and cut-kernel percentage were not noticed.

Keywords: sweet corn, water deficit, different growth stage, ear yield, kernel quality

## Effect of number of plowing on okra productivity in suphan buri province, Thailand

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Plowing has been an important feature of technological development in agriculture, in particular in food production. The study has been conducted to identify effect of number of plowing on okra productivity by using t-test analysis in Suphan Buri Province, Thailand. Structure questionnaires were conducted to gather data from 82 okra growers in Suphan Buri Province. Descriptive statistics were used to describe socio-economic variables of the respondents as presented. Besides, t-test analysis was employed in relation between number of plowing and okra growers' productivity. The results revealed that there is no significant difference was found between one-time plowing and three times plowing on okra productivity.

Keywords: Okra, okra productivity, okra growers, plowing, t-test

#### **SESSION 6 ANIMAL BIOTECHNOLOGY**

## Cattle identification and traceability systems in botswana and its challenges

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Cattle population in Botswana is estimated to be approximately 2.1 million. About 80% of the national herd is owned by smallholder farmers with 1-20 cattle in open grazing areas, whereas the remaining 20% is found in fenced farms. It is estimated that up to 75% of beef is exported to the European Union (EU), 15% to South Africa and 10% to Norway. This makes livestock identification and traceability to be necessary requirements for beef exports, especially to the lucrative markets of EU and Norway. Identification of animals by marking their bodies was first recorded 3800 years ago in the Code of Hammurabi. Some reasons for identifying livestock include production management, control of disease outbreaks, establishment of ownership, export requirements, as well as, consumer demands. Cattle have for a long time been identified in Botswana using ear notching, hot iron branding and also by zonal branding. Electronic identification methods such as Livestock Identification and Traceability System (LITS) that used reticular bolus was introduced in 2001 after it faced implementation challenges. It was replaced by Botswana Animal Identification and Traceability System (BAITS) that uses electronic ear tags in 2013. About 83% of cattle in Botswana have been using electronic ear tags. Some major challenges in the implementation of BAITS are failure of farmers to report tagged animals, loss of ear tags, frequent equipment breakdown, ICT challenges leading to slowness of the system, lack of internet service especially in the rural areas, delays in inputting data at district level and farmers' low literacy level and apathy. Farmer education needs to be intensified in order to have more cattle identified using BAITS to enable their slaughter at abattoirs giving rise to increased throughput and beef exports.

Keywords: BAITS, beef exports, Bolus, Botswana, cattle, electronic ear tags, LITS.

# Effects of Garcinia cowa leaf powder supplementation in diets on the productive performance of broilers

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This experiment was conducted to evaluate the effects of *Garcinia cowa* leaf powder diet supplement on the productive performance and survival rate of broilers. The results showed that the supplementations gave highly significant differences in weight gain, average daily gain, and feed conversion efficiency of broilers (P<0.01). The supplemented with 4 and 5 percent of *Garcinia cowa* leaf powder expressed significant lower final weight, weight gain, average daily gain, and feed conversion efficiency than the supplemented with 1, 2, and 3 percent of *Garcinia cowa* leaf powder in the diets. Although the supplemented with 1 percent of *Garcinia cowa* leaf powder had higher final weight, weight gain, average daily gain than the supplemented with 0, 2 and 3 percent of *Garcinia cowa* leaf powder in the diets, the differences was not significant. In addition, the supplementations gave no significant differences in feed intake and survival rate (P>0.05). Hence, *Garcinia cowa* leaf powder could be supplemented in the broiler diets not more than 3 percent as a viable alternative to antibiotics growth promoters.

Keywords: Garcinia cowa, leaf powder, broiler performance

## The effect of sex, age, and final live weight on carcass traits of fattening dairy cattle

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This study was conducted to investigate the effect of sex, age, and final live weight of fattening dairy cattle on carcass traits. One hundred and sixty-seven fattening dairy cattle were intensive raised during January to May 2019 by members of Beef Cluster Cooperative Limited until their body weight reached to slaughter weight, 500 kg. After slaughter, data of carcass traits, such as final live weight, hot and chilled carcass weight, marbling score, chilling loss was measured and recorded. The percentages of hot and chilled carcass weight were calculated based on final live weight. Percentage of chilling loss was also calculated based on hot carcass weight. Descriptive statistic was used to analyse data distribution. To determine the studied factors affecting marbling score, chi-square test was used. The effect of sex, age, and final live weight on the others carcass traits was analyzed by using general linear model. Results showed that most of the animals (63.25 %) obtained marbling score 2, and about 19.27 % of them obtained the score 1. Marbling score was not affected by all studied factors (P>0.05). Sex of animals had highly significantly affected to hot and chilled carcass weight (P<0.01). Averages of both hot and chilled carcass weight of male were greater than those of female. All studied traits (P<0.05), except chilling loss (P>0.05) were influenced by age of animals. Averages of both hot and chilled carcass weight, both in kg and in percent of the animals with 3 yrs age showed the highest, but did not statistically differ from those of animals with 4 yrs age. Almost all of the studied traits were highly significantly affected by group of final live weight (P<0.01). As final live weight increased, both hot and chilled carcass weight in kg and in percent decreased.

Keywords: Carcass traits, Fattening dairy cattle

Intake and digestibility of mix-herbal supplements block for bali cattle fed agricultural by-product in bengkulu, Indonesia

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The objectives of this research were to analyze the effect of Mix-Herbal Supplementation Block (MHSB) fed agricultural by-product on Bali cattle intake and digestibility. Twelve growing Bali steers, 1.5-year old with Body Condition Score of 5 (maximum of 9) and average body weight of 175.7 kg were used. Three groups of 4 steers each, evenly matched for live weight, were fed with different supplementations, SO: no supplementation, SCL: supplemented with *Curcuma longa* (50 mg/kg live weight/day), and SZO: supplemented with *Zingiber officinale* (50 mg/kg live weight/day). SCL and SZO group were also given a similar amount of *Nigella sativa* and *Melastoma malabatricum*. Feed, consisting of tofu by-product, and Palm Oil Sludge and Elephant grass, was given *ad libitum* for all groups. The experiment was conducted over 21 days consisting of a 14-day preliminary period and a 7-day measurement period. During the measurement period, feed intake and fecal output were daily measured. The result indicated that MHSB did not significantly (P>0.05) affect intake and digestibility in all treatments. This study showed that the amount of mixherbal supplements could be given in a higher amount to the Bali cattle.

**Keywords:** agricultural by-product, Bali cattle, digestibility, mix-herbal.

Essential oils from Zingiber mekongense gagnep, Myristica fragrans Houtt and Curcuma zedoaria roscoe as larvicidal agents against Aedes albopictus (skuse)

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This research investigated the larvicidal effects against the fourth instar larvae of *Aedes albopictus* of three essential oils extracted from *Zingiber mekongense* Gagnep, *Myristica fragrans* Houtt and *Curcuma zedoaria* Roscoe (EOs). These effects were compared to that of a synthetic larvicidal agent, temephos 1% w/w (Sai GPO-1%). The three essential oils at concentrations of 1%, 5% and 10% were tested by using a dipping method against the fourth instar larvae of *Ae. albopictus*, and the mortality rates were recorded at 0.08, 0.16, 0.5, 1, 6, 12, and 10% and 10% concentration provided the strongest larvicidal activity against *Ae. albopictus* and yielded the highest mortality rate of 100% with 10% value at 1.09% and 10% value of 10% values for *Z. mekongense* and *M. Fragrans* EOs were 10% value was the same at 10% values for *Z. mekongense* and *M. Fragrans* EOs were 10% value of 10% value of 10% value of 10% value of 10% value was the same at 10% value of 10% value

Keywords: Aedes albopictus, larvicidal activity, plant essential oils

Effects of sodium chloride replacement with potassium chloride and flavor enhancers on the physico-chemical and sensorial qualities of low-fat chicken frankfurter sausage

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The effects of sodium chloride (NaCl) replacement with potassium chloride (KCl) and flavor enhancer on the physico-chemical and sensorial qualities of low-fat chicken frankfurter sausage was studied to reduce sodium content in the product. Three replications of low-fat chicken frankfurters were manufactured to compare seven treatments: control (1.2% NaCl), replacing NaCl by 25% 50% 75% KCl with 1% lysine (25KL, 50KL and 75KL, respectively) and replacing NaCl by 25% 50% 75% KCl with 1% yeast extract (25KY, 50KY and 75KY, respectively). The results showed that NaCl replacement by KCl together with both flavor enhancers did not affect to pH of batter, pH of sausage, and emulsion stability (P>0.05). Replacement of NaCl by KCl with both flavor enhancers in the product resulted in lower values of hardness, gumminess, and chewiness as increased the percentage of KCl (P<0.05). Moreover, the addition of yeast extract in replacing NaCl by KCl showed a lower lightness with higher yellowness than control and lysine groups (P<0.05). The flavor of 50KY and 75KY were lower sensorial liking score than control. Liking scores of taste, texture, and overall acceptability of 75KL, 50KY, and 75KY were lower than control. However, the lysine addition of 25KL and 50KL showed similar flavor, taste, and overall acceptability scores as compared with control. In conclusion, the use of 50% replacement of NaCl by KCl and with 1% lysine (50 KL) was optimum formulation to reduce the sodium content in low-fat chicken frankfurter sausages while maintaining quality characteristics of low-fat frankfurters.

Keywords: Sodium chloride replacement, Potassium chloride, flavor enhancer, low-fat frankfurter

#### Sous-vided restructured goat meat: changes in meat qualities during refrigerated storage

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The aim of this study was to evaluate the changes in meat qualities of sous-vided restructured goat meat during refrigerated storage. The samples were cooked using sous-vide technology at core temperature 60°C for 43 min and stored at 4°C for 49 days. The results showed that all microbial counts of sous-vide sample were significantly increased during storage. The aerobic and anaerobic mesophilic bacteria were higher than 6 log cfu/g for 35 days which their counts were below limited microbiological quality of cooked food in Food and Container Standard No. 3, Thailand. *Brochothrix thermosphacta* and pathogen counts as *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens* were below 1 log cfu/g. Additionally, *Salmonella* spp., *Listeria* spp., *Listeria monocytogenes* were not detected during storage. For physicochemical quality, pH value of sous-vide sample decreased during storage which due to increased lactic acid bacteria counts. Purge loss had a significant increase (P<0.05) throughout the storage. CIE L\* had a significant increase (P<0.05) whereas CIE a\* and CIE b\* had significant decreases (P<0.05) with increasing storage time. For texture profile analysis, hardness, gumminess, and chewiness had significant increases (P<0.05) as storage time was increased. After grilling, there were significant increase for grilling loss and significant decreases for CIE a\* and CIE b\* values (P<0.05) throughout storage time. Thus, shelf-life of sous-vided restructured goat meat was 35 days during storage at 4°C.

**Keywords:** shelf-life, sous-vided meat, restructured meat, cold storage

Effects of heart fatty acid biding protein (H-FABP) gene, housing system, and sex on carcass and meat quality of commercial crossbred pigs

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This study was conducted to find the genetic polymorphism and frequency of genotypes of H-FABP gene and to investigate the effects of H-FABP genotypes, housing system, and sex on the carcass and meat quality of commercial crossbred pigs. Eighty-seven pigs (Landrace x Large White) x (Duroc or commercial New Line) were divided into two groups, then raised in two different housing systems: Evaporative cooling system and Thai conventional housing system. The pigs were slaughtered at 100 kg live body weight at the standard slaughterhouse. The chilled carcasses were dissected following the Thai style cutting for investigate the carcass quality; lean percentage (LP), fat percentage (FP), LSQ, Loin Eye Area (LEA), and intramuscular fat (IMF). The M. *longissimus dorsi* muscle was used for examinaning the meat quality traits: pH<sub>1</sub>, pH<sub>24</sub>, T<sub>1</sub>, T<sub>24</sub>, color (L, a\*, b\*), and drip loss. Blood samples were collected for DNA extraction and identified the genotypes by the PCR-RFLP technique using the *Hae*III, *Msp*I, and *Hinf*I as the restriction enzyme. The results showed three H-FABP *Hae*III genotypes (DD, Dd, and dd), three H-FABP *Msp*I genotypes (AA, Aa, and aa), and the H-FABP *Hinf*I (HH). The allele Frequency of *Hae*III, *Msp*I and *Hinf*I polymorphism were as follow: D=0.55 and d=0.45; A=0.68 and a=0.32, while H=1. There was no effect (p> 0.5) of H-FABP genotypes on carcass quality in this study. It has been shown that no significant effect of H-FABP genotypes on meat quality traits (T<sub>1</sub>, T<sub>24</sub>, pH<sub>1</sub>, color (L, a\*, b\*), and drip loss. However, it was found that the pH<sub>24</sub> of both genotypes Dd and dd were lower than that of genotype DD. Similarly, the pH<sub>24</sub> of both genotypes Aa and aa were lower than that of AA. Whereas sex (gilts and barrow), gilts had the muscle temperature lower than that of the barrows.

Keywords: H-FABP gene, carcass quality, meat quality, crossbred pig

Biological, farm, and slaughter month factors affecting dairy carcass traits and the relationship between the traits, thoracic cavity depth, and carcass length

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The objectives of this study were to investigate biological (sex and age of animal), farm and slaughter month factors influenced on carcass traits of dairy cattle, and to study relationship of the traits with thoracic cavity depth and carcass length. A total of fiftynine dairy cattle was raised intensively in members' farms of Beef Cluster Cooperative Limited until their body weight reached to slaughter weight about 500 to 550 kg, then they were transported to a private commercial slaughter house. Before slaughter, farm and animals' identifications, sex, slaughter date, and final live weight were recorded. Age of animals was estimated by counting number of pair of permanent incisors. After slaughter, skin was weighed. Clean carcass was halved, and they were weighed, as hot carcass weight. Dressing percentage was calculated based on hot carcass weight. The thoracic cavity depth (TCD) and carcass length were measured. Least squares method was used to analyze the factors affected the carcass traits. The relationship between the traits studied with TCD, and CL was analyzed using Pearson correlation. Results showed that the biological factors did not influence on all studied traits. The factor of farm affected most traits, except TCD, skin weight (SKW), and percentage of skin weight (PSK). Slaughter month had affected all the traits, except PSK. For the relationship studied, it was found that the final live weight had highly significantly positive correlated with all studied traits (P<0.01), except TCD, and PSK. Hot carcass weight had highly significantly positive correlation with dressing percentage, CL, and SKW (P<0.01), except PSK. Dressing percentage did not correlate with TCD and CL. However, the negative correlation between TCD and CL (r = -0.325) was found, whereas the TCD did not relate with SKW and PKW. However, the CL had negative correlate with PSK (r=-0.287), while the relationship between SKW and PSK was 0.586.

Keywords: Carcass traits, relationship, dairy cattle, Thailand

Influence of sex, age, slaughter weight, and farm on carcass composition of fattening dairy cattle

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The objective of this study was to determine the influence of sex, age, slaughter weight, and farm on dairy beef carcass composition. Data from a total of one-hundred and forty-six carcasses from fattening dairy (minimum 87.5% Holstein Friesian) cattle were conducted. After slaughter, the carcasses were chilled for 7 days at 4°C, then, they were cut into 4 quarters. Each quarter was weighed, and then it was deboned and fat was trimmed. Lean meat, bone, fat, and tendons of each quarter were weighed and recorded. Chilled carcass weight, total weight of lean meat, bone, fat, and tendons weight were calculated. Percentages of lean meat, bone, fat, and tendons based on chilled carcass weight, and proportions of muscle:bone, and muscle:fat were also computed. General linear model was used to determine the factors of sex, age, slaughter weight, and farm affected to the carcass composition. Results showed that sex had highly significantly influenced to total tendons weight and percentage of tendons weight (P<0.01). Furthermore, the factor had significantly affected to total weight, total lean meat weight, and total bone weight (P<0.05). Age influenced on total bone weight, total fat weight of lean meat, bone, fat, and tendons were highly significantly affected by slaughter weight group (P<0.01), while the factor had significantly influenced percentage of fat (P<0.05). The cattle with slaughter weight more than 650 kg had higher averages of chilled carcass weight, total of lean meat, bone, fat, and tendon weight and percentage of fat than other groups. The factor of farm had highly significantly effected chilled carcass weight and total fat weight (P<0.01), and it also influenced on total weight of lean meat and bone, and percentages of bone and of fat (P<0.05).

Keywords: Dairy carcass composition, Thailand

#### Aquacultured tropical eels in bengkulu province of Indonesia and its potentials

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Based on reported studies, about nineteen species and subspecies of eel genus *Anguilla* inhabit around the world. Five species among them are inhabiting in the Indonesian waters. Those tropical eels just spread out from east, centre, to west of Indonesian archipelagos in various species composition and pattern of abundance. However, mostly Indonesian people did not recognize the importance of eels as a kind of fish or as a luxury food, but just a kind of snake that completely forbidden for consumption among the majority Muslim of Indonesian society. At present, Indonesian tropical eels become a remarkable luxury and famous food resources around the world especially in the region of East Asian countries where the eels has long been esteemed as an important food fish having a unique taste beside its highly traditional values. East Asian regions including Japan, Korea, China, Hongkong and Taiwan were the most highly consumer of eels in the world. In order to avoid decreases in tropical eel resources, we need to understand the basic, the causes, and the mechanisms, and also to develop technology in order to maintaining stable population of eel in the tropic. Aquaculture of single species caught from the nature maybe problem for such a great species diversity of the Indonesian tropical eels. The potentials of cultured tropical eels in Bengkulu Province are high. Tropical eels collected from nature survive and grow very well.

Keywords: aquaculture, tropical eel, Bengkulu

#### Paternity testing of crossbred beef cattle with 15 microsatellite markers

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The objectives of this study were to determine the possibility of 15 microsatellite markers for paternity testing, and to compare accuracy of the markers with pedigree records. Blood sample at jugular vein or ear vein were collected from thirty crossbreds (Native-Brahman Cow x Charolais Sire). As recorded in pedigree, all crossbreds were offspring of 4 sires. Frozen semen of the sires was supported by Pon Yang Kham Livestock Breeding Cooperative NSC. Ltd. DNA from both blood and semen were extracted using DNA blood kit and DNA tissue kit, respectively. Quality and quantity of extracted DNA were measured using spectrophotometer. Polymerase Chain Reaction technique used amplified DNA with 15 microsattellite primers: BM1818, BM1824, BM2113, CSRM60, CSSM66, ETH10, ETH225, HEL1, INRA023, INRA037, INRA063, TGLA53, TGLA122, TGLA126, and TGLA227. PCR-products were tested using 1% Agarose gel, with DNA 100 bp Ladder standard marker, and stained with GelRed. Alleles of each microsatellite marker was separated using Capillary Electrophoresis with DNA1000 kit chip. Allele size of each marker within or between chips was analyzed using Bioanalyzer software. Cervus version 3.0.7 was used to analyse allele frequency, identity analysis, simulation test, and parentage assignment analysis, then paternity and accuracy were tested. Results found that alleles from only 9 microsatellites were detected, which were BM1818, BM1824, BM2113, CSSM66, ETH10, ETH225, INRA023, INRA037, and INRA063. The highest number of alleles (23 alleles) was found in CSSM66, while the lowest number (13 alleles) was found in ETH 10. BM1818 had allele size between 239 to 316 bp, while the smallest sizes, 129 to 179 bp, was detected in BM2113. Twenty-nine pairs of sireoffspring had positive of LOD score, except B066 with Carlos-sire the LOD score was negative. In conclusion, these 9 microstellites could be used for paternity testing.

Keywords: Paternity Testing, Microsatellite Markers, Beef cattle, Thailand

# Prevalence of trematode metacercariae in cyprinoid fish from chiang rai province, Thailand

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Liver flukes are parasitic worms that live in the bile ducts and the liver of infected human and animals. It needs at least one or more intermediate hosts. Liver fluke transmitted by eating raw fish can be found in the intestines of many specific hosts and other specialized hosts. People get infected with liver flukes by eating raw fish with a metacercaria. This study aimed to investigate the prevalence of trematode metacercaria in cyprinoid fishes in Nong Luang wetland area, Chiang Rai province. Data were collected between April and December 2016, from total 11 species of 360 cyprinoid fishes. The prevalence of metacercaria in cyprinoid fishes was 39.17% (141/360), mostly of metacercaria found in fish species; *Henicorhynchus siamensis* 100% (7/7), *Systomus rubripinnis* 94.44% (17/18), *Barbonymus gonionotus* 76.74% (33/43), *Rasbora tornieri* 55.56% (5/9), *Osteochilus vittatus* 50% (1/2), *Puntius brevis* 30.39% (31/102), *Anematichthys repasson* 28.95% (33/114), *Luciosoma bleekeri* 25% (3/12), *Labiobarbus siamensis* 21.57% (11/51). With the average intensity per one fishes 33.14, 34.47, 17.45, 3.8, 32, 17.39, 61.79, 6.64, 3, respectively. The most common metacercaria detected in this study was *Haplorchoides* spp. followed by *Centrocestus formosanus* and unidentified type of metacercaria ,respectively. Moreover the findings revealed that seasonal changes related with the prevalence of metacercaria in cyprinoid fishes. The highest prevalence of metacercaria can be found in winter season. These results indicated that eating raw freshwater fish presents a high risk in acquiring fish borne zoonotic trematode infection.

Keywords: prevalence, trematode, metacercariae, cyprinoid fish, Nong Luang Wetland

#### Decreasing greenhouse gas emissions from cattle

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Livestock is a significant contributor of green house gases, especially through the emissions of nitrous oxide and methane, and the greenhouse gas emissions from cattle represent about 65% of the total livestock emissions. it is crucial to find more sustainable ways to keep cattle and supply food for the increasing population. Enteric fermentation is a normal part of the ruminant digestive process in which volatile fatty acids are formed in the rumen. These volatile fatty acids are the main source of energy for ruminants. In the process of forming fatty acids, methane and carbon dioxide are also formed and removed from rumen by eructation. The emissions from enteric fermentation represent over 40% of all emissions from the cattle production chains, but the amount of emissions can be decreased by increasing the forage quality and digestibility. Other dietary factors can affect emissions from cattle too, but making changes into the diets can be difficult, because the changes can easily affect the health of the herd. Most of the emission mitigation strategies aim to increase animal productivity. Animal welfare and health affect productivity, so improving those is also an effective way of reducing emissions, because sick animals need more energy and time to achieve the production goals. Animal welfare is also increasingly important for consumers, so improving the practices to better support animal welfare on the farm is not only beneficial to the animals, but also the producer.

#### Influence of duroc sire lines on carcass and meat quality traits of commercial crossbred pigs

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Carcass and meat quality traits of commercial crossbred pigs sired by two Duroc lines were determined and compared. Two Duroc sire lines were imported or new sire line and country herd sire line or old sire line for producing three-way crossbred commercial finishing pigs (Duroc x Large White x Landrace). Five castrated male and five femal pigs from each pig sire line were selected and slaughtered. The carcass and meat quality traits were measured and analysed. The offsprings from imported Duroc sire had significantly lower cold carcass percentage (71.86 % and 74.99 %, P<0.001) and body fat percentage (12.40 % and 15.48 %, P<0.01), but significantly higher bone percentage (15.40 % and 13.81 %, P<0.05) than those from in country herd Duroc sire line. No significant different in meat quality traits were found between two sire lines. Castrated male pigs had higher marbling score than female (P < 0.05). There were interaction between sire line and gender in all studied traits.

Keywords: carcass quality, meat quality, duroc sire line, commercial crossbred pig

Mosquito-repellent activity of *Illicium verum* hook.f., *Amomum villosum* lour. and *Amomum krervanh* pierre. essential oils against *Aedes albopictus* (skuse)

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Mosquito repellent activities of essential oils derived from *Illicium verum* Hook.f., *Amomum villosum* Lour. and *Amomum krervanh* Pierre. against *Aedes albopictus* females were compared with that of a chemical repellent (DEET; N, N-diethyl-3-methylbenzamide). The experiment consisted of three treatments of essential oil concentrations at 1%, 5% and 10%. All experiments were arm-in-cage testing done under laboratory conditions. For each treatment, 0.1 mL of each concentration of each essential oil was applied to an area of the tester's forearm. The tester wore a glove with a 3x10 cm² hole and stretched the arm through the diaphragm into the cage. Each test continued for 3 minutes. If no mosquitoes came to bite the arm in that area, it was pulled back, and the test was repeated every 15 minutes until some mosquitoes bit the arm or landed on the exposed area twice, then the test ended. The results showed that the three essential oils at the concentrations of 10% and 5% provided the same 15-minute protection from *Ae. albopictus*. The essential oils at 1% also provided some protection, a 3-minute protection. Comparing among essential oils, we found that the essential oils at 5% concentration provided the most effective and safe protection against *Ae. albopictus*, with a protection time of 15-minute, the higher concentration, 10%, only provided the same protection rate. DEET at concentrations of 1%, 5% and 10% provided protection times of 15-minute, 30-minute and 45-minute, respectively. We can conclude that the essential oils extracted from *I. verum, A. villosum, A.krervanh* have a full potential for effective protection against *Ae. albopictus* females. Although they are less effective than the synthetic chemical repellent, they are much safer. They are good alternatives to it and should be further developed into commercial repellent products.

Keywords: Aedes albopictus, repellent activity, plant essential oils

## SESSION 7 GENERAL SCIENCE AND TECHNOLOGY

Antimicrobial characteristics of wool fibers treated with chitosan and propolis nano composite and dyed with natural dye extracted from red prickly pear

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Nanotechnology provides a new concept for improvement of the antimicrobial activity of the textile fibers. Natural dye extracted from red prickly pear using microwave heating was used for dyeing wool fibers. Chitosan and propolis nano composite was applied as treatment of wool fibers before dyeing using microwave and ultrasonic methods. Dyes concentration and pH factors, color strength, color data and fastness properties of the dyed wool fibers were investigated. The results obtained indicated that wool fibers pretreated with the tested nano materials exhibited higher results than the untreated. Nano composite of chitosan and propolis show antimicrobial activity against some pathogenic fungi and bacteria. The results obtained indicated that the antimicrobial activity for natural dyes under investigation were good and enhanced by treatment with Nano composite. The morphologies structure of the untreated and pretreated wool fibers were examined by scanning electron microscopy (SEM). The untreated wool fibers have a rough surface. The pretreated wool fibers were swelling compared to the untreated fibers. The diameter of the fibers increased and has smooth and even surfaces. The changes in the surface morphology due to the effect of pretreatment with and nano composite.

Keywords: nano chitosan, propolis, red prickly pear, natural dye, antimicrobial activity

Intermediaries and local actors in agricultural technology and management in peri-urban Bangkok

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Agricultural technology and management are supposed to improve through decentralization, devolution and multi-stakeholder approach. Processes and outcomes, however, are far from being straightforward. Much depend on a variety of contingencies and contexts, and the actors, networks, and interactions involved. This research focuses on the decentralization of agricultural technology and management in peri-urban Bangkok, a place similar to other middle-income developing countries characterized by rapid industrialization, weakness formal state institutions and procedures, and intensity of pollution. The research illustrates that the decentralized agricultural technology and management have pushed to the forefront and sharpened a number of pre-existing problems such as capacity deficit, and created new problems such as disconnects between planning and actions in government regime. However, its most significant contribution has been in creating political space where various stakeholders have become active, respond and address their concerns in various ways vis-à-vis environmental and agricultural-related problems. Thus, there is nothing inevitable in having positive outcomes resulting from decentralization in terms of improving protection and livability of critical areas being planned. Improving ecological sustainability and viability of local livelihoods in these areas through devolution and participatory approach under the decentralization is by no means certain. Neither do local governments and local communities automatically become more capable in effective agricultural technology and management. The shift instead creates spaces potent with possibilities for a meaningful and constructive engagement between and among local state and non-state actors that can lead to synergies and positive outcomes. And for this to become the likely trajectory, the role of extra-local intermediaries is crucial. It is in this sense, that decentralization, albeit loaded with risks of failure, hold a long-term promise for state and society collaboration in agricultural technology and management at the local level where degradation, impacts and improvements matter most to people.

Keywords: intermediaries, local actors, agricultural technology and management, peri-urban Bangkok.

#### Development of mushroom beverage with high β-glucan content from local mushrooms

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Mushrooms are fungi with a high nutritional value. β-glucan, in particular, is an interesting functional component of mushrooms. This compound has been shown to have powerful medicinal properties. Our study, therefore, was focused on finding local edible mushrooms with high β-glucan content. From our previous research (Mongkontanawat and Wongekalak, 2015) together with our current study, fifteen species of native mushroom were analyzed for their β-glucan content. It was found that the highest β-glucan content was exhibited in *Schizophyllum commune* Fr. (39.08  $\pm$  5.82 %w/w). However, there was only a non-significant difference ( $p \le 0.05$ ) from *Pleurotus ostreatus* (Fr.) Kummer and *Pleurotus sajor-caju* (Fr.) Sing (37.01  $\pm$  1.35 and 34.02  $\pm$  3.06 %w/w, respectively). These species were, therefore, chosen to demonstrate product development of high β-glucan content in mushroom drink. Mixture design was used in this evaluation. Our results found that treatment 1 (S. *commune* Fr: P. *ostreatus* (Fr.) Kummer: P. *sajor-caju* (Fr.) Sing; 60:20:20) showed the highest significance ( $p \le 0.05$ ) of β-glucan content. The data strongly suggests that the levels of phenolic compounds, β-glucan content and radical scavenging antioxidant activity are dependent on the amount of S. *commune* Fr. In summary, S. *commune* Fr. could be used in a potential medicinal mushroom drink. Furthermore, this investigation provides a guideline for the selection of native mushrooms that could be used to develop a new functional drink in the future.

**Keywords:** β-glucan, mushroom, *Schizophyllum commune* Fr., product development

## The effects of reduced sugar on macaron quality

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Macaron is one of the most luxurious and delicious dessert in the world. It is originally from Italy. Macaron consists of 3 main ingredients which are egg white, ground almond, and sugar (granulated sugar for meringues making and icing sugar). They are round shells and filled with filling such as jam, buttercream or ganache. The purposes of this study was to determine the effects of the reduction of granulated and confectioner' sugar from the standard recipe by using Response Surface Methodology: RSM with Central Composite Design (CCD). Granulated and confectioner'sugar contents were studied in the range of 120-150 g. Thirteen treatments were studied on the effects of reduction of both sugars on the firmness and cohesiceness of batter, the fractubility, hardness and cohesiveness of nacaron shell. Higher granulated sugar and confectioner' sugar affected less firmness but more cohesiveness on magaron batter. For magaron shell quality, higher granulated and icing sugar caused more fractubility, hardness and cohesiveness of nacaron shell. Both sugars showed the significantly pronounced effects on textural attributes of magaron shell, especially on its brittleness, hardness, and cohesiveness. The amount of sugar could be reduced from 150 g to 130.46 g and icing sugar from 150 g to 125.66 g.. Finally, sensory evaluation was tested using 9-point hedonic scale. The reduced sugar macaron shell impacted on the feet height and chewiness that professional panellists gave the liking score less and more, respectively as compared to the macaron made from the standard recipe. The reduced and non-reduced sugar macarons obtained the overall liking score not significantly different (p>0.05) from the macaron shell made from the standard recipe.

Keywords: macaron, sugar reduction, meringue, icing sugar, sugar syrup, healthy

Investigation of rice blast resistant genes in Thai eliterice varieties (*Oryza sativa* L.) for improvement of broad-spectrum blast disease resistance variety

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Rice blast disease caused by the fungus *Pyricularia oryzae* is one of the most devastating diseases of rice growing areas worldwide. The control of the disease can be achieved by generating resistance variety using rice variety containing broad spectrum blast resistance gene as material in breeding program. Many of Thai indigenous rice varieties have been reported their properties such as disease resistance, stress tolerance and environmental adaptation and could be used as good genetic resources. Huai variety (GS19567) was found to contain more than one blast resistance genes which one of them was mapped on chromosome 11. The objective of this research was to investigate the 8 blast resistance genes included *Pi9*, *Pi54*, *Pia*, *Pi50*, *Pigm(t)*, *Pi-ta*, *Pik* and *Pib* by using gene specific primers in Huai and other 40 Thai elite rice varieties. The results obtained from polymerase chain reaction revealed that all tested varieties contained at least one blast resistant gene. The most frequent finding resistant gene was *Pib*, while *Pib* was missing in Huai variety. Therefore, in order to enhance broad spectrum blast disease resistance in sustaining blast resistance variety, the resistance gene *Pib* is recommended to be used as candidate gene in breeding program.

Keywords: rice blast disease, Pyricularia oryzae, resistance genes

Inulin extraction from jerusalem artichoke (Helianthus tuberosus L.) tuber powder and its application to yoghurt snack

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In this study inulin was extracted from Jerusalem artichoke tuber powder (JATP). The different extraction ratios of JATP and water; 1:10, 1:20 and 1:30 (w/w) were performed at 85 °C for 30 minutes. All extracted samples were concentrated to be approximately 19 °Brix before quality determination and its application to yoghurt snack. The results showed that at a ratio of 1:20 (w/w) the concentrated JA extract contained the highest inulin content 33.14±0.39 mg/ml and 2.06±0.01 mg/ml of reducing sugar. For yoghurt mixture formulation, concentrated JA extract was used as an ingredient. Yoghurt was mixed with sugar, concentrated JA extract and juice, namely Tomato juice (TJ) 20%, Lemon juice (LJ) 20%, Passion fruit juice (PJ) 20%, the combination of TJ 10% with LJ 10%, TJ 10% with PJ 10% or LJ 10% with PJ 10%. The sensory evaluation appeared that treatment added PJ20% got significantly higher scores between 7.03 to 7.93 in aspects of color, flavor, sweetness, sourness and overall acceptability indicating that panelists almost like sample very much. The sugar content in PJ yoghurt mixture formula was substituted by four sweetners including maltitol, xylitol, prebiotic syrup and JA syrup. It was found that the sugar substitution with JA syrup in PJ yoghurt mixture caused insignificantly different in color, flavor, sweetness, sourness and overall acceptability from control (sugar). Yoghurt fortified with concentrated JA inulin, PJ and JA syrup was foamed and dried by foam-mat drying technique. The addition of 35% foaming agents solution (2% methocel and 1.5% carboxymelthyl cellulose) produce stable foam. The resulting foam were squeezed into circle shape and dried at 70 °C 2 h to form JA inulin yoghurt snack. The moisture content, aw and pH of dried snack were 5.73±0.08 %wb, 0.39±0.02 and 4.24±0.01 respectively. JA yoghurt snack contained inulin 129.78±5.86 mg/g and reducing sugar 146.444±6.47 mg/g.

Keywords: foam-mat drying, inulin, Jerusalem artichoke, juice, yoghurt snack

# The utilization of seaweeds as an inexpensive source of new drugs agent

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The increasing human population have same trend in the rate of infection by pathogenic bacterial. The uses of synthetic antibacterial chemicals continuously have a negative effect. The pathogenic bacterial developed resistance in high doses. Alternatively, the discovery of new antibacterial agent is urgent need. The searching of new drugs agent as antibacterial have been looking from marine organisms, especially seaweeds. The aims of this research were to measured the clear zone of seaweeds against bacterial test and to evaluate the ability of seaweed as antibacterial agent. The samples of Seaweeds were collected from Pantai Panjang, Bengkulu, Indonesia using purposive sampling method. Maceration method used in this research to extract the antibacterial agent from seaweeds. Agar diffusion method was used as antibacterial activity assay against *Escherichia coli, Salmonella typhii* and *Staphylococcus aureus* as bacterial test. Totally, there are three species of seaweeds that found in the location e.g *Halimeda discoidea*, *Halimeda micronesica*, and *Caulerpa taxifolia*. The antibacterial assay presented *H. discoidea* and *C. taxifolia* have the antibacterial activity against all bacterial test with clear zone that categorized into moderate and high. Meanwhile, *H. micronesica* have the potential as antibacterial agent against *E. coli* and *S. typhii* with clear zone that categorized into moderate and high. In the future, it could be a potential new drugs agent especially for antibacterial agent.

**Keywords:** antibacterial, clear zone, drugs, resistance, seaweeds.

## The influence of fattening and ageing periods on meat quality of charolais crossbred steers

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The objective of this study was to determine the effect of fattening (12 and 15 months) and ageing (10, 14 and 21 days) periods on meat quality of Charolais crossbred steers. The animals in this study were 12 Charolais crossbred steers which 6 of them were started fattening at the average weight of 371.6 kg at 628 days of age for 12 months and other 6 of them were started fattening at the average weight of 390.0 kg at 582 days of age for 15 months. They were slaughtered at the end of fattened period and their *Longissimus dorsi* muscle were collected and aged for 10, 14, and 21 days then determined meat quality. The 2x3 factorial with CRD experimental design was used to study the effect of different fattening and ageing period on color, shrinkage loss, cooking loss, shear force, and collagen content. The results showed that there were no interaction between fattening and ageing periods on all studied traits. Also the fattening and ageing periods did not affect meat quality traits. This study demonstrated that shear force value decreased with increasing ageing time.

**Keywords:** Charolais crossbred steer, fattening, ageing, meat quality.

## Development of strawberry gummy jelly with reduced sugar content from strawberry syrup

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This research aimed to develop gummy jelly strawberry from concentrated strawberry syrup that is the by-product of frozen strawberry industry. The suitable basic recipe of strawberry gummy jelly was selected and then the concentration of strawberry

syrup in strawberry gummy jelly was determined. The results indicated that the use of 100% concentrated strawberry syrup in strawberry gummy jelly had the highest consumer acceptance. The levels of sucrose replacement by sweetener (sucralose) in low-calorie strawberry gummy jelly were studied. The sucrose was replaced by sucralose at the percentage of 0 25 50 75 and 100 (w/w). The results suggested that the use of 50% sucralose for sucrose replacement in strawberry gummy jelly had the highest consumer acceptance and had lower calorie than control (sucrose) (P<0.05).

Keywords: gummy jelly, sucralose, reduced sugar

Physicochemical quality improvement of ready to cook baby corns using calcium propionate immersion

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Texture, taste and nutritional values are the main factors affecting quality of baby corn during storage. The aim of this study was to improve physicochemical quality of ready to cook baby corns using calcium propionate (Ca-propionate) immersion. The baby corns were immersed in 1.0% Ca-propionate for 5 min and then stored at 4 °C for 6 d. The investigated physicochemical quality attributes were texture, total sugars content, pectin substances contents, antioxidant activities and bioactive compounds contents. It was found that Ca-propionate treatment retarded the loss of texture which was concomitant with the lower EDTA-soluble pectin and higher  $Na_2CO_3$  soluble pectin concentrations when compared to untreated baby corns. Ca-propionate treatment did not obviously affect total sugars content and both antioxidant activity and free radical scavenging activity of the baby corns compared to untreated samples. However, Ca-propionate treatment induced bioactive compounds such as total phenols, flavonoids and ascorbic acid contents of ready to cook baby corns during storage. Ca-propionate immersion is an alternative in improving the quality especially texture and nutritional values of ready to cook baby corn during cold storage.

Keywords: baby corn, calcium propionate, texture, nutritional value

Natural products of fine particles derived from *Chaetomium* spp to inhibit fusarium wilt of tomato

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Fusarium solani was proved to cause wilt of tomato var Sida. It is confirmed by morphology and molecular phylogenic identification on the basis of ITS1-5.8S-ITS2 ribosomal gene sequence acquisition and analyses. It is pathogenic confirmed by pathogenicity test. The antagonistic fungi were identified by morphology and molecular phylogeny as Chaetomium cochliodes and Chaetomium globosum. Ch. cochliodes and Ch. globosum were proved to against Fusarium solani by dual culture method. Thease antagonists expressed ability to inhibit the growth and spore production of F. solani causing wilt of tomato var sida. The methanol crude extract of Ch. cochliodes inhibited spore production of F. solani which the ED<sub>50</sub> of 229 ppm, and followed by EtOAc crude extract and hexane crude extract which the ED<sub>50</sub> values which were 319 and 973 ppm, respectively. Hexane crude extract of Ch. globosum inhibited the spore production of F. solani at the ED<sub>50</sub> of 200 ppm, and followed by crude EtOAc crude extract and methanol extract which the ED<sub>50</sub> values of 314 and 378 ppm, respectively. The nautral products of fine particles constructed from the tested Chaetomium gave significantly inhibited F. solani at all tested crude extracts. Fine particle-CCOM showed the highest spore inhibition of F. solani which the ED<sub>50</sub> of 8.78 ppm, and followed by fine particle -CCOH and fine particle CCOE which the ED<sub>50</sub> values were 9.21 and 9.70 ppm, respectively. However, fine particle CGM actively expressed antifungal activity of F. solani which the ED<sub>50</sub> of 1.48 ppm, and followed by fine particle -CGH and fine particle-CGE which the ED<sub>50</sub> values were 3.41 and 3.48 ppm, respectively.

Keywords: Fusarium solani, Chaetomium cochliodes, Chaetomium globosum, Fusarium wilt

## Formulation of sweetened condensed rice-milk fortified with cereals in retortable pouch

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The effect of different cereal varieties on quality of sweetened condensed rice-milk products was studied. Job's tears (J), almond (A) and white sesame (S) were cereals used in this research. Six formulas of sweetened condensed rice-milk were developed including three recipes that mixed with single cereal J, A or S and other three recipes added two cereal combination of JA, JS and AS. All treatments were packed in retortable pouches and sterilized at 121°C for 18 minutes. The results showed that the addition of single cereal in sweetened condensed rice-milk had lower viscosity than those of cereal combination formulas (*p*<0.05). As sweetened condensed rice-milk formula had the highest viscosity of 2,103.33 cP that was insignificant and comparable with commercial sweetened condensed milk (control) 1,890 cP. The total soluble solid, water activity (a<sub>w</sub>) and pH values of all developed samples were in the ranges of 23.90 - 26.63 °Brix, 0.966-0.971 and 6.07-6.48, respectively. The coffee test was performed at 80 °C and the infusions were made by pouring 200 mL of hot water over 1.6 g, 3.0 g and 5.0 g of coffee. The 4 g of control and six samples of sweetened condensed rice-milk fortified with cereals were added to coffee infusions. The higher strength of coffee infusion did not effect on the amount of residues significantly. The amount of residues in coffee inufusion added control and six samples of sweetened condensed rice-milk fortified with cereals were 0.01-0.03 g and 0.04-0.08 g, respectively. Sweetened condensed rice-milk fortified with cereals were 0.01-0.03 g and 0.04-0.08 g, respectively. Sweetened condensed rice-milk fortified with cereals were 0.50-0.09 g, respectively. Sweetened condensed rice-milk fortified with cereals were 0.50-0.09 g, respectively. Sweetened condensed rice-milk fortified with cereals were 0.50-0.09 g, respectively. Sweetened condensed rice-milk fortified with cereals were 0.50-0.09 g, respectively. Sweetened condensed rice-milk fortified with cereals were 0.50-0.09 g, respectively.

**Keywords:** cereals, coffee test, retortable pouch, sweetened condensed rice-milk

# Development of sterilized durian cake roll

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Cake roll is a sponge cake filled with cream or jam that is a popular dessert. Durian is a tropical fruit that has been called "King of fruit", which is very popular among Asian people. Cake roll is very perishable food. Preservatives had been mostly used for extending shelf life in commercial sponge cake. Chemical addittives is the trend of avoidance for current consumers. This research aimed to develop the durian cake roll using serilization process for extending shelf life of the cake roll. The first study aimed to improve the durian cream for withstanding at 121°C for 15 minutes of serilization process. Two types of commercial modified starch (SMS and SPF) were studied with concentrations of 6, 8 and 10%. More concentration of both moditied starches caused more firmness in cream. Sensory evaluation showed more liking score on odor, taste and overall acceptance in cream with SMS modified starch. The second part is improving the cake. Three hydrocolloids with different concentrations (HPMC 0.8, 1.0%, CMC 0.5, 1.0%, Xanthan gum 0.5 and 1.0%) were used to study to improve the sterilized durian cake roll. CMC of 1.0 % affected more liking score on taste, texture and overall acceptance with the significantly lowest hardness on texture measurement. The change of durian cake roll stored at 35 and 55°C for 4 weeks, the result showed the tendency of increase in hardness and decrease in springiness of sterilized durian cake roll, by more storage temperature and longer storage time, the effects showed more pronounced. Microbiology analysis by total plate count, yeast and mold count of both stored at 35 and 55°C for 4 weeks were lower than 1 x 10¹ CFU within the regulated standard.

Keywords: durian cake, hydrocolloids, modified starch, sterilization, longer shelf life

#### Evaluation of agricultural input for cultivation of organic green okra in the field

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Result showed that the total yield or fresh weight of organic treatment was significantly different when compared to the natural control. It is proved that application of the tested bio-products as organic fertilizer, liquid bio fertilizer, *Chaetomium*, Bot-F, Nanoelicitor, Bio insect, Ya-Ra-Gua, Nano-chitosan, and Nutrient food as agricultural inputs which research derived from Bio-control Research Unit, KMITL, Bangkok, Thailand. Organic green okra cultivation has continuously increased in yield of green okra when compared to the natural control. Percent increase in total fresh weight yields per week in organic treatment was increasing with 82.6%, 32.3%, 73.8%, 105.5%, and 120.4% 146.5% and 166.0% accordingly. It was in seventh week with 166.0% increase that gained the highest percent increase while second week was the lowest increase with 32.3%. The results clearly show that fresh weight yield in organic treatment was increased compared to natural control as shown in table 11. Result showed that total yield of green okra for six weeks in organic treatment was significantly different at P=0.05 when compared with the natural control. Green okra yield for six weeks in organic treatment was 430.10kg and the natural control was 215.48 kg. With this, it is increased of 99.60% in yield for organic treatment when compared to natural control.

Keyword Organic Agricutural, okra

## Antifungal activity of Chaetomium isolate CNC2 against Alternaria spp causing leaf blight of kales

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Chaetomium sp CNC2 isolated from forestry soil in China proved to be antagonized Alternaria alternata causing leaf blight of Kales in China in bi-culture antagonistic evaluation. The crude metabolites from Chaetomium sp CNC2 were extracted by hexane, ethyl acetate and methanol that expressed antifungal activity against A. alternata. The detail results will be presented at the conference.

Keywords: biocontrol, antagonist, Chaetomium, kales, leaf blight

Product development and training centre (PDTC): food product development-hub of the province quirino, Philippines

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The Product Development and Training Center of the Quirino State University is regularly funded by the Department of Science and Technology Region 2 to perform its function of developing products from the different agricultural produce of the province. It is mandated to do product development, utilization and Technology transfer to its clients. The center has now enhanced with its equipment and facilities through fund from its agency linkages such as DOST, DA-BAR, PCAARRD and DA-02(Organic). Different lines of products were now developed and obtained its ownership through its registration to the Intellectual Property office of the Philippines under the Utility Model.

Keywords: PDTC, product development, technology transfer, utility Model

#### Shortage of sugarcane workers, nong na kham district, khon kaen province of Thailand

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This research aims to study the shortage of sugarcane workers from the opinions of operators and workers by using quantitative research. For the data collection, the questionnaire was used to collect data from 33 samplings of sugarcane operators and the sample groups of 300 sugarcane workers in the area. The sampling data was analyzed by using descriptive statistics such as frequency, %age, mean, and standard deviation. The results of the study presented that the opinions of sugarcane operators towards factors affecting the labor shortage in all aspects was at a moderate level ((x) = 2.80). For the change in population structure, it was at the highest level ((x) = 3.48), followed by the economy which was in the medium level ((x) = 3.23), and the least was the management (physical welfare) at the low level ((x) = 2.09), respectively. Then, opinions of all labor workers were at the moderate level ((x) = 3.16). For the change in population structure, it was at the highest level ((x) = 3.45), followed by policy and welfare in the medium level ((x) = 3.25), and the least was the aspect of the job as it was in the medium level ((x) = 2.98), respectively. From the results of the study, it can be seen that the opinions of sugarcane operators and workers on factors affecting the structure of the population were ranked as the no.1.

**Keywords:** opinion, sugarcane workers, labor shortage, sugarcane cultivation

#### Bioactivity test of Chaetomium isolate CNC1 to control Pestalotia sp causing leaf spot of orchid

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The active strain of *Chaetomium* CNC1 isolated from forestry soil in China. It was confirmed to inhibit *Pestalotia* spp causing leaf spot of Orchid from Thailand by dual agar culture test. It revealed that the colonies of Chaetomium grew over the pathogren's colony within 30 days incubation. The tested *Chaetomium* proved to be inhibited the colony growth and spore of *Pestalotia* sp. Crude hexane, ethyl acetate and methanol extracts resulted to inhibit colony growth and spore of tested pathogen.

Keywords: biocontrol, antagonist, Chaetomium, Orchid, leaf spot

#### POSTER SESSION1 PLANT SCIENCE AND SOIL MANAGEMENT

Genetic analysis of seed dormancy QTLs in yardlong bean [Vigna unguiculata (L.) walp ssp. unguiculata cv.-gr. sesquipedalis]

## Yoshida, A. K.\* and Mayer, A.

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Seed dormancy is one of the key trait as the result of domestication in yardlong bean [ $Vigna\ unguiculata\ (L.)$ ] Walp ssp.  $unguiculata\ cv.$ -gr. sesquipedalis]. The objective of this study was to identify QTL controlling seed dormancy-related traits of yardlong bean using  $BC_1F_2$  population from a cross between yardlong bean accession JP81610 and wild cowpea accession JP89083. SSR-based linkage map developed from  $BC_1F_1$  population was used for QTL analysis of seed dormancy-related traits including seed germination, electrolyte leaching and water absorption of seeds. Composite interval mapping (CIM) identified two QTLs for electrolyte leaching of seeds with phenotypic variance explained (PVE) of 8.71 and 9.83 % and alleles from yardlong bean increased the electrolyte leaching. CIM detected three QTLs for water absorption of seeds with PVE of 6.45-11.53%, and alleles from yardlong bean increased water absorption. No QTL detected by CIM for seed germination. The results of this study will be useful for marker-assisted selection development and further Vigna breeding programs.

Keywords: yardlong bean, Seed dormancy, Quantitative trait loci

The influence of plant growth reglurators for plant regeneration of chinese chaste (Vitex negundo L.)

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The aim of this study was to develop a productive protocol for optimum node induction and regeneration of chinese chaste (*Vitex negundo* I.) Micropropagation of Chinese chaste to preserve and increase plant species to be used for medical. In these studies, we compared the effect of MS medium (Murashige and Skoog, 1962) supplemented with 0.5, 1, 2 and 3 mg/L of 6-Benzylaminopurine (BAP), *meta*-Topolin (*m*T), Thidiazuron (TDZ) and without Plant Growth Regulators (PGRs). The result shown, the highest percentage of shoot regeneration was 100% at 1 mg/L BAP. The maximum of shoot induction was 4 shoots at 0.5 mg/L BAP after culture for 8 weeks. For rooting, the effect of MS medium combined with 0.5, 1, 2 and 3 mg/L of Indole-3-Butyric Acid (IBA) and Naphthalene acetic acid (NAA) found that the best of root induction was 90% at 3 mg/L IBA and the maximum of root induction was 108 roots on 2 mg/L NAA after culture for 8 weeks. Finally, the plantlets were successfully transferred into the plastic pot. In this experiment, a successful regeneration method for plantlet derived from node was developed and node was found to be a highly regenerative explant source of chinese chaste.

Keywords: Chinese chaste (Vitex negundo L.), node, root induction, shoot regeneration

#### In vitro propagation from nodal segments of Arachis glabrata cultivar Florigraze

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Arachis glarata Benth. or rhizome perennial peanut is forage plants with higher nutrient value. Growing in nature, necessary the use of rhizomes for propagation, therefore the inability to produce seeds. In this research, it is necessary to procure nodal segments to propagate *in vitro* by 0.2% of HgCl<sub>2</sub> plus antibiotic and ceffotaxime and preservative for plant tissue culture media active (PPM) at 15 min is the most effective procedure for disinfecting plant parts (survival of 73.33%). Shoot induction used to the nodal segments of *Arachis glabrata* cultivar Florigraze were cultured on MS medium combination with plant growth regulators in cytokinin group at different concentrations 0.5, 0.75, 1.2

Keywords: activated chacoal, Arachis glabrata cultivar Florigraze, nodal segment, shoot induction.

Heterotrophic and coliform loads, and *E. coli* and *Salmonella* detection in farm soils and organic fertilizers from selected organic farms in southern luzon, Philippines

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Bacterial pathogen contamination in organic farms has raised the alarm for risks associated with foodborne diseases. Pathogens have been reported usually for organically grown crops. With the objective of determining whether selected bacterial pathogens contaminating the vegetables are also found in potential farm reservoirs such as the farm soil and organic fertilizers used, this study analyzed samples collected for heterotrophic count, coliform load, and for the detection of *E. coli* and *Salmonella* strains in four selected "practicing organic" farms in Laguna and Quezon provinces in the island of Luzon, Philippines. Putative *E. coli* and *Salmonella* isolates obtained from the farm soils and organic fertilizers mainly used were purified, and initially phenotypically characterized for eventual molecular identification.

Keywords: heterotrophic count, coliform count, E. coli, Salmonella, organic farms, farm soil, organic fertilizers, Philippines

Lignin biosynthesis genes (OsPAL and Os4CL3) sequencing of native upland rice varieties from pala u village, Thailand

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Lignin, a complex polymer, is a major component of plant secondary cell walls and act for culm strengthening. Biosynthesis of lignin derived from various genes and their regulators to produce monolignols (H, G and S subunits) in cytoplasm and transport to cell wall. Previously, it has been shown that rice growth and development depends on lignin biosynthetic genes, and mutations in these genes may cause lodging. This study aimed to identified the Phenylalanine Ammonia-Lyase (OSPAL) and 4-Coumarate-coenzyme A

ligase 3 (*Os4CL3*) genes from 7 varieties of native up land rice cultivated from Pala-U village, Prachuap Kiri Khan province. mRNA samples were extracted from seedling of seven rice varieties, and RT-PCR and DNA sequencing were performed. The results indicated that all seven *OsPAL* gene sequences showed higher similarity to *Oryza sativa Japonica* (99.4-100%) than *Oryza sativa Indica* (98.7-99.4%). For *Os4CL3* gene, all seven sequences revealed 100% sequence identity to both *Oryza sativa Japonica* and *Oryza sativa Indica*. These lignin biosynthesis genes information from native upland rice varieties in Thailand were established as the basic information for further genetic conservation and breeding improvement.

Keywords: upland rice, OsPAL, 4CL3

Factors affecting the sale of ripe mango of nam dok mai mango collaborative farming in bang phli district, samut prakan province, Thailand

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This study aimed to determine the production behavior and analyze the relationships and, factors that affect farmers, whether it would change the increasing sales of ripe mangoes and SWOT analysis of Nam Dok Mai Mango Collaborative Farming, Bang Phli District, Samut Prakan Province in Thailand. We used structured questionnaires and conducted in-depth interviews of 22 large agricultural members. We found out that farmers grow Nam Dok Mai mangoes in collaborative farming, and numbers of males and females were equal. Mostly people were aged more than 60 years old and graduated from primary school and had experiences on growing mangoes for 6-10 years. Farmers have the area of planting mangoes around the fishpond, and most of them receive fertilizer support from the district agricultural office. They can do harvesting on most of the products throughout the year, and each can collect as many as 2 times per year. Most of the mango cultivars are mangoes number 4, In terms of sales, it is focusing primarily on sales in front of the farm. The labors are family labors and they must rent the areas. In addition, farmers are not likely to expand the planting area and increased sales of ripe mangoes because there is no exact support market, and there is labor shortage. In terms of factors affecting the change of ripe mango sales is the age factor, educational factors, and mango cultivation behavior.

Keyword: Nam Dok Mai Mango, collaborative farm, production behavior, SWOT analysis

Times for callus propagation of indica rice (Oryza sativa L.) cultivar Sangyod in suspension culture

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The calli formation of indica rice (*Oryza sativa* L.) cultivar Sangyod were propagated in the liquid culture media containing 4.10 gL<sup>-1</sup>of modified Chu/Gamborg (NB) basal medium, 2 mgL<sup>-1</sup>of 2,4-dichlorophenoxy acetic acid (2,4-D), 1 mgL<sup>-1</sup>of 1-Naphthalene acetic acid (NAA), 30 gL<sup>-1</sup>of sucrose and 1 gL<sup>-1</sup>of L-proline. Then maintained in shake flasks at  $25\pm2^{\circ}$ C, 100 rpm under light conditions for 27 days. The day of  $12^{th}$  suspension culture, it is the best suitable time for callus propagation that indicated by the doubling time (t<sub>d</sub>) parameter was 11.73 day<sup>-1</sup> in fresh weight and also showed the highest cell density was grown up to 66.83 gL<sup>-1</sup> F.W. and 4.98 gL<sup>-1</sup> D.W., which referred to exponential phase. It was achieved the productivity in 2.61 gL<sup>-1</sup>day<sup>-1</sup> F.W. and yields ( $Y_{x/s}$ ) of cell mass was 1.08 g<sub>Cell</sub>g<sup>-1</sup><sub>glucose</sub> F.W. The specific growth rate ( $\mu$ ) was 0.06 day<sup>-1</sup> in fresh weight. This data will be useful to well-know the suitable time for repeated subculturing on callus growth to increase the productivity of high cell density cultures and it is an important for further study the physiological and morphological differentiation of cell suspension culture, improve plant regeneration system, approaches to increase productivity and selection of tolerant or mutant strains.

Keywords: callus propagation, cell suspension culture, exponential phase, Sangyod rice

#### Improvement of Dendranthemum grandiflora cv. canter with colchicine in vitro

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The induced mutation of chrysanthemum with ray florets were soaked in colchicine at concentration of 0, 0.10, 0.15 and 0.20% for 12 and 24 hours on MS medium (Murashige and Skoog. 1962) supplemented with 2 mg/l NAA and 4 mg/l Kinetin for inducing calli for 12 weeks. The shoots were regenerated from callus when cultured on MS medium (Murashige and Skoog. 1962) for 8 weeks. The plant growth, size of stomata and number of chloroplasts per stomata were not statistically different in each treatment. However, the ray florets were received high levels of colchicine is less to survivor rate. The plantlet of chrysanthemum was transfered to pot plant containing soil and chopped coconut. Five of ten plants showed the flowers color changed from purple into a pinkish-orange at the 0.10% colchicine for 12 hours. The plants obtained from soaked of 0.15 % colchicine for 12 hours with the highest number of flowers per plant, 72 flowers per plant.

Keywords: chrysanthemum, mutation, colchicine, tissue culture

Pathogenicity of *Pyricularia oryzae* on elite rice cultivars and geographical distribution of avirulence genes causing blast disease in Thailand

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Pyricularia oryzae is the plant pathogenic fungi for rice blast disease. The fungi cause heavy damage to susceptible rice varieties and cause of low rice productivity. In Thailand, there is very little information about pathogenicity and avirulence gene of rice blast pathogen. The purpose of this study was to determine avirulence gene of *P. oryzae* and their pathotype on elite rice cultivars. Fifty blast isolates were isolated from blast samples which were collected from 19 provinces. Investigation of the pathotype of each isolate was conducted using 31 near isogenic lines (NILs). The results showed that Avr-Pita-2 was the most discovered in isolates from the North (83%), Avr-Pikp and Avr-Pi7(t) were the most discovered in isolates from the North East (94%), while 8 avirulence genes including Avr-Pikp, Avr-Pikh, Avr-Pi1, Avr-Pi7(t), Avr-Pi2, Avr-Pi19 and Avr-Pi20 were the most discovered in isolates from the South (88%) and Avr-Pi9 was the most discovered in isolates from the Central region of Thailand (71%). Isolates BRM60001 that carried the most amount of Avr gene was unable to infect any elite rice cultivars, while isolates carried as less as 2 Avr genes could infect few elite rice cultivars. Isolate CRI59004.1 carried Avr-Pik (Avr-Pikh, Avr-Pikh, Avr-Pi7(t)), Avr-Pi9, Avr-Pish, Avr-Pib, Avr-Pita-2 and Avr-Pi19, could infect the greatest numbers of elite rice cultivars. The distribution information of Avr gene in *P. oryzae* will be useful for rice blast forecast and for appropriate blast isolates being choosen for rice blast disease resistance breeding programs.

**Keywords:** rice blast disease, *Pyricularia oryzae*, blast fungus, elite rice cultivars, near isogenic lines, geographical distribution of avirulence gene of blast fungus.

#### Effect of β-mannanase on nutrient composition of palm kernel meal (PKM) mixed with yeast cake

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Palm kernel meal (PKM) is one of an important by-product generated from the palm oil industry. PKM has been used as feed for ruminants; however, its use for non-ruminants is limited due to the high fiber content. Since PKM contains β-mannan as the main fiber components, thus in this study, feed enzyme Hemicell<sup>TM</sup> HT composing of β-mannanases is used for improving the nutritive composition of yeast cake premixed-PKM. PKM mixed with yeast cake were treated with β-mannanase at 0, 0.02%, and 0.04% followed by incubation for 0, 12, and 24 h at 40 °C. Proximate analysis data of crude fiber (%CF) and crude protein (%CP) were obtained. The results showed that %CF were decreasing when PKM were treated with β-mannanases in time- and concentration-dependent manner. Supplementation of β-mannanases at 0.02 and 0.04% significantly reduce the fiber content in 12 h incubation (P<0.05). The best improved PKM was obtained when 0.04% enzymes and 40% of yeast cake was added for 24 h which contained %CF equal to 4.84±0.85 and %CP equal to 40.86±1.87. In conclusion, improvement of PKM using enzymes and yeast cake increased %CP and reduced %CF which could be further used as diets for non-ruminants.

**Keywords:** Palm kernel meal, β-mannanase, feed improvement

The effect of composting manures on growth of green oak, frillice iceberg and green cos (Lactuca sativa L.)

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The composted livestock manures were used as main source of organic fertilizers for many years in plant production. Organic fertilizers increase the yield and quality of agricultural crops similar to inorganic fertilizer. This study aimed to determine the effects of different compost manures on the growth of Green oak, Frillice Iceberg and Green cos. The experiment was conducted at Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang from September to November, 2018. Randomized Complete Block Design (RCBD) with three replications and six plants per each were designed. Four fermented composting manures from cow, chicken, goat manures and bio-fermented fertilizer were compared with chemical fertilizer (24-7-7) and no fertilizer (control). The results showed that chicken manure gave the height on plant growth of three salad species as well as chemical fertilizer, whereas cow, goat manures and bio-fermented fertilizer gave the lowest of plant growth similar to no fertilizer. Plant height, leaf width, SPAD unit and root length of green oak applied with chicken manure was 29.50 cm, 11.02 cm, 17.28 SPAD unit and 16.25 cm respectively. Frillice Iceberg showed the highest on plant height, plant canopy, number of leaves per plant, leaf length, SPAD unit, root length, plant fresh weight and root fresh weight as 11.63 cm, 18.25 cm, 8.50 leaves, 11.31 cm, 30.24 SPAD unit, leaf length, SPAD unit, root length, plant fresh weight and root fresh weight) of green cos applied with chicken manure was 11.63 cm, 18.25 cm, 8.50 leaves, 11.31 cm, 30.24 SPAD unit, 19.93 cm, 63.58 g and 53.77 g, respectively.

Keywords: Chicken manure, Cow manure, Goat manure, Organic fertilizer

The effects of abscisic acid and sorbitol on the slow growth in vitro preservation of mahesak (Tectona grandis L.)

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The conservation of teak (*Tectona grandis* L.) through *in vitro* preservation techniques by using osmotically active compounds and growth retardants need to be studied. To find out how to slow down growth of Mahesak or teak (*Tectona grandis* L.), shoots of Mahesak were used to prepare shoot tips and node explants cultured on Woody Plant Medium (WPM) medium supplemented with 0.25 mg·L<sup>-1</sup> 6-benzylaminopurine (BAP) containing 30 g·L<sup>-1</sup> sucrose for shoot and callus induction. The experiment had chosen calli and node explants were cultured on Murashige and Skoog (MS) and WPM media supplemented with 1, 2 and 3 mg·L<sup>-1</sup> abscisic acid (ABA) containing 30 g·L<sup>-1</sup> sucrose and hormone-free MS and WPM media containing 0.1, 0.2 and 0.3 M sorbitol for reduced the growth rate. Calli and node explants were transferred to WPM medium supplemented with 0.25 mg·L<sup>-1</sup>BAP for callus and node explant regrowth. Results showed that preservation of calli on MS and WPM media supplemented with 0.2 and 0.3 M sorbitol and node explants cultured on WPM medium supplemented with 0.2 and 0.3 M sorbitol were able to inhibit the growth and maintain quality calli and node explants for up to 12 weeks. Callus and node explant survival from slow growth preservation were able to regrowth successfully (100%) tested on WPM medium supplemented with 0.25 mg·L<sup>-1</sup> BAP containing 30 g·L<sup>-1</sup> sucrose after for 2 weeks.

Keyword: Growth retardants, Mahesak (Teak), preservation, slow growth, Tectona grandis L.

Effects of plant growth regulators and type of media on shoot proliferation of neck orange (Citrus reticulate Blanco)

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Neck orange (*Citrus reticulate* Blanco) is one of the most popular cultivars cultivated in Southern part of Thailand. The propagation method of using branches is dangerous when infected with diseases. Thus, the aim of this study was investigate the effect of benzyladenine (BA) and naphthalene acetic acid (NAA) on shoot proliferation of Neck orange. The seeds of neck orange surface were sterilized with sodium hypochlorite and then cultured on Murashige and Skoog (MS) medium for 2 months. Afterwards, the shoot was cut into Shoot 2-3 cm length, and cultured on MS Medium supplemented with to BA (0-2 mg/L), NAA (0-1 mg/L), 3 % sucrose and with or without 0.2% activated charcoal for 3 months. The result revealed that MS medium with 1.5 mg/L BA and 1 mg/L NAA gave the highest number of shoot (10.6 shoot/explant) and shoot length (4.9 cm) then study type of media (solid, semi-solid and liquid medium) on shoot proliferation. The result revealed that MS medium (solid medium) with 1.5 mg/L BA and 1 mg/L NAA gave the highest number of shoot (7.43 shoot/explant). Thus, the plant growth regulators in plant tissue culture are used to increase the shoot proliferation in order to fulfill the farmers' demand.

**Keywords:** Neck Orange, Plant growth regulators, Shoot proliferation

## Selection of appropriate ornamental plant species for outdoor vertical garden

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The objective of this study was to select appropriate species of plants for outdoor vertical garden using hydroponic system. The nutrient solutions was supplied to the plants on felt panel system. Twelve species of ornamental plants were studied. The EC value of nutrient solutions in the hydroponic system was 0.8. The light intensity, survival rate and beautifulness score of plant were recorded. As the results, eight plant species with 80% survival rate were observed. In addition, an average of beautifulness score of plant species was 3. However, the beautifulness score higher than 4 were observed in four plant species including 1) Schefflera sp., 2) Tradescantia spathacea, Sw., 3) Tradescantia pallida (Rose) D. Hunt and 4) Rhipsalis cereuscula. These four ornamental plant species would be considered as an ornamental plant species for outdoor vertical garden in the future.

Keywords: Hydroponic cultivation, nutrient solution, felt panel system

#### Effect of foliar application of chitosan on growth and fruit quality of 'densuke' and 'kinnaree' watermelons

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Watermelon (*Citrullus lanatus*) cultivars 'Kinnaree' (most popular Thai cultivar) and 'Densuke' (most expensive black Japanese cultivar) were grown under field conditions in Thailand following vertical training system. The plants were sprayed with 25, 50, 100, 200 and 400 ppm chitosan after one week from transplanting and weekly thereafter until one week before harvest (after eight weeks from transplanting), with distilled water spray as control. Chitosan spray did not significantly affect plant height, number of nodes per plant and fruit color, firmness, total soluble solids, titratable acidity and sensory quality in both cultivars. It increased the number of leaves and chlorophyll content of 'Kinnaree' plants but this did not translate into increased fruit growth. In 'Densuke' plants, chitosan spray increased leaf size and resulted in more rapid growth of the developing fruit measured as increases in fruit polar and equatorial diameter. At harvest, chitosan spray increased 'Densuke' fruit size resulting in heavier fruit (more than 1.0 kg) than the control (0.75 kg) while it did not affect 'Kinnaree' fruit size and weight (0.9-1.0 kg per fruit). Chitosan also increased flesh thickness of 'Densuke' fruit with more intense red color (lower L\* and higher a\* values) than the control. Chitosan at 25 ppm was sufficient to cause the above effects in both cultivars.

Keywords: Cultural practice, preharvest treatment, sensory evaluation, vertically trained system

Comparative efficacy of plant extracta, petroleum oil and insecticides to control citrus leaf-miner (*Phyllocnistis citrella Stainton*) in neck orange (*Citrus reticulate Blanco*)

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The comparative efficacy of plant extracts, petroleum oil and insecticides to control citrus leaf-miner (*Phyllocnistis citrella* Stainton) on neck orange (*Citrus reticulata* Blanco) were evaluated to determine the best method of controlling citrus leaf-miner. 2 years old neck orange orchard in Thung Song district, Nakhon Si Thammarat province, Thailand, was osed from 18 May to 8 June 2018 in the experiment whose treatments were laid out using RCBD with 4 replications. The 6 treatments were 1) Thai neem extract 0.1% at the rate of 5 ml/L. 2) tobacco extract at the rate of 30 g/L. 3) petroleum oil 83.9% EC at the rate of 2.50 ml/L. 4) imidacloprid 10%

SC at the rate of 0.4 ml /L. 5) cyflutrin 5% EC at the rate of 0.25 ml /L. and 6) control (non-treated). The numbers of citrus leaf-miner were recorded before and after spraying methods (three-times one week apart). The results showed that spraying the 3 times using imidacloprid had the highest effectiveness at 83.29% followed by cyflutrin, petroleum oil, tobacco extract and Thai neem extract at 81.46, 78.96, 77.91 and 31.23%, respectively, compared with control (non-treated)

Keywords: plant extracts, petroleum oil, citrus leaf-miner

## Callus and hairy root induction of Melaleuca cajuputi powell

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Callus and hairy root induction for *Melaleuca cajuputi* Powell was studied. Nodal explants were washed through running tap water for 60 min. The lowest percentage of contamination at 49.63% and highest percentage of survival at 37.95% were obtained when bud explants were sterilized with 70% ethanol for 1 min and 1.8 % NaOCl for 30 min. The bud explants were cultured on Murashige and Skoog (1962) (MS) medium supplemented with 0, 0.02, 0.04, 0.06, 0.08 mg/l 2.4-D combination with 0, 0.02, 0.04, 0.06, and 0.08 mg/l TDZ for callus induction. The results showed that the explants cultured on MS medium supplemented with 0.06 mg/l TDZ was suitable for callus induction giving the biggest size at 56.2x 26.1cm of width and length. Culturing buds on MS medium supplemented with 0.02 mg/l TDZ had the highest average shoot number. For roots cultured in liquid MS medium supplemented with 0.04 mg/l TDZ, 0.04 mg/l 2.4-D and has MS medium controlled hairy root proliferation. The highest weight of hairy root was obtained in MS medium supplemented with 0.04 mg/l 2,4-D treatment.

Keywords: Callus induction, Hairy root, Thidiazuron, 2,4-Dichlorophenoxyacetic acid

Effects of 6-benzylaminopurine and meta-topoline on micropropagation of Dendrobium chrysanthum Lindl.

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Dendrobium chrysanthum Lindl. under family Orchidaceae and classified as rare and threatenned species were of high demand in market. It is no surprise that a number of this orchid in nature are decreasing significantly. This study aimed to propagate Dendrobium chrysanthum Lindl. by tissue culture technique to increase quantity of this orchid in short period. Mature seeds of Dendrobium chrysanthum Lindl. were grown on solid and liquid Gamborg's B5 medium with plant growth regulators concentration of 0, 0.5, 1, 1.5 and 2 mg/L 6-benzylaminopurine (BAP) or meta-topoline (mT) for 8 weeks. Protocorms were induced by seed culture for regeneration. The solid Gamborg's B5 medium at 2 mg/L mT provided the highest average number of shoots at 1.6 shoots per explant and number of leaves at 3.7 leaves per explant. At 1.5 mg/L mT, e highest average length of shoots at 9.82 mm and 0.5 mg/L mT provided the greatest number of roots at 0.9 roots per explant with the longest root at 3.75 mm. For liquid Gamborg's B5 medium showed the highest average number of shoots at 1.5 mg/L BAP as same as with 1.5 mg/L mT and medium without plant growth regulator and 1 mg/L mT had the highest average length of shoots 13.98 mm and the highest number of roots per explant was 0.8 roots per explant similarly with 2 mg/L mT but gave the different of the maximum length of roots was 3.04 mm. The percentage of protocorms were induced shoots 100 percent for all medium.

Keyword: Dendrobium chrysanthum Lindl., mature seeds, protocorm, regeneration, seed cultured

#### POSTER SESSION 2 ANIMAL & FISHERY SCIENCES

Growth and yield of african catfish (Clarias garriepinus) as influenced by density and grown in turbid water pond

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Two experiments were conducted to determine the effects of density on growth and yield of African catfish (*Clarias garriepinus*) grown in turbid water. Experiment 1, the catfish were cultured in turbid soil ponds of 12 square meters with average initial weight  $2.91 \pm 0.15$  gram and average length 8.19 + 0.21 centimeters. The densities were 20, 40 and 60 fish per square meter, respectively. Feeding fish was done by floating food pellets of 30 percent protein for a period of 90 days. Results showed that there was no statistical difference (p> 0.05) in the average growth rate but fish data at the density level of 40 per square meter with average weight, length, feed conversion ratio and specific growth rates were higher than other groups. The second experiment involved raising African catfish in a cage of 2x2x1.5 cubic meters in turbid water. African catfish had an average initial weight of  $2.95 \pm 0.19$  gram and average length of  $8.27 \pm 0.01$  centimeters. Densities varied from 20 to 40 and 60 fish per square meter. Fish were fed through floating food pellets of 30 percent protein for a period of 90 days. The rate of growth was found no statistical difference (p>0.05). Nevertheless, fish data at the density level of 40 per square meter with average weight, length, feed conversion ratio and specific growth rates were higher than other groups. From this study, it was found that the optimum density for raising African catfish in turbid water both the earthen pond and the cages was 40 per square meter.

Keywords: turbid water, African catfish (Clarias garriepinus), earthen pond and cages culture

Effectiveness of long pepper (*Piper retrofractum* Vahl) extracts against adult of cowpea weevil (*Callosobruchus maculatus* Fabricius) and southern cowpea weevil (*Callosobruchus chinensis* Linnaeus)

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The efficacy of hexane, acetone and ethanol extracts from dried fruits of long pepper (*Piper retrofractum* Vahl) against adult of cowpea weevil (*Callosobruchus maculatus* Fabricius) and southern cowpea weevil (*Callosobruchus chinensis* Linnaeus) was investigated. The contact method of applying different extracts and concentrations on filter paper and compared with the control groups (Tween-20 in water and insecticides, fipronil) was performed. The mortality rates of adult at 24 and 48 hours and the toxicity levels ( $LC_{50}$  and  $LC_{90}$ ) were calculated. The results showed that the hexane extract from long pepper had the highest toxicity to the cowpea weevil with  $LC_{50}$  at 24 and 48 hrs were 8.7 and 4.8  $\mu g/cm^2$ , respectively and also gave highest toxicity to the southern cowpea weevil with 9.6 and 8.0  $\mu g/cm^2$ , respectively. It showed higher significantly toxic activity than that of acetone and ethanol extracts. The hexane extract from long pepper was further tested for the repellent efficacy against the cowpea weevil and the southern cowpea weevil. It was found that hexane extract of long pepper at the concentration of 3.15  $\mu g/cm^2$  was effective in expelling the cowpea weevil at 5 hrs with the repellent index (%RI) equal to 44%. While the extract at concentration of 2.36  $\mu g/cm^2$  was effective to repel the southern cowpea weevil at 3 hrs with %RI equal to 40%. This study revealed that the hexane extract from long pepper can be used to control weevils, specifically for seed coating.

**Keywords:** Long pepper, extract, stored product pests, contact method, repellent

#### Concentrated pineapple extract, Na-Cid<sup>o</sup>, facilitates the digestion of soybean meal and shrimp diet

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Soybean meal has been used as an alternative plant protein source to replace fishmeal in shrimp diet for decades. However, the use of soybean meal is concerned because of its low-digestibility in digestive tract of shrimp and antinutritional factors found in plant derived protein. In addition, the residues of undigested feedstuff have been considered to cause inflammation in the digestive tract of shrimp leading to white feces syndrome (WFS). This disease negatively affects the production of Pacific white shrimp (*Litopenaeus vannamei*) culture in Southeast Asia including Thailand. This work aimed to study the ability of the concentrated pineapple extract, Na-Cid\*, to increase the digestibility of soybean meal and shrimp diet via its protease activity. The treatment conditions were tested in a range of pH 4.0–10.0 and temperature of 25–50°C. The digested protein was determined in term of soluble protein released into the reaction mixture. In addition, the amount of released protein in each condition was compared between the treatments with and without Na-Cid\*. The results obviously showed that Na-Cid\* could facilitate the digestion of soybean meal and shrimp diet over a wide range of pH and temperature. This study demonstrated the capability of the concentrated pineapple extract in increasing the *in vitro* digestibility of soybean meal protein. This finding may provide the potent pretreatment procedure to solve the problem regarding the protein digestible of feedstuff leading to the reduction of undigested residues remained in digestive tract of shrimp and the appearance of WFS consequently.

Keywords: Concentrated pineapple extract, Digestion, Na-Cid\*, Pacific white shrimp (Litopenaeus vannamei)

Prevalence and genetic diversity of *Trypanosoma evansi* infections causing abortions among cattles and buffaloes in eastern border area of Thailand-Cambodia

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Trypanosoma evansi, a protozoan blood parasite in animals, causes surra disease and easily leads to abortion in cattle and buffaloes. The objectives of this research were to investigate the prevalence and genetic diversity of *T. evansi* infections causing abortions among cattle and buffaloes in eastern border area of Thailand-Cambodia. A polymerase chain reaction method was evaluated for detection of *T. evansi* DNA in cattle and buffaloes of each border district in Sa Kaeo province using the set of primer TBR1 and TBR2. The results demonstrated that the PCR product was 164 bp in length. The overall prevalence of *T. evansi* infection in cattle and buffaloes of Ta Phraya, Khok Sung, Aranyaprathet and Khlong Hat districts in Sa Kaeo province was 19.67% (12/61), 38.57% (27/70), 45.16% (70/155) and 35.45% (25/92), respectively. The satellite DNAs (TBR primer) were analyzed and revealed that it could demonstrate the genetic diversity of *T. evansi* of cattle and buffaloes. Tree construction based on the satellite DNAs in each district of border areas confirmed the close relationship between cattle and buffalo. The results found that trypanosome minor variations might be due to livestock system, a pasture or forest grazing. These feeding are difficult to get rid of insects that are disease vectors such as tabanidae, flies, and mosquitoes as well as also easy to spread or transmit trypanosome.

Keywords: Trypanosoma evansi, cattle, buffalo, Eastern Border Area of Thailand-Cambodia

#### The effect of supplementary food on the adult growth of Zophobas morio fabricius

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The types of supplementary foods suitable for growth in the adult stage of super worm (*Zophobas morio* Fabricius) are investigated. 1,000 super worm larvae at 25 days old were prepared initially and reared in plastic trays with wheat bran as the main food. They were fed every 5 days with sufficient main food and compared with providing additionally supplementary food such as jicama, pumpkin, banana, cucumber, watermelon peal, winter melon, mulberry leaf, carrot and cantonese. At the super worm age of 70 days, they were randomized for 50 worms per experiment by separating 1 worm in a glass. Single rearing with wheat bran and the same type of supplements food was performed until entering the pupa and become an adult. Survival rate and proportion of sex were determined. The results showed that the super worms fed with supplementary food had the survival rate of 82-96%, whereas, without providing supplementary food had only 72% survival rate. The super worms reared with winter melon as a supplementary food showed the highest female number with the sex ratio of male: female equal to 33.3: 66.7 followed, by rearing with cucumber and cantanese as a supplement with the ratios of 37.5: 62.5 and 42.9: 57.1, respectively.

Keywords: super worm, wheat bran, supplementary food, survival rate, sex ratio

# Stock identification of short mackerel (*Rastrelliger brachysoma*) from the gulf of Thailand using morphometric and meristic parameters

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The variation of morphometric ratio and meristic parameters among five locations of short mackerel (*Rastrelliger brachysoma*) from the Gulf of Thailand (Chonburi, Samut Songkhram, Prachuap Khiri Khan, Chumphon, and Surat Thani provinces), during the period from June – November 2018 were investigated. The results revealed that there was a significant difference in folk length/head depth, folk length/great depth, head length/great depth, head length/folk length, folk length/pre-pelvic length of fish in different areas of the Gulf of Thailand. In meristic, gill raker on left-hand side, gill raker on right-hand side and finlet of 1st dorsal fin was statistically significant among the locations of the Gulf of Thailand. While, short mackerel population from Chumphon and Prachuap Khiri Khan province, morphometric ratio and meristic in all parameter not statistically different.

**Keywords:** morphometric, meristic, short mackerel, the Gulf of Thailand

## Appropriate feed formulation for rearing super worms (Zophobas morio Fabricius)

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The different feed formulations for rearing super worms (*Zophobas morio* Fabricius) were evaluated. The experiment was carried out initially by using 20 days old super worms containing 50 grams (by average weight of 46.39 mg/larva) put in plastic tray with size 28X42X9.5 cm. The insect was fed with different KMITL formulations, with 21 and 24% protein content every 5 days. Different amounts of food and supplement of pumpkin were given at the same amount of the formulation. The moisture was allowed by

means of an aerosol spray of water 2 times daily at 09.00 am and 16.00 pm. The growth of super worms was recorded and remain food was collected every 5 days until reached to 70 days. Then, the feed conversion ratio (FCR) and survival percentage were calculated. The results showed that the super worms fed with the formulas CP00 (wheat bran), CP21 (protein 21%) and CP24 (protein 24%) together with the food given unlimitedly, the worm was growing rapidly. When the worm reached to 70 days, its weight was not different from that of feeding with limited food supply at 1,110 grams. Every experiment presented more than 80% survival percentage of super worms. While the FCR value of super worms fed with limited dietary of 1,110 gm was equal to 1.43-1.63, which was lower than that of the unlimited feed supply (2.60-2.68). KMITL formulations of CP21-21 and CP24-21 should be used to feed super worms with a limit dieted of 1,000-1200 grams over 70 days of rearing period.

Keywords: super worm, KMITL formulation, survival percentage, feed conversion ratio

#### Molecular and morphological identification of banana thrips in chantaburi province, Thailand

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Thrips is a major pest causing peel damage in banana cv. 'Kluai Khai'. Thrips identification to the species level using morphological characters is complicated. Therefore, other methods need to be used to accurately confirm the identification. In this study, we used morphological and molecular techniques to identify banana thrips species. Thrips were collected from banana field at Maung, Tha Mai and Makam districts in Chanthaburi province. Morphology structure was used to identify distinct species of thrips. Nucleotide sequencing analysis of mitochondrial cytochrome C oxidase subunit I (COI) gene was used to confirm thrips species identification. The morphological and DNA barcoding results indicated Thrips hawaiiensis was the most common thrips species in all study areas.

Keywords: DNA Barcoding; Thrips hawaiiensis; banana cv. 'Kluai Khai'

# The effect of okara in diets on productive performance of broiler

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Soy milk residue or okara is a by-product from soy milk industries. Three hundred sixty broiler chicks were individually weighed and divided randomly into three treatment groups consisting of 120 chicks each, which was further subdivided into four replicates of 30 chicks. Three treatment groups diets were formulated in which okara used at 0%, 5 % and 10 % (T1, T2 and T3). The body weight and feed consumption in each replicate of three groups was recorded weekly for a total period of 7 weeks experimental feeding period. The results showed that the differences in their growth performances were not statistically significant (P>0.05) among the various groups. The average daily gain was 60.75, 63.00 and 60.75 g/bird/day. The average total feed consumption of seven weeks was 6091.00, 6160.00 and 6199.00 g/bird, average feed conversion ratio was 2.04, 2.01 and 2.06 respectively. The feed cost/kg weight gain (FCG) were 28.78, 28.87 and 30.11 Baht/kg, respectively. Up to 5 % Okara substitute of protein supplied by corn meal, soybean meal and rice barn in the tested diets with non-significant (P > 0.05), without negative effect on growth performance of broiler was obtained.

Keywords: soy milk residue, growth performance, broiler, okara, by-product

Effects of anatomical hook location to the mortality rate of short mackerel (Rastrelliger brachysoma) from nature as a broodstock

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The mortality rate of short mackerel (*Rastrelliger brachysoma*) caught from nature with a hook as a brood stock was evaluated. The experiment was conducted on the Gulf of Thailand of the Koh Teab, Pak Khlong sub-district, Pathio, Chumphon province to evaluate the effect of anatomical hook location on short mackerel. We examine the impact on anatomical hook location about dorsal, ventral, upper jaw, lower jaw, left maxillary, and right maxillary on mortality rates. The frequency of these anatomical locations in 0.69%, 0.69%, 18.18%, 42.30%, 23.07% and 15.03% (2, 2, 52, 121, 66 and 43 heads), while the fish hooking to the dorsal and ventral will die immediately. Mortality rates of short mackerel in 1st day was estimated for each of four anatomical hooking locations (upper jaw, 26.92%; lower jaw, 12.40%; left maxillary, 56.10%; and right maxillary, 41.86%), in 2nd day was upper jaw, 13.15%; lower jaw, 16.03%; left maxillary, 37.93%; and right maxillary, 58.10. Accumulated mortality for a period of 7 days, it was found that the mortality rate of short mackerel in each anatomical hooking location i.e. left maxillary and right maxillary led to overall death, while the upper jaw and lower jaw were 40.38% and 67.77%, respectively. Selecting a short mackerel by the hook to be used as a brood stock should a fish with an anatomical hooking location on the upper jaw and lower jaw.

**Keywords:** mortality rate, anatomical hook location, short mackerel

#### POSTER SESSION 3 BIOTECHNOLOGY AND FOOD SCIENCE

Bacillus velezensis SD03 - potential biocontrol agent for diseases of potato

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Biological control with microbial antagonists has emerged as a promising alternative that can reduce pesticide usage along with safe production of vegetable and a lower environmental impact. Microorganisms isolated from agricultural field soil were identified by rDNA technology, screened for their beneficial traits and utilized for developing strategies of biological control of diseases of potato. *In vitro* antagonistic activities of bacteria against phytopathogens (*Fusarium graminearum*, *Sclerotium rolfsii*, *Streptomyces tendae*, *Ralstonia solanacearum* and *Pectobcterium carotovorum* subsp. *basiliense*) were confirmed to their application in laboratory and nursery. Bacillus strain SD03 has shown dramatically inhibitory effect on phytopathogens in vitro and in vivo. Bacillus strain SD03 was identified as *Bacillus velezensis* through physiological biochemical characters and nucleotide sequence analysis based on 16S rRNA. PCR detection and nucleotide BLAST analysis showed that the genome of B. velezensis SD03 contains lipopeptides biosynthesis related genes of iturin A and bacillomycin D. Additionally, SD03 strain was capable of producing amylase, siderophores as well as phosphate solubilization. In the meantime, plant hormone (IAA) was checked in SD03 strain by colorimetric method in the amount of 374.78 µg/ml. Greenhouse experiment, potato tubers were treated in suspension of SD03 strain before planting into the soil. It has provided the germination of potato seedlings increase crop yields and diseases suppression. *B. velezensis* SD03 was found to be a promising biocontrol agent for the control of potato pathogens.

**Keywords:** potato, *Bacillus velezensis*, *Fusarium graminearum*, *Sclerotium rolfsii*, *Streptomyces tendae*, *Ralstonia solanacearum* and *Pectobcterium carotovorum* subsp. *basiliense*.

#### Development of grass jelly processing using modified starches and higher efficient extraction method

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Grass jelly is one of the most famous desert in Asian countries. The traditional processes of preparing grass jelly are boiling grass jelly stalks in sodium carbonate for 3-8 hours. After that the solution is percolated from grass jelly extract and then mixed with tapioca starch solution to form grass jelly. Since this process spends a lot of time and energy, this study aimed to improve the grass jelly processing by using modified starches and higher efficient extraction method. The results found that boiling grass jelly stalks using pressure cooker at 117°C for only 30 minutes followed by boiling under atmospheric pressure for 60 minutes gave the total solid content (%) in grass jelly solution not significantly different from the traditional boiling for 6 hours. The native pea starch and 3 types of modified starches (KSA1502, ET50, and ESH15) at 5 % by solution weight were further studied for producing better texture of grass jelly. The grass jelly from KSA1502 had hardness, springiness and gumminess not significantly different from the control (tapioca starch) and also its appearance was the most similar to the control. Finally, the grass jelly solution was extracted by reducing grass jelly stalks by 30% and using 2% KSA1502 with gelatin 2.5 % together with varying tapioca starch to 4 levels as 0, 1, 2 and 3% by solution weight. It was found out that the formula using 2% KSA1502 along with 1% tapioca starch and 2.5% gelatin by weight received the best sensory scores in all attributes and also had the least differences among the other formulas in its texture from the control.

Keywords: grass jelly, modified starch, gelatin, tapioca starch, pressure

Antagonistic *Bacillus* sp. AAHMRU15 and yeast extract product, beta-sac plus\*, inhibit the growth of AHPND—Causing *Vibrio parahaemolyticus* (VP<sub>AHPND</sub>)

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Acute hepatopancreatic necrosis disease (AHPND) has caused huge losses in Pacific white shrimp (*Litopenaeus vannamei*) culture in Thailand since 2013. The causative agent of AHPND is a specific strain of *Vibrio parahaemolyticus* (VP<sub>AHPND</sub>) that carries plasmids with the genes encoding the Photorhabdus insect-related (Pir) A and Pir B toxins. One of the promising practices to control this bacterium is using probiotic and natural products derived from microorganisms and plants. The antagonistic bacterium belonging to the genus *Bacillus*, named *Bacillus* sp. AAHMRU15, was previously isolated and characterized in our laboratory. In this study, the inhibitory efficacy of this bacterium was tested against 4 strains of VP<sub>AHPND</sub> (VPO1, VPO2, VPO3, and VPO4). The antagonistic activity of AAHMRU15 was clearly demonstrated by cross streak technique. In addition, the inhibitory efficiency of AAHMRU15 against VP<sub>AHPND</sub> was evaluated through agar well diffusion compared between those cultured in the different conditions, individually cultured and in combination with 2% (w/v) of the yeast extract product, Beta-Sac Plus\* (BS). We found that the yeast extract product increased the antibacterial activity. Moreover, the real-time growth of VP<sub>AHPND</sub> cultured in the cell-free media containing extracellular products of B15 alone (CFS) or in combination with Beta-Sac Plus\* (CFS+BS) was monitored. The results indicated that the antagonistic *Bacillus* sp. AAHMRU15 and Beta-Sac Plus\* were able to inhibit the growth of VP<sub>AHPND</sub>. The obtained data introduce the potential tool to control the pathogenic bacteria in white shrimp culture through biologically, environmentally friendly and safety practice. Our findings provide an alternative way to reduce the use of antibiotics which supports the concept of ecologically sustainable aquaculture and safety food.

Keywords: Antagonistic Bacillus sp., Vibrio parahaemolyticus-causing AHPND (VPAHPND), Beta-Sac Plus\*, Litopenaeus vannamei

#### Effects of sterility value on qualities of chinese braised culled steer beef in retort pouch

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This study was done to estimate the effects of sterility value on qualities of Chinese braised culled steer beef in retort pouch. Chinese braised beef was prepared from culled steer beef top round and packed in four layeres laminated retort pouches. The samples were sterilized by a steam/air retort at 121°C under three different  $F_0$  values (8, 10 and 12 min). Time and tempareture of sterilization process were colleted using an Ellab Sterilization Monitoring System. The results showed that their cook values were 72.42, 87.05 and 95.04 min, respectively. Microbiological analysis and sterility test prooved that samples produced by all  $F_0$  values were safe for microbiological quality. However, parameters of texture profile (hardness, gumminess, springiness and chewiness) decreased as  $F_0$  value was increased (P<0.05). Similarly, shear force decreased with increasing  $F_0$  value (P<0.05). The CIE L\* value increased and CIE a\* values decreased as  $F_0$  value was increased. For sensory evaluation, semi-trained panelists mostly impressed this product produced by  $F_0$  value of 10 min (P<0.05). Therefore, the development of Chinese braised beef in retort pouch was satisfied with  $F_0$  value of 10 min.

Keywords: braised beef top round, toughness beef, retort pouch, mouist heat sterilization, Fo value

#### Isolation and identification of cyclic lipopeptides from Paenibacillus ehimensis

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Lipopeptides are low- molecular-weight compounds formed by cyclic or linear peptides linked to a lipid tail or other lipophilic molecules. They constitute a structurally diverse group of secondary metabolites produced by a wide variety of bacteria, fungi and yeasts. Biosynthesis of lipopeptides is carried out by multi-modular mega- enzymes classified as nonribosomal peptide synthetases. Lipopeptides consist not only of the 20 canonical L-amino acids, but also nonproteinogenic amino acids. Microbial lipopeptides exhibit a broad array of bioactivities from antimicrobial to antitumor properties. Phytopathogens such as fungi, bacteria, yeasts, and viruses are responsible for major crop losses during agricultural production, and they also affect the quality and safety of fresh and processed food. Until recently, control of pathogens has relied on chemical fungicides and antibiotics. However, public concerns about the potential side effects on human health and environment have stimulated search for new biological agents that meet current health and safety standards. Aerobic endospore-forming bacteria belonging to genera Bacillus and Paenibacillus have been recognized for their antagonistic properties, which may be exploited in biocontrol of plant and animal diseases, as well as, in the prevention of decay of woody construction materials. Paenibacillus species are common in natural habitats and can produce antimicrobial compounds (peptide and other antibiotics, cyclic lipopeptides, bacteriocins and volatiles) and cell wall degrading enzymes. Many of the lipopeptides produced by these bacteria are known for decades and represent a potential "gold mine" of antibiotics. This list includes polymyxins, octapeptins, polypeptins, iturins, surfactins, fengycins, fusaricidins, and tridecaptins, as well as, some novel examples, including the kurstakins. In addition to biocontrol activity, various species of Paenibacillus promote plant growth by atmospheric nitrogen fixation, mineral solubilization and production of phytohormones. We isolated from soil antifungal P. ehimensis strain and studied mechanism of its antifungal action. It secreted not only several cell-wall-degrading chitinolytic enzymes but also low-molecular weight antifungal metabolites. The fraction was purified with water-methanol extraction followed by a chromatography on a C18-support. The analysis with LC-MS showed presence of two main series of homologous compounds, family of depsipeptides containing a hydroxy fatty acid, three 2,4-diaminobutyric acid (Dab) residues, five hydrophobic amino acids and one Ser/Thr residue, and cyclic lipopeptides of bacillomycin L and fengycin/plipastatin/agrastatin families. The prevailing compounds in this group are bacillomycin L-C15, fengycin/plipastatin A-C16 together with their homologs

responsible for the majority of fungal growth inhibition by *P. ehimensis*. It was found that the substances secreted by the bacterium make damages into cells of fungi affecting them to swell and finally die.

**Keywords:** isolation and identification of lipopeptides, antifungal, *Paenibacillus*.

A survey on acid hydrolysis in analyzing the monosaccharide composition of exopolysaccharide from Ophiocordyceps sinensis

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Exopolysaccharide (EPS) is a heterogeneous group of polysaccharides which has diverse biological activities; in which monosaccharide composition is one of the crucial factors determining the biological activity of EPS. In the analysis of monosaccharide composition by GC-FID technique, the determination of proper acid is a significant step to help cut the glycosidic bond which is less stable in an acidic environment, great hydrolysis of polysaccharide chain to monosaccharide level, to help form Acetyl derivatives and bring reliable results. The present study investigates the hydrolysis ability of 3 acids: H<sub>2</sub>SO<sub>4</sub>, HCl, and TFA for hydrolyzing EPS of *Ophiocordyceps sinensis* (*O. sinensis*). Hydrolysis ability was assessed by thin-layer chromatography and high-performance liquid chromatography (HPLC), then the product was treated by acetylation and finally analyzed by GC-FID to determine the monosaccharide composition of the EPS. The results showed that hydrolysis by H<sub>2</sub>SO<sub>4</sub> gave higher hydrolysis efficiency and was more suitable than hydrolysis by HCl and TFA in the pretreatment of EPS sample for monosaccharide composition analysis by GC-FID. H<sub>2</sub>SO<sub>4</sub>-treated EPS detected 5 types of monosaccharides: rhamnose, arabinose, mannose, glucose, and galactose; mainly mannose, galactose, and glucose; mannose portioned the highest percentage. The study has set the fundamental for further analysis of the chemical structure and biological activities of EPS.

Keywords: EPS, hydrolysis, GC-FID, HPLC, monosaccharide, O. sinensis

Optimization of culture parameters of selenium-enriched Ophiocordyceps sinensis biomass by response surface methodology

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The aim of this study was to optimize the medium composition and culture conditions of 25 mg/l selenium supplementing *Ophiocordyceps sinensis* by response surface methodology. The results showed that the optimum medium composition for Se enrichment of *O. sinensis* was found at 53.48 g/l saccharose, 11.17 g/l peptone, 1.05 g/l KH<sub>2</sub>PO<sub>4</sub>, and the biomass and selenium content was 24,93 g/L, 960,31  $\mu$ g/g, respectively. The obtained optimum conditions for *O. sinensis* consisted of the following: daylight, temperature 20 °C, initial pH value 6.1, and the maximum biomass and total Se yield in fungi could reach 26,45 g/l, 1068  $\mu$ g/g, respectively. The result of these experiments indicated that response surface methodology is a promising method for the optimization of selenium-enriched *O. sinensis* fermentation process, and which is the basis for further studies of the bioactivity of this fungal extracts.

Keywords: biomass, Ophiocordyceps sinensis, optimization, selenium, response surface methodology

#### In vitro antioxidant and anti-inflammatory activities of Cordyceps neovolkiana DL0004 extracts isolated in Vietnam

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Cordyceps neovolkiana was obtained at Lam Dongprovince, Vietnam. In the course of our continuous search for more antioxidant and anti-inflammatory compounds for community health, we initially screened the antioxidant resistance of *C. neovolkiana in vitro* according to the reducing power assay, and examining the ability to free radicals according to DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay. At the same time, we also evaluated the *in vitro* anti-inflammatory ability according to the model of protecting the denaturation of albumin by heat as well as the ability to inhibit Xanthine oxidase - an enzyme that catalyzes the oxidation reaction of purine to uric acid causing arthritis. From the 12 extracts, studying the antioxidant and anti-inflammatory activities *in vitro* obtained the following results: the crude EtOH giving the highest reduction ability ( $\Delta$ OD<sub>1000µg/ml</sub> =0,08±0.0008), the EtOAc showed the free radical activity with IC<sub>50</sub> 107.01 ± 0.0743µg / ml. The n-BuOH expressing ABTS free radical activity with IC<sub>50</sub> values of 111.91 ± 0.195 µg / ml, respectively. Xanthin oxidase inhibitory activity of the PS is 7.31 ± 0.15 µg / ml and the EtOH capable of protecting BSA denaturation by heat with IC<sub>50</sub> is 430.35 ± 4.3703 µg / ml.

**Keyword:** Cordyceps neovolokiana, DPPH, ABTS, Xanthin oxidase, Serum albumin.

Study on arbuscular mycorrhizal fungi related to soil p, soil om and soil ph in cassava field from Thailand

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Two-hundred and ninety-five soil samples were collected from cassava field in Thailand to study distribution of arbuscular mycorrhizal (AM) fungi under root zone of cassava. The AM fungal spores were counted for spore number  $100g^{-1}$  soil, morphotyped classified and AM fungal colonization determined after pot culture. Soil P, soil OM and soil pH were analyzed to calculate the correlation between each parameter and AMF spore number. The result showed that cassava has been planted in wide soil pH in Thailand. They could be grown in acidic soil to alkaline soil (pH 3.0-8.5). Soil P in cassava field was 0.05- 91.91 mg kg $^{-1}$ . Soil OM was 0.22-4.49%. AMF spore number under cassava field was 25- 1,880 spores  $100g^{-1}$  soil classified as eighteen AMF species. *Glomus* sp. and *Acaulospora* sp. were always found under cassava field. This research showed no relationship between Soil OM and AMF spore number and no relationship between Soil pH and AMF spore number. However we found negative relationship between Soil P and AMF spore number (AMF spore number = 426.11– 4.12Soil P,  $r^2 = 0.075**$ ).

Keywords: AMF, Cassava, Soil P, organic matter, pH

#### Floods increase soil microbial activity in paddy soil: a case study in sakon nakhon province, Thailand

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Floods is an important problem that occur frequently in specific areas. Flooding in agricultural areas cause of yield lost and sometimes plant mortality. Moreover, water that flow through residential and industrial areas can bring many contaminants to agricultural fields. This study focused on different flooding history on soil chemical and biological activities. Soil sample were collected from 3 paddy areas with different flooding history; full area flooded, partial area flooded, and non-flooded. The results showed that the different flooded histories effect on some soil properties. Partial area flooded had the highest soil electrical conductivity with 0.06 mS/cm, but in the range that do not affect plant growth. Organic matter in all areas of study were in the low level. Total nitrogen was in low level under partial area flooded, but in medium level under full area flooded and non-flooded. Total phosphorus in all field of study were in low level with less than 4 ppm. Total soil potassium was in low level under full area flooded but in high level under partial area flooded and non-flooded. The microbial activity found the highest soil respiration under full area flooded with 0.16 mg CO<sub>2</sub>/g soil, however soil respiration was in the low level. The highest bacterial population, fungi population, FDA activity, urease, dehydrogenase and protease activities found under full area flooded condition. However, soil pH, microbial biomass carbon, microbial biomass nitrogen and acid-phosphatase activity were not statistically different. No pesticide in carbamate, organochloride and organophosphate groups were observed. The amount of mercury, arsenic, lead and cadmium were lower than the standard set of Thailand. Flooded did not reduce soil quality in the areas of studies, on the other hand, microbial activities that benefit plant growth increased.

Keywords: floods, soil chemical property, microbial activity, soil enzyme

#### Antioxidants and product development of bread crust

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Recently, it was discovered that Maillard reaction can provide antioxidant products. The brown crust of sandwich bread, a by-product from bakery industry, has a higher Maillard reaction products than other parts of bread. The objectives of the research was to study the relationship between brown color of bread crust and antioxidant activity and to develop a waffle cone produced from bread crust, which has an added value and health benefits. The main ingredient was bread crust, which was baked at 60°C to control aw equal to 0.4. RVA result showed that bread crust powder had different viscosity behavior compared with all-purpose wheat flour. The maximum viscosity of crust powder was 20 times lower than all-purpose flour. After that, bread crust was separated into 5 groups by its brown shade, bread crust was then analyzed in terms of the Browning Index (BI) and antioxidant activity by DPPH assay. The results showed that all bread crust exposed BI of 37.67-50.70 and antioxidant activity (%Inhibition) of 11.6-30.3%, respectively. For product development part, two factors was studied that was the waffle cone formulas and the ratio of all-purpose flour and bread crust powder contents. Both factors affected waffle cone in terms of moisture content, water activity, texture, durability time, color and ability to maintain cone shape. The 3 best formulas was selected for sensory evaluation. The results showed that Strange's formula can be used to substitute 50% bread flour for whole wheat flour without affecting the sensory 9-point hedonic scale score and give the overall preference score was 6.5 point. Strange's cone formula with a Browning Index of 48.47, had also the highest antioxidant activity of 60.96%.

Keywords: Baking, Maillard reaction, DPPH, Waffle

#### Effect of tomato powder on quality of Chinese sausage

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This research studied the effect of tomato powder on quality of reduced nitrite Chinese sausage. There were three experiments. The first experiment was quality comparison of the Chinese sausages, with four levels of tomato powder (0.2, 0.6, 1.0 and 1.4%.w/w) and 0.6.w/w sodium nitrite. The control treatment was 0%.w/w tomato powder and 1.22%w/w sodium nitrite. The appropriate amount of tomato powder was selected by color measurement  $a_w$ , pH, hardness, springiness, cohesiveness, and 9-point hedonic scale sensory evaluation. It was found that the Chinese sausage with 0.2% w/w tomato powder and 0.6%w/w sodium nitrite made the highest overall sensory score and no significant difference from control(p>0.05). The second experiment was the proximate analysis and nitrite residue analysis were conducted and compared with control treatment. The result showed that the quality of Chinese sausage with 0.2%w/w tomato powder and control treatment had no significant difference (p>0.05). However, nitrite residue in Chinese sausage with 0.2% w/w tomato powder was less than control treatment. The third experiment was shelf life study. It was conducted at atmosphere and vacuum package conditions, and stored at 32-35°C and 4°C. It was found that the best condition of storage was vacuum packaging at 4 °C that could preserve Chinese sausage for 12 days.

Keywords: tomato powder, reduced nitrite, Chinese sausage

#### Antifungal and antibacterial activities of essential oil from som keaw (Citrus nobilis) in Thailand

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Som Keaw (*Citrus nobilis*) is a member of family Rutaceae and Thailand, Samutsongkram province is the best area for growing this orange. The antifungal and antibacterial activities of essential oil from Som Keaw were tested against plant pathogens on both of fungi and bacteria in various concentrations. The antibacterial activity of essential oil extract from leaf of Som Keaw was tested by paper disc diffusion method against *Erwinia chrysanthemi* and *Xanthomonas axonopodis*. The essential oil from leaf was displayed antibacterial activity higher than the essential oil extract from peel with inhibition zone at 10,000 ppm as 10.67 mm and 11.33 mm, respectively. Furthermore, the pure essential oil from peel 20  $\mu$ L displayed the highest against *E. chrysanthemi* and *X. axonopodis* with the inhibition zone as 20.00 mm and 14.00 mm, respectively. The antifungal activity was tested by vapour phase technique against five plant pathogenic fungi (*Colletotrichum gloeosporioides, Curvularia lunata, Fusarium sacchari, Pythium* sp. and *Rhizoctonia solani*) at 5, 10 and 20  $\mu$ L/ disc. The results illustrated that the essential oil from peel extract completely inhibited the mycelial growth of *Pythium* sp. and *8. solani*. However, the essential oil from leaf and peel of Som Keaw were of high antifungal activity against all of tested fungi (41.49-100% inhibition).

Keywords: essential oil, orange, plant pathogens, Som Keaw (Citrus nobilis)

# GC-MS analysis and biopesticide properties of different crude extracts of Annona squamosa and Annona muricata

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Annona squamosa and A. muricata are members of the family Annonaceae, commonly known as sweetsop and soursop, respectively. This study aimed to investigate the secondary metabolites present in ethanolic extract of A. squamosa and A. muricata and their biopesticide properties. Ethanolic crude extracts of fresh leaves, dried leaves and seeds of A. squamosa and A. muricata were subjected to phytochemical screening by GC-MS. The mass spectra of identified compounds from ethanolic extract showed that the most common compounds in leaves of the two species were neophytadiene, hexadecanoic acid, and trans-caryophyllene. Hexadecenoic acid and ethyl oleate were identified in seeds of A. muricata, while only methyl-p-tert-butyl phenylacetate was identified in seeds extract of A. squamosa. Crude extracts were tested for their antifungal activities against postharvest plant phatogens and insecticidal activities against brown planthopper, Nilaparvata lugens (Stål), and cassava mealybug, Phenacoccus manihoti Matile-Ferrero. Our results showed low inhibitory effect (less than 20%) against pathogenic fungi Insecticidal test against cassava mealybug of all crude in brown planthopper treated with 5% seed extract of A. squamosa, indicating its potential as an alternative botanical insecticide, but further studies should be conducted to improve yield and quality of the extract.

Keywords: secondary metabolite, soursop, sweetsop, insecticidal activity, antifungal activity

 $Pre liminary study on chlorophyll \it a \it concentration and phytoplankton diversity in huai jorakhemak \it reservoirs, buriram province, Thailand$ 

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The objective of this study was to investigate chlorophyll a concentration and phytoplankton diversity In Huai Jorakhemak Reservoirs, Buriram Province, Thailand. The samples were collected from 6 stations with 5 times between August to October 2018. The results showed that Chlorophyll a concentration ranged between 2.757-3.430  $\mu$ gl<sup>-1</sup>. The highest chlorophyll a concentration was obtained in September 2018 with mean value 3.43 $\pm$ 0.67  $\mu$ gl<sup>-1</sup>. Species diversity of phytoplankton found 27 species. The most species diversity was Division Chlorophyta and there were 18 species, the common species were *Botryococcus* sp. and *Sirogonium* sp. From the results of this study, it showed that the water quality in Huai Jorakhemak Reservoirs, Buriram Province, Thailand is in oligotrophic status.

**Keywords:** chlorophyll a, phytoplankton, species diversity, reservoirs.

#### Isolation of cellulase producing bacteria using sunflower meal as substrate

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Twenty-three samples from soil and dung were enriched in sunflower meal (SM) broth at  $30^{\circ}$ C, 150 rpm for 2 days for selection bacteria using SM as substrate. All cultures were screened of cellulase producing bacteria on carboxymethyl cellulose (CMC) agar. Sixty-one isolates showed cellulose assimilation and were divided into eleven groups based on their colonial morphology. Total bacteria were studied cellulase production when were cultured in broth medium as CMC and SM as substrate. They showed cellulase activity at  $0.04 \pm 0.01$  to  $3.28 \pm 0.09$  and  $0.03 \pm 0.00$  to  $3.26 \pm 0.06$ , respectively. The isolate PR3-5-1 produced the highest cellulase activity from two substrates.

Keywords: cellulose, substrate, feed, bacteria and Bacillus

Evaluation of the antifungal activity of various agricultural residue extracts to control seed borne pathogens of maize in Thailand

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The aim of this study was to evaluate the antifungal potential of various agricultural residue extracts against seed borne pathogens of maize (waxy corn) in Thailand. *In vitro* Seeds of maize were treated with three locally available agricultural residues that are left in an agricultural field; *Musa sapientum* (banana) peel, *Garcinia mangostana* (mangosteen) peel and *Anacardium occidentale* (cashew nut) testa. Agricultural residues were extracted with three different solvents (hexane, ethyl acetate and water). Crude extracts of the agricultural residues were treated to maize seed at different concentration (250–4000 ppm) using seed coating technique. Among the treated maize seeds, the most effective agricultural residue extracts showed significantly lower percentage of fungal infection were banana peel and mangosteen peel extracted by hexane compared to other treatments. Furthermore, the cashew nut testa extract had no potential antifungal activity against fungal pathogens. The seed germination test demonstrated banana peel extracts had no effect to maize seed germination as shown by non-significant different of percent germination in all the treatments. As a result, the agricultural residue extracts can be used as an alternative choice to control seed borne pathogens of maize.

**Keywords:** biological control, crude extract, biocide, seed germination

Quality characteristics and storage stability of reduced-fat Thai fermented pork sausage, sai krok e-san, with konjac gel during chilled storage

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In order to evaluate the quality and shelflife of reduced-fat Thai fermented pork sausage (Sai Krok E-san), the effect of replacing pork backfat with konjac gel on the changes in qualities during chilled storage were studied. Products were formulated as control sample (30% pork backfact) and reduced-fat sample (50% of substitution pork backfat with konjac gel), stuffed in hog casing, and fermented at ambient temperature for 3 days. Thereafter, products were taken at 0, 1, 2, 3, and 4 weeks of storage for analysis of weight loss, pH, color, texture profile analysis, protein degradation, lipid oxidation, microbiological analysis, and sensory evaluation. The results showed that weight loss, pH, color, and protein degradation between control and reduced-fat products were not significantly different (P<0.05). However, reduced-fat product with konjac gel exhibited a superior texture in terms of higher values of hardness, cohesiveness, gumminess, springiness, and chewiness than control (P<0.05). During 2 weeks of storage, lipid oxidation

was lower in reduced-fat sample than those in control (P<0.05), thereafter increased with similar content. Although lactic acid bacteria (LAB) were not influenced by the reformulation (P<0.05), LAB in sample gradually increased during storage range from 8 log CFU/g at the begining to 9 log CFU/g at 4 weeks. The sensory panel stated that both products had acceptable sensorial quality, if product were kept less than 3 weeks of storage. The longer periods resulted in a higher rancid flavor, especially for control sample.

Keywords: healthy meat products, fermented meat product, glucomannan konja

Development of banana flavor carrageenan jelly drink fortified with banana peel extracts

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Abstract This research aims to develop banana flavor carrageenan jelly drink fortified with banana peel extracts product. Start with selecting the type and amount of gelling agent by using sensory evaluation techniques in the term of appearance, color, banana flavor, taste, texture and overall liking with 9-point hedonic scale and the optimum intensity of banana flavor was evaluated with 5-point category scale. The results showed that the suitable gelling agent is carrageenan at level of 0.4 percent by weight, but also found that banana flavor that is not enough after pasteurized jelly drink at 73°C 15 min. After that, Study on the amount of banana and artificial banana flavoring were varying by reducing the amount of bananas from the usual formula three levels of 0, 50 and 75 percent and adding artificial banana flavoring two levels are 0.01 and 0.02 percent, then evaluated sensory characteristics with 9-point hedonic scale. The results showed that the most suitable formula of product is banana reducing 50 percent from usual formula, and adding artificial banana flavor 0.02 percent. Banana flavor carrageenan jelly drink fortified with banana peel extracts has a hardness value was 0.59 Newton and the color L \* a \* and b \* was -2.86 31.06 and 22.81 respectively. The appropriate banana peel extracts content for adding in product was 1 percent with no difference from formulas without the extracts. In addition, it was found that banana peel extract gave 2,2-Diphenyl-1-1 picryhydrazyl (DPPH) radical scavenging activity (7.92 g sample/mol DPPH)

Keywords: Jelly drink, Banana peel extract, Banana, Gelling agent

The usage of rice bran flour and pea protein in pork patties and pork meatballs

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This study aimed to replace meat and back fat with rice bran flour or pea protein in pork patties and pork meatballs. The experiment compared two levels of rice bran flour (5 and 10% w/w) and two levels of pea protein (5 and 10%.w/w) in the pork patties and pork meatballs. The control treatment was 0%w/w rice bran flour or pea protein. The qualities of these products were evaluated by cooking yield, diameter reduction, juiciness, moisture and fat retention, texture profile analysis, color, and sensory evaluation. It was found that the higher the amount of extenders, the higher was the cooking yield, moisture retention and fat retention whereas, the diameter reduction and juiciness decreased. The texture evaluation factors of all treatments were higher than the control treatment(p<0.05). For the color parameters, increased in pea protein causes the lightness, redness, yellowness, and protein content of the product to rise. Also, the increase of rice bran flour causes fiber, ash, and carbohydrate to rise, but the lightness reduces. In addition, using rice bran flour and pea protein can reduce fat content. Sensory evaluation results suggested that using rice bran flour and pea protein at 5% w/w in pork patties and pork meatballs had an overall acceptability similarly to the control. In conclusion, at 5% w/w or below each, rice bran flour and pea protein are suitable as extenders for pork patties and pork meatballs.

Keywords: patties, meatballs, rice bran flour, pea protein

# Full Text

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Essential oils from Zingiber mekongense Gagnep, Myristica fragrans Houtt and Curcuma zedoaria Roscoe as Larvicidal agents against Aedes albopictus (Skuse)

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**Abstract** Comparatively, *C. zedoaria* EO at 10% concentration provided the strongest larvicidal activity against *Ae. albopictus* and yielded the highest mortality rate of 100% with  $LC_{50}$  value at 1.09% and  $LT_{50}$  value of <0.01 h. The  $LT_{50}$  values for *Z. mekongense* and *M. Fragrans* EOs were 0.25, 0.52 h. respectively, while their  $LC_{50}$  value was the same at 3.00%. On the other hand, 1% w/w temephos (positive control) exhibited an  $LT_{50}$  value of 1.49 h., whereas 70% v/v ethyl alcohol (negative control) caused no mortality of *Ae. albopictus* larvae at all during the 48 hours of testing period. These results revealed that essential oils extracted from these plants can control mosquitoes well. They are good alternatives to temephos because they are friendly to the environment, humans and animals and should be further developed into commercial products.

Keywords: Aedes albopictus, Larvicidal activity, Plant essential oils

#### Introduction

Since the last few decades, it has been realized that mosquitoes not only transmit malaria but also many other serious pathological conditions such as dengue, chikungunya, yellow fever, Japanese Encephalitis (JE), filariasis and so on. Recently, Zika virus has created havoc in Africa, Southeast Asia, Pacific Island and Brazil (Hung *et al.*, 2019). Botanical insecticides in general and essential oils, in particular, have emerged as promising, environmentally friendly alternatives. There are around 12 species of Erechtites (Asteraceae). They are indigenous in Australia, North America, South America, New Zealand, and West Indies. Many plant species have been found to contain toxic substances against insects during the last half of the century. These plants are from various families, and some chemicals that they contain have adverse effects on insects (Hung *et al.*, 2019).

Mosquito-borne diseases are serious and prevail in countries where malaria is still a constant threat to 3.3 billion people (Benelli and Pavelec, 2018; Benelli and Beier, 2017). Most importantly, no medicines or vaccines are currently available for providing treatment against arboviruses, such as the virus of dengue fever. Drugs against Zika virus, malaria, and chikungunya currently employed in the fight against these parasites are seriously ineffective due to rapidly developing resistance by *Plasmodium parasites* (Benelli and Pavelec, 2018; Benelli *et al.*, 2017b), while the freshly developed Plasmodium falciparum vaccine (RTS, S/ASO1/Mosquirix) provides only temporary protection, as reported in a children treatment study by Gosling and Seidlein, (2016).

Adult, fully-grown mosquito population is harder to manage and control by insecticides, mainly because adult mosquitoes tend to develop resistance to insecticides after a short period of exposure. Therefore, the most effective control program is likely to be controlling mosquitoes when they are still in larvae or pupae stages (WHO, 2017; Soonwera and Phasomkusolsil, 2016). Insect management programs that apply insecticides to mosquito population in their larvae or pupae stages are called Larviciding and pupiciding methods. These methods also help preventing chikungunya dengue and other diseases vectored by mosquitoes.

Prevention from bites of sucking arthropods is based on the use of chemical pesticides. However, they hurt human health and the environment (Hicks et al., 2017; Silver et al., 2017). In addition, currently they are becoming

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ineffective, due to the rapidly developing resistance of the targeted vectors (Benelli and Pavelac., 2018; Naqqash *et al.*, 2016).

Accordingly, it is urgent to expand a natural product vector management strategy for controlling mosquito populations. Lately, considerable attention has focused attention on essential oils as alternative agents due to their low toxicity to humans and the environment. and high biodegradability in the environment. EOs extracted from plants provide real-world applicability and high efficacy. They act as insecticides through several mechanisms of action (e.g., inhibiting P450 cytochromes, binding to GABA receptors, inhibiting cholinergic system or modulating octopaminergic system), but exert low toxicity to humans and animals including friendly to the environment (Pavela and Benelli, 2016).

Manifold bioactive ingredients of EOs with varied action mechanisms can substantially decrease resistance in mosquitoes. It is reported that approximately 17,500 aromatic plants species have reported containing EOs; in particular, Asteraceae, Lauraceae, Lamiaceae, and Myrtaceae are major plant families is a plant extracting essential oils (Huang *et al.*, 2019).

In general, essential oils have been considered as useful insecticides, with low mammalian toxicity and rapid degradation in the environment. Essential oils derived from various plants show different bioactivities against mosquito species. These activities range from ovicidal, larvicidal, pupicidal activities to adulticidal activity as well as oviposition deterrence and repellency. Some plants that are known as excellent sources of essential oils with insecticidal properties include those in the Rutaceae family. In the Rutaceae family, plants in the genus Zanthoxylum provide a variety of secondary metabolites including alkaloids, aromatic and aliphatic amides, lignans and coumarins with important phytochemical and biological activities. (Soonwera. and Phasomkusolsil, 2017; Aurelie et al., 2016).

Larvicidal activity is very important in vector management because larvae that are in the growth stage are the easiest to destroy. However, this kind of management program works well only when the water resource is a small area where larvae and pupae mosquitoes live in the stagnant water. Several previous papers report that essential oils extracted from various plants have larvicidal and pupicidal effects against mosquitoes. A lot of products from food, cosmetic and pharmaceutical industries are currently made from highly volatile essential oils extracted from plants (Soonwera and Phasomkusolsil, 2017). These essential oils have also been considered as crucial ingredients for natural insecticidal products that can be extracted from almost every part of a plant such as the seed, leaf, twig, flower, fruit, bark, bud, stem, and root. Since natural insecticides are made from plants oils, they are minimally toxic to mammalian species and quickly degrade in an environment with minimal impact to it.

This research aimed to determine the larvicidal and pupicidal properties against *Aedes albopictus* of essential oils extracted from three medicinal plants: *Zingiber mekongense* Gagnep, *Myristica fragrans* Houtt, and *Curcuma zedoaria* Roscoe.

#### Materials and methods

#### Mosquitoes

In this research, the *Ae. albopictus* mosquito subjects were reared in the laboratory of the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. Their larvae were fed with fish food pellets (HIPRO\*) and raised under the conditions of 26-28°C, 70-80% relative humidity, and 12:12 h. (light: dark) photoperiod. Stage-4 larvae were used in the tests for larvicidal and pupicidal activities.

#### Plant materials

Zingiber mekongense, Myristica fragrans and Curcuma zedoaria were obtained from sources in Thailand. All plant samples were correctly identified by a plant taxonomist from the Plant Production Technology Section, Faculty of Agricultural Technology, KMITL. Different plant parts were extracted for essential oils by a water distillation method. One kg of plant material was placed in an extraction column connected to a round-bottomed distillation flask containing distilled water. The flask was heated to almost 100° C, then the water was boiled until the distillation was complete at 4-6 h. The distilled fraction was removed of water with anhydrous sodium sulfate and kept in a refrigerator at 4 °C until further use. All of the extracted plant EOs were diluted with ethyl alcohol to 3 solutions at

concentrations of 1%, 5%, and 10% and maintained under laboratory conditions before subsequent uses in various assays.

#### Chemicals

Chemical insecticide (positive control) and Ethyl Alcohol (negative control)

- 1) Temephos (1% w/w temephos; Sai GPO-1°) was employed as the positive control. Sai GPO-1°(1% w/w temephos) was a common chemical larvicide in Thailand. It was bought from the Government Pharmaceutical Organization, 75/1, Rama VI Rd, Ratchathewi, Bangkok 10400, Thailand.
- 2) Ethyl Alcohol 70% v/v (Alcohol Siribuncha\*) was employed as the negative control. It was bought from Siribuncha Co., LTD, 50/4 Mu 7, Banggruay-sainoi Rd., Nonthaburi province, Thailand.

### Larvicidal Bioassay

The larvicidal bioassay used was a standard assay of the World Health Organization (WHO,2017). Ten mosquito larvae (fourth instar larvae or pupal stage) of  $Ae.\ albopictus$  were put in a 150 ml glass jar containing 99 ml of distilled water and 1 ml of test solution. One percent w/w Temephos (Sai GPO-1\*) was employed as the positive control and 10% ethyl alcohol was used as the negative control. During the assay, the larvae were not fed with anything. Larval mortality and pupal mortality were recorded at 0.08, 0.16, 0.5, 1, 6, 12 and 24 h. The Lethal time for 50% mortality (LT<sub>50</sub>) was calculated according to probit analysis and significant differences were analyzed by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) with SPSS for windows software version 16.0.

#### Results

The results of the larvicidal assay for 1%, 5% and 10 % essential oils were recorded as mortality rate listed in table 2, 3 and 4, respectively, and  $LT_{50}$  as well as  $LC_{50}$  are listed in table 1.

The mortality rate was higher with increasing concentration of essential oils. *C. zedoaria* provided an LT<sub>50</sub> of less than 0.01 h. against *Ae. albopictus*, while *Z. mekongense* and *M. fragrans* EOs provided an LT<sub>50</sub> of 0.25 and 0.52 h, respectively. At 5% concentration, *C. zedoaria* and *Z. mekongense* provided an LT<sub>50</sub> of 0.02 and 0.36 h, respectively, while *M. fragrans* EO was the least effective with an LT<sub>50</sub> of 0.48 h. At 1% concentration, *Z. mekongense* EO did not induce any mortality against *Ae. albopictus* larvae (Table 2), but *C. zedoaria* EO and *M. fragrans* EO provided an LT<sub>50</sub> of 1.06 and 6.04 h, respectively. Ten percent essential oil from *C. zedoaria* provided the highest larvicidal effect against the larvae of *Ae. albopictus* within the shortest period (LT<sub>50</sub> < 0.01 h.) and had the lowest LC<sub>50</sub> of 1.09%, followed by the essential oils from *Z.mekongense* and *M. fragrans* that had an identical LC<sub>50</sub> of 3.00%.

**Table 1.** 50% lethal concentration ( $LC_{50}$ ) and 50% lethal time of three essential oils, negative, and postsitive control against fourth instar larvae of *Ae. albopictus* 

Treatment	LC <sub>50</sub> (%)	LT <sub>50</sub> (h)	(LCL-UCL) <sup>1/</sup> / concentra	ation(%)
rreatment		1 %	5 %	10 %
70% <i>v/v</i> Ethyl alcohol (Negative control)	NA <sup>2/</sup>	NA	NA	NA
1% <i>w/w</i> Temephos (Postsitive control)	NA	1.49 (1.36-1.62)	1.49 (1.36-1.62)	1.49 (1.36-1.62)
C. zedoaria EO	1.09 (-)	1.06 (0.97-1.20)	0.02 (-0.25-1.41)	<0.01(-1.07-0.03)
Z.mekongense EO	3.00 (-)	NA	0.36 (0.30-0.44)	0.25 (0.15-0.35)
M. fragrans EO	3.00 (-)	6.04 (5.49-6.78)	0.48 (0.42-0.55)	0.52 (-0.17-0.16)

<sup>1/95%</sup> CL means 95% confidence limit; LCL= lower confidence limit; UCL= upper confidence limit

**Table 2.** Larvicidal activity of three essential oils at 1% concentration , negative and postsitive control against fourth instar larvae of *Ae.albopictus* 

Treatment -			%	Mortality ± SD at s	pecified time(h)		
rreatment	0.08	0.16	0.5	1	6	12	24
70% v/v Ethyl alcohol	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0b <sup>1/</sup>	0.0 ±0.0c <sup>1/</sup>	0.0 ±0.0	0.0 ±0.0
1% w/w Temephos	$0.0 \pm 0.0$	0.0 ±0.0	$0.0 \pm 0.0$	20.0 ±27.4b	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0
C. zedoaria EO	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	46.0 ±15.2a	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0
Z.mekongense EO	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	0.0 ±0.0b	$0.0 \pm 0.0c$	0.0 ±0.0	$0.0 \pm 0.0$
M. fragrans EO	$0.0 \pm 0.0$	0.0 ±0.0	$0.0 \pm 0.0$	0.0 ±0.0b	48.0 ±38.4b	100.0 ±0.0	100.0 ±0.0
C.V.(%)	NA <sup>2/</sup>	NA	NA	60.30	28.80	NA	NA

<sup>1/</sup> Mean mortality in a different column followed by a different letter is significantly different (P< 0.05 by ANOVA and DMRT)

<sup>2/</sup> NA means not available

<sup>2/</sup> NA= not computed from this Probit analysis.

**Table 3.** Larvicidal activity of three essential oils at 5% concentration, negative and postsitive control against fourth instar larvae of *Ae.albopictus* 

Tractment			% Moi	tality ± SD at spe	cified time(h)		
Treatment	0.08	0.16	0.5	1	6	12	24
70% <i>v/v</i> Ethyl alcohol	0.0 ±0.0b <sup>1/</sup>	0.0 ±0.0	0.0 ±0.0c	0.0 ±0.0c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
1% w/w Temephos	0.0 ±0.0b	0.0 ±0.0	0.0 ±0.0c	20.0 ±27.4b	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
C. zedoaria EO	46.0 ±21.9a	100.0 ±0.0	100.0 ±0.0a	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
Z.mekongense EO	0.0 ±0.0b	0.0 ±0.0	84.0 ±13.4b	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
M. fragrans EO	0.0 ±0.0b	0.0 ±0.0	100.0 ±0.0c	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
C.V.(%)	51.00	NA <sup>2/</sup>	12.80	17.00	NA	NA	NA

<sup>1/</sup> Mean mortality in a different column followed by a different letter is significantly different (P<0.05 by ANOVA and DMRT)

**Table 4.** Larvicidal activity of three essential oils at 10% concentration , negative and postsitive control against fourth instar larvae of *Ae.albopictus* 

			% Mortalit	y ± SD at spec	ified time(h)	)	
Treatment	0.08	0.16	0.5	1	6	12	24
70% v/v Ethyl alcohol	0.0 ±0.0c <sup>1/</sup>	0.0 ±0.0c	0.0 ±0.0	0.0 ±0.0c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
1% <i>w/w</i> Temephos	0.0 ±0.0c	0.0 ±0.0c	0.0 ±0.0	20.0 ±27.4b	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
C. zedoaria EO	88.0 ±26.8a	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
Z.mekongense EO	0.0 ±0.0c	72.0 ±21.7b	100.0 ±0.0	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
M. fragrans EO	36.0 ±5.5b	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
C.V.(%)	35.20	15.10	NA <sup>2/</sup>	16.60	NA	NA	NA

<sup>1/</sup> Mean mortality in a different column followed by a different letter is significantly diffent (*P*< 0.05 by ANOVA and DMRT)

### Discussion

<sup>2/</sup> NA= not computed from this Probit analysis.

<sup>2/</sup> NA= not computed from this Probit analysis.

krervanh Thai (*Amomum krervanh*), greater galangal (*Alpinia galanga*), and basil (*Ocimum basilicum*). Earlier studies showed that 1-8cineole was toxic to *Plutella xylostella* and exerted a moderate toxicity to *Cotesia pultellae*. 1,8-cineole and thymol have been reported to be insecticides against varroa codling moth (*Cydia pomonella*), larvae of American cockroach (*Periplaneta americana*), and northern house mosquito (*Culex pipiens*) larvae (Bullangpoti, 2018). Essential oil-based repellents are safe for human health, animals, and the environment. Temephos, an organophosphate, was an approved chemical larvicide that was commonly being used in Malaysia, In the past 43 years, Temephosto has been used to control the mosquito larva. (Rahim *et al.*, 2017). In Malaysia, malathion, permethrin, deltamethrin, and temephos were commonly being used for controlling mosquito vectors. In Malaysia, has reported a metabolic resistance mechanism, overproduction of cytochrome P450, responsible for pyrethroid resistance in *A. albopictus* (Ishak *et al.*, 2016). Development in the activity of the metabolic resistance mechanism developed into crossresistance between pyrethroids and organophosphate. Several studies have reported insecticides resistance of Aedes albopictus (Rahim *et al.*, 2017). Herbal products are one of the best alternatives to synthetic chemicals for mosquito control. EOs can be safe and eco-friendly alternatives to synthetic pesticides. These results are encouraging for prospective development of new natural mosquitocidal products from plant oils (Soonwera and Phasomkusolsil, 2017).

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#### Effect of growing media and 20-20-20 chemical fertilizer on yield performance of melon in plastic house

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**Abstract** The result indicated that melon which grown in rain tree leaves: coir: manure (2:1:1) plus 20-20-20 7 gram per pot gave the highest fruit weight and the most TSS content with the mean of 1345.90 gram and 12.58 °brix respectively and showed significantly different among treatment combination. Melon those grown in coir plus 20-20-20 9 gram per pot gave the lowest fruit weight with the mean of 779.70 gram. The melon received from those grown in coir only gave the most palatability with the mean of 27.00 score. This study also indicated that growing media and 20-20-20 fertilization rate effected on well quality and quantity of melon grown in plastic house.

Keywords: growing media, fertilizer, melon, yield components, yield quality

#### Introduction

Melon (*Cucumis melo* L.) is a member of cucurbitaceae which includes cucamber, pumpkin, watermelon, squashe, gourds. (IPGRI, 2003) It's fruit which is rich in carbohydrates, minerals, dietary fiber, flavonoids and vitamins. In Thailand melon cultivation as open field often failed due to many factors such as insect pest probloms which require excessive pesticide application, low yield, poor fruit quality over cost production and impact of environmental constraints. Nation (2016) Melon cultivation under protective structure is required in order to avoid mentioned problems and improved their quality. Food safty and market acceptability are the ultimate goal. Melon cultivation need a proper mineral which impact on growth development and their quality (EL-Desuki *et al.*, 2000). The sweetness of fruita could also effected by harvesting date and proper mineral management also (Ferrante *et al.*, 2008).

#### Materials and methods

4x4 Factorial in Completely Randomized Design (CRD)with four replications.

Factor A represented the growing media; A1 = coir (C), A2 = coir dust: coir: manure (CD:C:M), A3 = rice husk: coir: manure (RH:C:M) and A4 = rain tree leaves: coir: manure (RTL:C:M) and then combining for each growing media in the ratio of 2:1:1.

Factor B represented chemical fertilizer there are 4 rates (NPK, 20-20-20) where B1 (3 g), B2 (5 g), B3 (7 g) and B4 (9 g) with 16 treatment combinations.

7-day seedling of melon #204 orange flesh seedling was transplanted in black plastic pots 15 inch diameter in container filled up with growing media is mixed at a ratio of 2: 1: 1. Growing 1 plant per pot, after 7 day all treatments were treated with 20-20-20 complete fertilizer + water 2 liter per 3 days until 52 days after fruit setting the fruit were harvested for data collection. All treatments were placed under plastic house.

The data of fruit components were recorded as followed There are fresh weight (g), width of fruit (mm), length of fruit (mm) and data of fruits quality, comprising of thickness of flesh (mm), thickness of peel (mm), flesh firmness (N), total soluble solid (°brix), titratable acidity (%), pH juice and palatability test. All the data were analyzed of variance (ANOVA) and mean comparisons by Tukey; HSD method.

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Figure 1. Growing media A = Coir (C), B = Coir Dust:Coir:Manure (CD:C:M), C = Rice Husk:Coir:Manure (RH:C:M), D = Rain Tree Leaves:Coir:Manure (RTL:C:M)

#### Results

#### Yield components

This study indicated that melon fruit was harvested on 52 days after fruit setting; firmness, TSS, TA and juice pH ware analyzed the data shown in table 1. Melon fruits those grown in various growing media the result showed that melon fruits grown in RTL: C: M (2:1:1) had the most fresh weight and second best fruit was grown in RH: C: M (2:1:1) while the lowest fruit weight received from those fruit grown in C with the mean of 1252.50 grams, 1184.70 grams and 956.60 grams respectively and showed significantly difference among treatment.

Regarding with growing media indicated that therefore growing media impact to fruit width fruit length and thickness of flesh. The most of these parameters received from melon grown in RTL:C:M (2:1:1) with a mean of 133.54 mm, 130.61 mm and 113.52 mm. respectively while the lowest fruit width, fruit length and thickness of flesh got from those grown in C with the mean of 124.96 mm, 119.84 mm and 103.06 mm respectively and showed significantly among treatment.

Regarding with 20-20-20 fertilization rate showed that melon fruits treated with 20-20-20 at 3,5,7 and 9 grams per pot had a range of fruit weight, fruit width, fruit length, flesh thickness and peel thickness with a range of 1113.40-1147.80 grams, 127.4-132.01 mm, 126.75-132.26 mm, 107.93-112.10 mm and 8.08-9.22 mm respectively and showed non-significantly different among treatment (table 2).

**Table 1.** Effect of growing media on yield components of melon grown in plastic house.

growing media	fruit weight(g)	width of fruit(mm)	length of fruit(mm)	thickness of flesh(mm)	thickness of peel(mm)
С	956.60c	124.96b	119.84b	103.06b	8.02
CD:C:M (2:1:1)	1123.10b	132.54a	133.15a	113.49a	9.10
RH:C:M (2:1:1)	1184.70ab	131.01ab	132.97a	111.24a	8.28
RTL:C:M (2:1:1)	1252.50a	133.54a	130.61a	113.52a	9.06
F-test	**	*	**	**	ns
C.V. (%)	10.64	5.74	7.38	7.35	23.57

<sup>\*\* =</sup> Significant difference at 0.01 level, \* = Significant difference at 0.05 level, ns = Non-significant difference, C = Coir, CD = Coir Dust, RH = Rice Husk, RTL = Rain Tree Leaves, M = Manure.

**Table 2.** Effect of 20-20-20 fertilizetion rate on yield components of melon grown in plastic house.

fertilization rate (g)	fruit weight(g)	width of fruit(mm)	length of fruit(mm)	thickness of flesh(mm)	thickness of peel(mm)
20-20-20 3g/pot/3 days	1124.00	127.40	126.75	107.93	8.08
20-20-20 5g/pot/3 days	1147.80	131.62	130.35	112.10	8.64

20-20-20 7g/pot/3 days	1131.70	132.01	127.21	109.91	9.22
20-20-20 9g/pot/3 days	1113.40	131.02	132.26	111.37	8.51
F-test	ns	ns	ns	ns	ns
C.V. (%)	10.64	5.74	7.38	7.35	23.57

<sup>\*\* =</sup> Significant difference at 0.01 level, \* = Significant difference at 0.05 level, ns = Non-significant difference, C = Coir, CD = Coir Dust, RH = Rice Husk, RTL = Rain Tree Leaves, M = Manure.

The interaction between growing media and 20-20-20 fertilization rate effected on fruit weight this study found that melon grown in RTL:C:M x 20-20-20 7 grams per pot per 3 days had the highest fruit weight with the mean of 1345.90 grams per pot per 3 days while the lowest fruit weight got from melon grown in C x 20-20-20 9 grams with the mean of 779.70 grams and showed significantly different among treatment. The fruit width, fruit length, flesh and peel thickness had no influence from growing media and the 20-20-20 fertilization rate which showed non-significantly different (table 3).

The melon grown in RTL:C:M x 20-20-20 7 grams per pot per 3 days had the most fresh weight with the mean of 1345.90 grams and give the most flesh thickness with the mean of 118.32 mm. while the second best received from those grown in RTL:C:M x 20-20-20 9 grams per pot per 3 days with the mean of 1336.80 and 117.62 mm. respectively (table 3). The lowest fruit weight and peel and pulp thickness got from those grown in C x 20-20-20 9 grams per pot per 3 days with the mean of 779.70 grams, 6.09 mm. and 93.81 mm. respectively and showed significantly different (table 3).

#### Fruit quality.

This study found that melon grown in RTL:C:M (2:1:1) and fruits were harvested on 52 days after fruit setting gave the most flesh firmness with the mean of 38.12 newton while the lowest flesh firmness got from those grown in C with the mean of 28.86 newton and showed significantly different (table 4). Total soluble solid (TSS) the highest TSS content of fruit juice received from those grown in RTL:C:M (2:1:1) with the mean of 12.27 °brix therefore the lowest TSS got from those grown in C with the mean of 10.29 °brix and showed significantly different. Regarding to growing media only the result of titratable acidity, juice pH the statistical analysis showed non-significantly different. Table 4 also indicated that there is non-significantly different and showed the mean of palatability score 19.06-21.38 score with well accepted from consumer.

**Table 3.** Effect of interaction of growing media and 20-20-20 fertilization rate on yield components of melon grown in plastic house.

treatment	fruit weight(g)	width of fruit(mm)	length of fruit(mm)	thickness of flesh(mm)	thickness of peel(mm)
C x 20-20-20 3g/pot/3 days	942.40cd	122.82	122.08	103.33ab	7.70
C x 20-20-20 5g/pot/3 days	996.20cd	127.78	121.91	106.42ab	9.57
C x 20-20-20 7g/pot/3 days	1108.30abc	132.71	124.96	108.68ab	8.70
C x 20-20-20 9g/pot/3 days	779.70d	116.52	110.39	93.81b	6.09
CD:C:M x 20-20-20 3g/pot/3 days	1204.10abc	132.64	130.18	113.46ab	8.77
CD:C:M x 20-20-20 5g/pot/3 days	1114.30abc	132.59	133.90	116.11a	8.02
CD:C:M x 20-20-20 7g/pot/3 days	1011.50bcd	131.21	129.64	107.69ab	10.22
CD:C:M x 20-20-20 9g/pot/3 days	1162.40abc	133.72	138.89	116.72a	9.39
RH:C:M x 20-20-20 3g/pot/3 days	1195.00abc	126.42	128.25	108.27ab	8.43
RH:C:M x 20-20-20 5g/pot/3 days	1307.70ab	133.89	136.61	114.46ab	7.69
RH:C:M x 20-20-20 7g/pot/3 days	1061.30abcd	129.54	126.66	104.94ab	8.18
RH:C:M x 20-20-20 9g/pot/3 days	1174.80abc	134.24	140.34	117.27a	8.83
RTL:C:M x 20-20-20 3g/pot/3 days	1154.50abc	127.74	126.48	106.66ab	7.44
RTL:C:M x 20-20-20 5g/pot/3 days	1172.80abc	132.21	128.97	111.40ab	9.29
RTL:C:M x 20-20-20 7g/pot/3 days	1345.90a	134.61	127.59	118.32a	9.78
RTL:C:M x 20-20-20 9g/pot/3 days	1336.80a	139.60	139.40	117.67a	9.74
F-test	**	ns	ns	*	ns
C.V. (%)	10.64	5.74	7.38	7.35	23.57

<sup>\*\* =</sup> Significant difference at 0.01 level, \* = Significant difference at 0.05 level, ns = Non-significant difference, C = Coir, CD = Coir Dust, RH = Rice Husk, RTL = Rain Tree Leaves, M = Manure.

**Table 4.** Effect of growing media on fruit quality of melon grown in plastic house.

growing media	flesh firmness (N)	total soluble solid(°brix)	titratable acidity (%)	pH juice	palatability test (score)
С	28.86b	10.29c	1.70	6.53	20.16
CD:C:M (2:1:1)	34.91ab	10.88bc	1.64	6.61	21.38
RH:C:M (2:1:1)	34.59ab	11.44b	1.88	6.64	19.06
RTL:C:M (2:1:1)	38.12a	12.27a	2.06	6.61	19.44
F-test	*	**	ns	ns	ns
C.V. (%)	26.26	6.36	26.95	2.53	17.16

<sup>\*\* =</sup> Significant difference at 0.01 level, \* = Significant difference at 0.05 level, ns = Non-significant difference, C = Coir, CD = Coir Dust, RH = Rice Husk, RTL = Rain Tree Leaves, M = Manure.

Regarding to effect of 20-20-20 fertilization rate on flesh firmness, total soluble content (TSS), titratable acidity, pH juice, and palatability score, a side from table 5. showed that TSS content and palatability score pronounce effected. The highest of TSS content and highest palatability gave a mean of 11.91 °brix and 22.50 score respectively, therefore the highest TSS got from the melon applied with 20-20-20 5 grams per pot while the palatability received from those melon applied with 20-20-20 3 grams per pot and showed non-significantly different, flesh firmness, titratable acidity and pH juice give a range of 30.57-37.62 newton, 1.76-1.90 percent and 6.54-6.69 respectively and showed non-significantly different (table 5).

Table 5. Effect of 20-20-20 fertilizer rate on fruit quality of melon grown in plastic house.

fertilization rate (g)	flesh firmness (N)	total soluble solid (°brix)	titratable acidity (%)	pH juice	palatability test (score)
20-20-20 3g/pot/3 days	36.51	10.86b	1.78	6.69	22.50a
20-20-20 5g/pot/3 days	37.62	11.91a	1.90	6.54	19.41ab
20-20-20 7g/pot/3 days	30.57	11.26ab	1.76	6.58	18.94b
20-20-20 9g/pot/3 days	31.78	10.84b	1.84	6.58	19.19b
F-test	ns	**	ns	ns	*
C.V. (%)	26.26	6.36	26.95	2.53	17.16

<sup>\*\* =</sup> Significant difference at 0.01 level, \* = Significant difference at 0.05 level, ns = Non-significant difference, C = Coir, CD = Coir Dust, RH = Rice Husk, RTL = Rain Tree Leaves, M = Manure.

The interaction between growing media and 20-20-20 fertilization rate effected on flesh firmness, total soluble solid, titratable acidity, pH juice palatability. Table 6 indicated that flesh firmness, titratable acidity and palatability had a range of 20.17-41.40 newton, 1.36-2.48 persent, and 15.38-27.00 score and well accepted with non-significantly differance and it also showed that TSS content of flesh juice showed significantly different. The highest TSS content had a mean of 12.78 °brix which received from melon those grown in CD:C:Mx 20-20-20 9 grams per pot while the lowest TSS got from the melon grown in C x 20-20-20 9 grams per pot with the mean of 8.68 °brix and showed significantly different therefore the highest and lowest pH juice got from the melon grown in RTL:C:Mx 20-20-20 9 grams per pot and CD:C:Mx 20-20-20 9 grams per pot with the mean of 6.68 and 6.31 respectively and showed significantly different among treatment. This study also indicated that flesh firmness titratable acidity and palatability score showed non-significantly different but well accepted palatability and showed significantly different.



Figure 2. Internal quality of melon effected by various growing media and 20-20-20 fertilization rate.

**Table 6.** Effect of interaction of growing media and 20-20-20 fertilization rate on yield components of melon grown in plastic house.

treatment	flesh firmness(N)	total soluble solid(°brix)	titratable acidity (%)	pH juice	palatability test (score)
C x 20-20-20 3g/pot/3 days	34.01	10.43cd	2.08	6.52ab	27.00
C x 20-20-20 5g/pot/3 days	38.44	10.45cd	1.68	6.71ab	17.63
C x 20-20-20 7g/pot/3 days	32.18	10.93bc	1.52	6.76a	19.13
C x 20-20-20 9g/pot/3 days	41.40	11.63abc	1.52	6.76a	21.75
CD:C:M x 20-20-20 3g/pot/3 days	33.04	11.50abc	1.60	6.66ab	22.25
CD:C:M x 20-20-20 5g/pot/3 days	35.89	11.70abc	1.68	6.56ab	19.88
CD:C:M x 20-20-20 7g/pot/3 days	40.33	11.68abc	1.36	6.64ab	19.00
CD:C:M x 20-20-20 9g/pot/3 days	41.24	12.78a	1.92	6.31b	19.50
RH:C:M x 20-20-20 3g/pot/3 days	28.20	10.55c	1.76	6.42ab	20.00
RH:C:M x 20-20-20 5g/pot/3 days	31.61	10.73c	1.76	6.61ab	21.63
RH:C:M x 20-20-20 7g/pot/3 days	32.15	11.20abc	2.24	6.61ab	19.25
RH:C:M x 20-20-20 9g/pot/3 days	30.30	11.95abc	1.76	6.67ab	15.38
RTL:C:M x 20-20-20 3g/pot/3 days	20.17	8.68d	1.68	6.54ab	20.75
RTL:C:M x 20-20-20 5g/pot/3 days	33.69	10.63c	2.48	6.55ab	18.50
RTL:C:M x 20-20-20 7g/pot/3 days	33.70	12.58ab	1.92	6.58ab	18.38
RTL:C:M x 20-20-20 9g/pot/3 days	39.56	12.10abc	2.16	6.68ab	20.13
F-test	ns	**	ns	*	ns
C.V. (%)	10.64	5.74	7.38	7.35	23.57

<sup>\*\* =</sup> Significant difference at 0.01 level, \* = Significant difference at 0.05 level, ns = Non-significant difference, C = Coir, CD = Coir Dust, RH = Rice Husk, RTL = Rain Tree Leaves, M = Manure.

### Discussion

This experiment indicated that growing media pronounced effect on fruit weight of melon this result mean that melon grown in RTL: C: M (2:1:1) showed highest fruit weight may due to the RTL had the nutrients more than other media supported by the study of EL-Desuki *et al.*, (2000) who study about the organic and mineral fertilization on growth, yield and quality of cantaloupe (*Cucumis melo* L.) Ferrante *et al.*, (2008) too.

It also showed that total soluble solid (TSS) content of those melon grown in RTL:C:M  $\times$  20-20-20 9 grams per pot had the TSS higher than the other growing media may be due to its had more N P K and interaction of RTL:NPK also this result supported by the stated of Mengel and Kirkby (1978), Shafeek *et al.*,(2015) who stated that the attribution of increasing in NPK availability and their uptake to obtained the goal.

Aside from these data suggested that melon should grown in media that mixed with optimized RTL or other organic growing media plus NPK fertilizer in order to cope up maximum quality.

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**Abstract** Chaetomium spp. isolate CNC2 isolated from forestry soil in China proved to be antagonized Alternaria alternata causing leaf blight of Kales in bi-culture antagonistic evaluation. The crude metabolites from Chaetomium sp CNC2 were extracted by hexane, ethyl acetate and methanol that expressed antifungal activity against A. alternata.

Keywords: biocontrol, antagonist, Chaetomium, kales, leaf blight

#### Introduction

Species of the genus *Brassica* are important vegetable crops grown in South east Asia, including Thailand, but their production is often limited by diseases (Tsou and Tsay, 1988). Diseases caused by the fungal genus *Alternaria* are among the most serious, and they have been reported from many countries including Australia (Sivapalan and Browning, 1992) and Taiwan (Wu, 1979) Spread of the disease during the growing season is by wind-blown or rainsplashed spores. Optimum conditions for sporulation are temperatures of 20 –30°C and a minimum wet period of 13 h, epidemics occurring when rainfall is frequent (Humpherson-Jones and Phelps, 1989; Fontem *et al.*, 1991). These conditions correspond to the climate in some tropical countries such as Thailand, where the average annual temperature is 26–28°C and there is daily rainfall during the growing season for vegetables. Nevertheless, the disease appeared to cause considerable damage to the crop in the country (Visethsung and Saranak, 1988). This experiment uses by *chaetomium* isolate CNC2 to biocontrol methods to inhibit the growth of fungal pathogens.

#### Materials and methods

#### Isolation and identification

Kale pathogens were isolated CAN-1 from leaf using moist chamber at room temperature, the signs of pathogens were transferred to water agar (WA) and then subcultured to potato dextrose agar (PDA) until get pure culture. The most frequency found pathogen would then prove for pathogenicity test followed the Koch's postulate method.

#### Pathogenicity test

The kale leaves were detached from healthy plant, and then surface sterilized with 70% ethyl alcohol and placed with the upper leaf surface in a sterile petri dish containing filter paper moist with distilled water to maintain high humidity. Wounding by sterile needle on the leaves for easy access of the fungus, then leaves were inoculated with mycelium discs of *Alternaria* spp. isolate CAN-1 on the wound. Non-inoculated controls were inoculated with an agar plug without the fungus. The petri dishes were incubated at room temperature for 7 days. Four replications of each treatment were used in the experiment. The result showed that isolate could infected in the leaves of kale and caused symptom. Disease severity (DS) scored as level 0 was no symptoms, level 1 was 1-10% leaf blight level 2 was 11-25 % leaf blight, level 3 was 26-50 % leaf blight, level 4 was 51-75 % leaf blight and level 5 was over 75 % leaf blight. (Wheeler, 1969).

#### Bi-culture test

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A mycelial disc of *Alternaria* spp. (5 mm diameter) was placed singly (as controls) or oppositely to a mycelial disc of each above antagonist on 9-cm-diameter Petri dishes, which contained PDA. After incubation at 25°C for 15 days, data were collected as colony diameter and number of sporangia produced by *Alternaria* spp. in both bi-culture and control plates. Numbers of sporangia were counted by using haemacytometer. Data were computed in a form of inhibition percentage of mycelial growth and sporangial production of the pathogen by using the formula below:

% Inhibition = 
$$100 \times (A - B)/A$$

, where A = colony diameter or numbers of sporangia of *Alternaria spp.* in control plates; B = colony diameter or numbers of sporangia of *Alternaria spp.* in bi-culture plates. Finally, variance and the treatment means were analyzed and compared by using Duncan's multiple range tests at 0.05.

### In vitro effects of antagonistic crude extracts on the growth of Alternaria spp.

The antagonistic substances were extracted from *Chaetomium* spp isolate CNC2 as crude extracts. These antagonistic substances were tested for their abilities to inhibit the growth of *Alternaria* spp. The crude extraction from antagonistic fungi was performed using the method of Kanokmedhakul *et al.*, (2006). *Chaetomium* spp. was separately cultured in PDB at room temperature (28-30°C) for 30 days. Fungal biomass were removed from PDB, filtered through cheesecloth and air-dried overnight. Fresh weight and dry weight of fungal biomass were weighed. Dried fungal biomass were ground with electrical blender, extracted with 200 ml hexane (H) and shaken for 24 hour at room temperature. The ground biomass was separated by filtration through Whatman No. 4 filter paper. The marc was extracted again with hexane using method described above. The filtrates were evaporated in vacuo to yield crude extract. The marc was further extracted with ethyl acetate (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. CNC2 cultured in PDB for 30 days. It exposed in air dry room tempearature 27-30°C for 5 days. Fungal biomass was the soaked in hexane for 3 daysfiltered to separate marc, the filtrate was performed in rotary vacuum evaporator yielded crude Hexane. The marc was then soaked in ethyl acetate for 3 days and filtered to get filtrate and passed through rotary vacuum evaporator yielded crude methanol.

#### Results

#### Isolation, Identification and Pathogenicity test of Alternaria spp.

CAN-1 were isolated and identified was confirmed is *Alternaria alternata* from leaf blight of kale with obvious symptom and get pure culture. The isolate CAN-1 was confirmed pathogenic isolate from pathogenicity test. The result showed that isolate CAN-1 could infected in the leaf of kale as seen in Table 1.

**Table 1**: Pathogenicity test on kale leaf

Treatmants	DS
Control	1
Alternaria alternata	3

Disease severity (DS) scored as level 0 was no symptoms, level 1 was 1-10% leaf blight level 2 was 11-25 % leaf blight, level 3 was 26-50 % leaf blight, level 4 was 51-75 % leaf blight and level 5 was over 75 % leaf blight.

#### Bi-culture antagonistic tests

Chaetomium spp. isolate CNC-2 was proved its abilities to inhibit the growth of A. alternata by using bi-culture tests. The result showed that CNC-2 gave significantly colony inhibition of A. alternata when compared to the control.

The number of spores that producing by the pathogen *A. alternate* was counted by using Hemacytometer. The results showed that CNC-2 significantly inhibited number of pathogen spores of 55% when compared to the control.

# Bioactivity test of crude extracts from Chaetomium spp. isolate CNC-2 against Alternaria spp. isolate CAN-1 causing laft blast of kales.

The crude extracts in CNC2 from hexane at concentrations of 10, 50, 100, 500, 1,000 ppm were tested inhibition of sporangia information of A. alternata which were 58.57 and 61.61 % in concentrations of 500 and 1000 ppm respectively (Table 2) (when compared to the control.) EtAOc at concentrations of 10, 50, 100, 500, 1,000 ppm were tested inhibition of sporangia information of A. alternata which were 58.73, 61.22, 75.15, 83.62 and 95.94 % in concentrations of 10, 50, 100, 500 and 1,000 ppm respectively Table 2 (when compared to the control.) MeOH at concentrations of 10, 50, 100, 500, 1,000 ppm were tested inhibition of sporangia information of A. alternata which were 56.39 and 67.30 % in concertrations of 500 and 1,000 ppm respectively Table 2 (when compared to the control.) Meanwhile ED50 values of CNC2-H, CNC2-Et, CNC2-M were 308.91, 7.92 and 272.85  $\mu$ g/ml respectively (Table 2).

**Table 2 :** Effect of erude extracts from CNC2 to inhibit *A. alternate* 

Crude extracts	Concentration (ppm)	Colony diameter (cm)	Growth inhibition (%)	Number of sporangium (×10 <sup>6</sup> )	Sporangium inhibition (%)	ED <sub>50</sub> (μg/ml)
Hexane	0	5.00°	O <sup>a</sup>	48.150 <sup>a</sup>	O <sup>a</sup>	
	10	4.60 <sup>b</sup>	8.00 <sup>l</sup>	44.325 <sup>ab</sup>	7.84 <sup>m</sup>	
	50	4.42 <sup>cd</sup>	11.50 <sup>j</sup>	43.225 <sup>ab</sup>	10.13 <sup>1</sup>	
	100	4.27 <sup>f</sup>	14.50 <sup>g</sup>	27.425 <sup>c</sup>	42.98 <sup>h</sup>	308.91
	500	4.25 <sup>fg</sup>	15.00 <sup>g</sup>	19.925 <sup>d</sup>	58.57 <sup>f</sup>	
	1000	4.12 <sup>h</sup>	17.50 <sup>e</sup>	15.575 <sup>de</sup>	67.61 <sup>d</sup>	
EtAOc	0	5.00°	O <sup>a</sup>	48.150 <sup>a</sup>	O <sup>a</sup>	
	10	4.37 <sup>de</sup>	12.50 <sup>i</sup>	19.85 <sup>d</sup>	58.73 <sup>f</sup>	
	50	4.17 <sup>gh</sup>	16.50 <sup>f</sup>	18.65 <sup>d</sup>	61.22 <sup>e</sup>	
	100	3.92 <sup>i</sup>	21.50 <sup>d</sup>	11.95 <sup>ef</sup>	75.15 <sup>c</sup>	7.92
	500	3.82 <sup>j</sup>	23.50 <sup>b</sup>	7.875 <sup>f</sup>	83.62 <sup>b</sup>	
	1000	3.57 <sup>k</sup>	28.50 <sup>ab</sup>	1.95 <sup>g</sup>	95.94 <sup>ab</sup>	
MeOH	0	5.00ª	O <sup>a</sup>	48.150 <sup>a</sup>	O <sup>a</sup>	
	10	4.95 <sup>ab</sup>	1.00 <sup>m</sup>	37.575 <sup>b</sup>	21.88 <sup>k</sup>	
	50	4.50°	$10.00^{k}$	35.975 <sup>b</sup>	25.20 <sup>j</sup>	
	100	4.30 <sup>ef</sup>	14.00 <sup>h</sup>	29.85 <sup>c</sup>	37.94 <sup>i</sup>	272.85
	500	4.10 <sup>h</sup>	18.00 <sup>e</sup>	20.975 <sup>d</sup>	56.39 <sup>g</sup>	
	1000	3.87 <sup>ij</sup>	22.50 <sup>c</sup>	15.725 <sup>de</sup>	67.30 <sup>d</sup>	
C.V.(%)		1.61		14.01		

Rage of four replications. Means followed by a common letter are not significantly different by DMRT at P = 0.05

### Discussion

The results, Chaetomium isolate CNC-2 is proved to act as biological activity against Alternaria spp. causing leaf blight of kales from crude extracts of CNC-2. The result showed that crude extracts from EtAOc, able to inhibit sporerangium the most and still have a low  $ED_{50}$  until satisfactory. Was successful to control Alternaria spp. It is useful resource as nature product to inhibit the pathogen which causing leaf blight of kales. It is not only reduced the production loss of growers but also reduced to pollute the environment as compared the traditional method. Previous research indicated that crude extracts of other species of Chaetomium spp. are also reported to believe antagonize to many plant pathogens. Sibounnavong et al. (2011). reported that Chaetomium lucknowense showed greater antifungal activity against F. oxysporum f. sp. lycopersici NKSCO2. The crude extract of endophyte Chaetomium

globosum No.04 which isolated from the medicinal plant Ginkgo biloba showed significant growth inhibitory activity against the phytopathogenic fungi *Rhizopus stolonifer* and *Coniothyrium diplodiella*. (Guizhen Zhang *et al.* 2013). Charoenporn *et al.* (2010). reported that oil bio-agent formulation from the antagonistic fungi of *Chaetomium globosum* and *Ch. lucknowense* showed their biological ability to control tomato wilt. The efficacy of *Chaetomium globosum* as a biocontrol agent against the late blight pathogen *Phytophthora infestans* was evaluated in potato plants by Shanthiyaa *et al.* (2013). Among eight *Chaetomium* isolates evaluated *C. globosum* isolate Cg-6 showed greater inhibition to mycelial growth of *P. infestans* in vitro. Phung et al. (2015). examined the in vitro and in vivo effects of *Chaetomium globosum, Chaetomium lucknowense, Chaetomium cupreum* and their crude extracts as biological control agents in controlling *Phytophthora nicotianae* causing root rot in citrus, and the result showed that *Chaetomium* species and their crude extracts strongly inhibited the growth of *Phytophthora. nicotianae* KA1.

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# Chanchum, W., Yasatae, R., Glahan, S. and Kramchote, S.\*

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**Abstract** The result found that melon increasing in height according to increasing of planting date. On harvesting day therefore significantly difference among treatment in TSS and TA content of juice. The most TSS content of juice extract with the mean of 16.33 °brix received from those melon fruit treated with KCL 5 gram + 50 day harvested and showed significantly difference among treatmant. The second and the lowest TSS content got from those treated with 36day+KCL 3g/pot 50days and 45 days harverted with the mean of 16.20 and 15.07 °brix respectipely. The fruit weight had a range 2293.30 to 2405.30 gram while there is less impact on the fruit volume, fruit circumstance flesh and peel thickness. The harvesting date 50 days shown more pronounce effect on TSS content of fruit juice than 45 days harvested on the other hand harvesting date also had influence of L \*and a \*on flesh melon grown in plastic house.

**Keywords:** melon, harvesting, potassium chloride, plastic house

#### Introduction

Food quality becomes mainly research approach. Melon (*Cucumis melo* L.) is rich in minerals, carbohydrates, vitamins and dietary fiber (IPGRI, 2003). The market acceptability are sweetness and palatability of melon that is needed for the customer especially, Thai people and Asian cluster (Lignou *et al.*, 2014). The proper mineral nutrition of plants is importance to improve yield components and quality in melon production. Several studies reported the importance of NPK application to melon plants. NPK application to melon plants increase their growth (EI-Deruki *et al.*, 2000) and improved yield and fruit quality such as fruit size, fruit weight and flesh thickness etc. Under plastic house condition, the melon plants applied with  $100 \text{kg N} + 64 \text{kgP2O5} + 64 \text{kg K}_2\text{O}$  showed the highest increasing in plant height, fruit weight size and quality (Mitchelle *et al.*, 2012; Castellano *et.al*, 2008) but TSS and moisture contents were not affected (Shafeek *et al.*, 2015). Mengel and Kirkby (1978) stated that the attribution of increasing in NPK availability and their uptake to obtain the goal with similarly to the studies of Ferrante *et al.*, (2008); Wang and Sun, (2008). The objectives this study to determine harvesting day and rates of photassium chloride to sweetness of melon.

# Materials and methods

A completely randomized design (CRD) with 6 treatments and 3 replications. Treatments were as follows: 1) 45 day before harvest + 15-15-15 at 9 g per pot per week, 2) 50 day harvested + 15-15-15 9 grams per pot per week, 3) 30 day + 15-15-15 9 grams per pot per week + KCL 3 g per pot per day 45 days harvested, 4) 36 day + 15-15-15 9 grams per pot per week + KCL 3 g per pot per day 50 days harvested, 5) 30 day + 15-15-15 9 g per pot per week + KCL 5 g per pot per day 45 days harvested and 6) 36 day + 15-15-15 9 g per pot per week + KCL 5 grams per pot per day 50 days harvested.

The 7 day of melon #20 orange flesh seedling was transplanted in white plastic bag 14 inch in diameter, in container filled up with soil + manure and mixed well. After one week all treatments were treated as treatment stated above.

The melon plants flowers on 20-27 days from transplanted. Hand pollination was done in early moring and one fruit was maintained per plant.

Data were collected as Fruit quality and quantity measurement. Plant growth was monitored weekly in term of height increment and leaf chlorophyll content. Plant height was determined using a measuring tape starting on the

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second weeks after transplanting up to two weeks brfore harvest. Chlorophyll content was measured using SPAD-502 chlorophyll meter (Minolta Camera Co., Japan) the leaf measured was leaf no.9 (about four weeks after transplanted up to four weeks before harvest).

On 50 days after fruit setting melon fruits were harvested and then the data below were recorded. Fresh weight (g), fruit volume (ml), fruit circumstances (cm), flesh thickness (mm), peel thickness (mm), fruit firmness (newton), TSS content (°brix), TA content (%) and L\* a\* and b\*.

#### Results

Wine height; according to this study melon wine increased in height corresponding to time increased. The increment more rapidly during 14 days to 35 days after planting or before fruit setting and then wine growing slow down up to fruit harvested. The height of wine showed non significantly difference the mean of wire height about 185.70 to 190.00 cm.

After fruit setting, fruit growth habitat raise rapidly up to these weeks on around 45 days often gradually deceased after transplanting then enlargement of fruit slow down according to increment of days after fruit setting; Fruit fresh weight and fruit volume, fruit circumstances, flesh and peel thickness, A side from table 1. indicated that all of these characteristics were not significantly difference, therefore such as fruit weight flesh and peel thickness were well accepted. The fruit fresh weight had a range of 2293.30 g. to 2405.30 g. while the flesh and peel thickness had a range of 26.11 mm. to 27.71 mm. and 7.69 mm. to 8.18 mm. respectively. This experiment also trend to show that more mature get more flesh and peel thickness and more fruit weight also.

Total soluble solid content of flesh juice (TSS) titratable acidity and fruit firmness are the importance characteristics of melon effected on the consumer buying decission. According to table 2 it indicated that TSS from those fruits applied with KCL and harvested on 50 days after fruit setting gave more TSS content and lesser fruit firmness. The highest TSS content got from melon flesh juice applied with KCL 5 g per pot per day and harvested on 50 days after fruit setting with the mean of 16.33 °brix while the lowest TSS content received from those applied with 15-15-15 9 g per week per pot and harvested on 45 days after fruit setting with the mean of 15.07 °brix and showed significantly difference. The titratable acidity of melon flesh juice had a range of 0.19 to 0.25 percent and showed non significantly difference.

**Table 1.** Shown fresh weight (g), fruit volume (ml.), fruit circumstance (cm.), flesh thickness (mm.) and peel thickness (mm.) melon grown in plastic house.

,e.e g. e p.ae	fresh	fruit	fruit	flesh	peel
					•
treatment	weight	volume	circumstanc	thickness	thickness
	(g)	(ml.)	e (cm.)	(mm.)	(mm.)
15-15-15 9g/pot/week + 45day	2293.30a	2273.30a	53.67a	26.11a	7.69a
15-15-15 9g/pot/week + 50day	2310.00a	2292.00a	54.33a	26.54a	7.93a
15-15-15 9g/pot/week + 30day + KCl 3g + 45day	2315.00a	2339.00a	55.33a	26.97a	7.95a
15-15-15 9g/pot/week + 36day + KCl 3g + 50day	2363.30a	2399.70a	56.00a	27.62a	8.15a
15-15-15 9g/pot/week + 30day + KCl 5g + 45day	2386.70a	2386.70a	55.67a	27.18a	8.02a
15-15-15 9g/pot/week + 36day + KCl 5g + 50day	2405.30a	2407.00a	56.33a	27.71a	8.18a
F-test	ns	ns	ns	ns	ns
C.V. (%)	5.93	6.63	3.67	8.75	5.45

**Note** ns =non significant difference

Palatability score; on harvesting date the firmness of fruit these harvested on 45 days after fruit setting showed more harder than those fruits harvested on 50 days (table 2). Palatability of flesh melon increased according to the increasing of days after fruit setting as persent in table 3. otherwise the flesh firmness decreased while the palatability increased as same as TSS of flesh juice increased due to the loose of calcium protate become soluble form. This table 3 also presents that melon fruit harvested on 45 days and 50 days were accepted in palatability and aroma also.

Table 2. Shown fruit firmness (newton), TSS content (°brix) and TA content (%) melon grown in plastic house.

treatment	fruit firmness (newton)	TSS content (°brix)	TA content (%)	
15-15-15 9g/pot/week + 45day	44.02a	15.07c	0.20b	
15-15-15 9g/pot/week + 50day	41.42a	15.33b	0.19b	
15-15-15 9g/pot/week + 30day + KCl 3g + 45day	42.22a	15.13c	0.22ab	
15-15-15 9g/pot/week + 36day + KCl 3g + 50day	41.17a	16.20a	0.22ab	
15-15-15 9g/pot/week + 30day + KCl 5g + 45day	41.97a	15.73ab	0.24a	
15-15-15 9g/pot/week + 36day + KCl 5g + 50day	41.12a	16.33a	0.25a	
F-test	ns	*	*	
C.V. (%)	10.65	2.8	9.93	

Note

ns = non significant difference statistically significant

**Table 3.** Shown L\*, a\* b\* and palatability score of flesh melon grown in plastic house.

treatment	I* of flesh	a* of flesh	b* of flesh	palatability	
	L OFFICSIT	a Officsii	b of ficsif	score	
15-15-15 9g/pot/week +	64.34a	11.92a	50.53a	4.00a	
45day	01.514	11.324	30.33d	1.000	
15-15-15 9g/pot/week +	64.74a	12.33a	25.62a	5.00a	
50day	04.744	12.55d	23.02u	3.00a	
15-15-15 9g/pot/week +	64.62a	12.38a	25.76a	4.50a	
30day + KCl 3g + 45day	04.02a	12.50a	23.70a	4.500	
15-15-15 9g/pot/week +	64.90a	12.44a	26.80a	5.00a	
36day + KCl 3g + 50day	04.30a	12.44a	20.80a	3.00a	
15-15-15 9g/pot/week +	64.72a	12.40a	26.28a	4.00a	
30day + KCl 5g + 45day	04.72a	12.40a	20.20a	4.00a	
15-15-15 9g/pot/week +	65.12a	12.60a	16.97a	5.00a	
36day + KCl 5g + 50day	03.12a	12.00a	10.97a	3.00d	
F-test	ns	ns	ns	ns	
C.V. (%)	2.85	8.01	6.33	0.00	

Note

ns =non significant difference at 0.05 level.

According to figure 1. and table 3. indicated that the internal and physical appearance of melon fruit harvested on difference date and various KCL fertilization were well accepted include L\* a\* and b\* of flesh and palatability score.

<sup>\* =</sup> significant difference at 0.05 level.

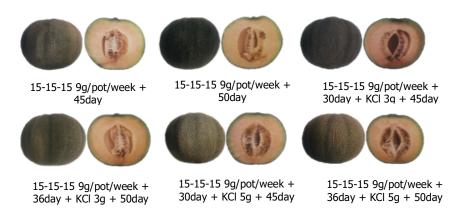


Figure 1. The internal and physical appearance of melon grown in plastic house.

#### Discussion

Melon fruits those supplementary with KCL showed more TSS content than the other treated with 15-15-15 only because of KCL will enhance the activity of enzymes those invert the media to sugar this finding similary to the study of Wang *et al.* (1996). Further more harvest maturity pronounced effect on TSS of melon juice more maturity give more TSS content. Those results similary to the study of Fellman *et al.* (2003) and the report of Song and Bangerth (1996) who study on Golden Delieious apple. The cultivation of melon should applied with KCL and harvest more maturity in order to receive more quality.

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#### Effect of β-mannanase on nutrient composition of palm kernel meal mixed with yeast cake

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**Abstract** The results of present study showed that % crude fiber were decreased when treated yeast-premixed palm kernel meal with β-mannanases in time- and concentration-dependent manner. Supplementation of β-mannanases at 0.02 and 0.04% significantly reduced the crude fiber content in 12 hours of incubation (p<0.05). The best improved palm kernel meal was obtained when 40% of yeast cake was mixed with palm kernel meal and 0.04% enzymes was added for 24 hours of incuabtion which contained % crude fiber equal to 4.84±0.85 and % crude protein equal to  $40.86\pm1.87$ . In conclusion, improvement of palm kernel meal using enzymes and yeast cake increased % crude protein and reduced % crude fiber which could be further use as diets for non-ruminants.

**Keywords:** Palm kernel meal, β-mannanase, Feed improvement.

#### Introduction

Improving the quality of an agro-by-products as a promising feed ingredient could help to sheer the competition for food between human and livestock species and maximize the value of those available by-products. Palm kernel meal (PKM) is a locally available agro-industrial by-product and relatively inexpensive feedstuff in many tropical countries (Perez *et al.*, 2000), including Thailand.

PKM contains a moderate level of crude protein (approximately 15 - 20%) but poor amino acid profile (deficient in lysine, methionine, and tryptophan) with a high level of fiber (approximately 13 to 20%) (Alimon, 2004; Abdollahi *et al.*, 2015). Thus, it is considered as a moderate quality feed ingredient for ruminant but not suitable for monogastric animals (Alimon, 2004). Since non-ruminant animals such as poultry and swine have simple stomachs which limit in the digestion of fiber, the incorporation of the improper amount of PKM in non-ruminant diets is reported to be associated with impaired performance parameters (Sundu *et al.*, 2006; Mardhati *et al.*, 2011).

PKM contains  $\beta$ -mannan as the main fiber components, making up between 30 to 35% on dry matter basis of PKM (Sundu *et al.*, 2006). It had been reported that PKM contained  $\beta$ -mannan content of up to 300 to 350 g/kg (Jackson, 2010).  $\beta$ -mannan is mostly consisted of water-insoluble glucomannan and a small amount of water-soluble galactomannan thus making it hard and water-insoluble (Knudsen, 1997; Sundu *et al.*, 2006). Several studies have shown that supplementation of exogenous fibrolytic enzymes on diets containing PKM could improve its nutritive values by a great potential in releasing unavailable nutrients and energy (Sekoni *et al.*, 2008; Chong *et al.*, 2008; Natsir *et al.*, 2018). Since the main fiber of PKM is  $\beta$ -mannan, the major enzymes used to improve the nutritive value of PKM are mannanases, together with other fibrolytic enzymes including  $\alpha$ -galactosidase and cellulase (Sundu and Dingle, 2003). However, studies investigating the effect of enzyme supplementation on PKM-containing broiler diets reported that fibrolytic hydrolysis to monomeric sugars is not very effective in the gastrointestinal tract of poultry and suggested that pre-treatment of PKM with enzymes before feeding could be an option in improving the nutritive values of PKM (Choct, 2006).

Besides the high fiber content, the moderate crude protein level and poor amino acid profiles also deteriorate the nutritive value of PKM. Single-cell protein (SCP) is the protein extracted from cultivated microbial biomass which can be used for protein supplementation replacing costly conventional protein sources like soymeal and fishmeal to improve protein quality in poor protein diets. Yeast *Saccharomyces cerevisiae* is well-known for its high protein content up to 40 - 50% and good amino acid profile with high essential amino acids especially leucine and lysine (Yamada and Sgarbieri, 2005). Yeast biomass is not only a source of proteins but also an excellent source of B-complex

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vitamins and nucleic acids (Ferreira et al., 2010). S. cerevisiae been accepted as safe as a food for humans and animals. Thus, yeast cake could be an excellent choice of protein source for improving the nutritive values of PKM.

The study aimed to investigate the efficacy of  $\beta$ -mannanase enzyme pre-treatment effect in lowering the crude fiber content of yeast-premixed PKM. We found that supplementation of  $\beta$ -mannanase effectively reduced the crude fiber while increased the soluble sugar content. Therefore, this improvement could be benefit to further application of PKM in non-ruminants.

#### Materials and methods

#### Sample sources

Palm kernel meal was perchesed from a reputable feed mill in Pranburi, Prachuap Khiri Khan Province, Thailand in 2017. The samples were stored in moisture free container until use. Hemicell<sup>TM</sup>-HT (Elanco, USA) and dried *Saccharomyces cerevisiae* yeast cake was provided by Behn Meyer Chemicals Co., Ltd. All reagents and solvents were analytical grade.

#### Preparation of 6-mannanase treated yeast-premixed palm kernel meal

Palm kernel meal were pre-mixed with yeast cake at 3 different ratios including 100:0, 80:20, and 60:40 palm kernel meal: yeast cake (PKM100, PKM80, and PKM60, respectively) in the plastic bag at a total weight of 200 g in each bag. Commercial  $\beta$ -mannanase (Hemicell<sup>TM</sup>-HT) was added at 0.00%, 0.02%, and 0.04% to every ratio of yeast-mixed palm kernel meal. The enzyme was mixed throughly before thightly closed the plastic bag. The  $\beta$ -mannanase treated yeast-premixed palm kernel meal were then incubated at 40°C in the temperature control incubator. Samples with different level of  $\beta$ -mannanase treatment were analysed for proximate composition and soluble sugar content at 0, 12, and 24 h after incubation. Each treatment was done in triplicates.

# Determination of proximate nutrient composition

Proximate composition including dried matter (DM), crude protein content (%CP) and crude fiber content (%CF) of all treatments and test ingredients, palm kernel meal, and yeast cake, were determined according to the method of Association of Official Analytical Chemists [A.O.A.C.] (1990). Each sample were analysed in triplicates.

#### Soluble sugar content analysis

Soluble sugar contents were determined by the 3,5-dinitrosalicylic acid (DNS) reducing sugar assay (Miller, 1959). One gram of each sample was dissolved in 10 ml of distilled water. The samples were shaken throughly for 10 min and centrifuged at 10,000 g for 10 mins. The pellet was discarded and the supernatant was transferred into a new clean tube. Supernatant (0.2 ml) was diluted with 0.8 ml of distilled water then 2.0 ml of DNS solution was added. Samples were boiled for 10 mins prior to the addition of 7.0 ml of distilled water. Absorption at 550 nm was read using microplate reader (SPECTROstar Nano, Germany) and sugar content was calculated using glucose as standard. Each sample was done in triplicates.

#### Statistic analysis

One-way analysis of variance (ANOVA) were calculated using R-program and comparison of means was performed by Tukey's multiple range tests. A value of P<0.05 was considered statistically significant.

### Results

## Nutrient composition of PKM, yeast cake, and yeast-premixed PKM

Prior to mixing, the nutrient compositions of PKM and yeast cake used in this study were analyzed individually. As shown in Table 1, PKM contained high fiber content of up to 30.07% compared to yeast cake which has low in fiber (0.42%). Crude protein analysis results demonstrated the yeast cake has a high amount of protein about 2-fold higher than that of PKM. PKM mixed with yeast cake showed higher protein content. PKM80 and PKM60 contained 26.53 and 32.60% of crude protein, respectively.

Table 1. Chemical	composition	(%DM)	of PKM	and v	veast cake
Table 1. Chemical	COMPOSITION	( /00101	<i>,</i> OI I IXIVI	anu	y Cast Carc

Chemical contents	PKM100	Yeast cake	PKM80	PKM60
Dry matter	94.88	94.40	94.79	94.58
Crude fiber	30.07	0.42	26.31	16.34
Crude protein	19.65	46.69	26.53	32.60

## Crude protein composition of 6-mannanase treated yeast-premixed PKM

PKM was mixed with yeast cake in order to improve its protein composition. Crude protein content (%CP) of yeast-premixed PKM was higher when increasing the ratio of yeast cake (21.40±2.62, 26.53±2.65, and 32.60±7.24 for PKM100, PKM80, PKM60 at 0 h with no enzyme, respectively). As shown in Figure 1, upon incubation time increase, the treatment of PKM100 plus 0.02% enzyme, PKM80 plus 0 and 0.02% enzyme and PKM60 plus 0 and 0.02% enzyme showed significant time-dependent increased of %CP (P<0.05). At 24 h, PKM100 supplemented with 0.02 and 0.04% enzyme showed %CP equal to 28.42±1.05 and 25.48±1.92 which were significantly higher than 0% enzyme (21.53±1.14). The highest amount of %CP was obtained from PKM60 incubated without enzyme for 24 h (45.45±3.91) (Figure 1).

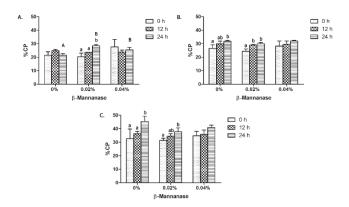


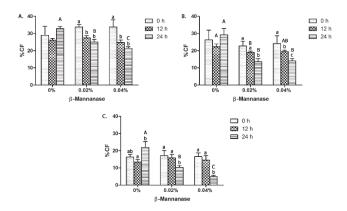
Figure 1. Crude protein composition (%DM) of β-mannanase treated yeast-premixed PKM at the ratio of PKM100 (A), PKM80 (B.) and PKM60 (C). The different small letter above the bar means significant different among incubation times of each enzyme level in each group of yeast-premixed PKM (P<0.05). The different capital letter above the bar means significant different among enzyme levels of the same incubation time in each group of yeast-premixed PKM (P<0.05).

## Crude fiber composition of 6-mannanase treated yeast-premixed PKM

Yeast-premixed PKM of 100:0, 80:20, and 60:40 ratio of palm kernel meal: yeast cake (PKM100, PKM80, and PKM60, respectively) were determined for  $\beta$ -mannanase effect on the reduction of fiber content in time- and dose-

dependent. Three different levels of enzyme including no enzyme (0%), recommended level (0.02%) and high level (0.04%) were applied to the yeast-premixed PKM and incubated in an air-free plastic bag up to 24 h.

Generally, the crude fiber content of the yeast-premixed PKM was lower when more amount of yeast cake was mixed in PKM (Figure 2). Enzyme treatment of PKM100, PKM80, and PKM60 shown that %CF were reduced significantly at 24 h of incubation compared to time zero when 0.02 and 0.04% of  $\beta$ -mannanase enzyme were added (Figure 2).



**Figure 2.** Crude fiber composition (%DM) of β-mannanase treated yeast-premixed PKM at the ratio of PKM100 (A), PKM80 (B.) and PKM60 (C). The different small letter above the bar means significant different among incubation times of each enzyme level in each group of yeast-premixed PKM (P<0.05). The different capital letter above the bar means significant different among enzyme levels of the same incubation time in each group of yeast-premixed PKM (P<0.05).

At 0 h incubation, yeast-premixed PKM showed no difference in crude fiber content regardless of the enzyme supplementation. The slight reduction in %CF was found at 12 h incubation of PKM80 treated with 0.02% and 0.04% enzyme. The %CF were significantly reduced from 22.23 $\pm$ 1.67 of no enzyme to 19.10 $\pm$ 0.69 and 19.41 $\pm$ 0.86 of 0.02 and 0.04% enzyme (P<0.05). A sharp difference of %CF at different enzyme levels were shown at 24 h of incubation. Percentage of a crude fiber of PKM100 treated with  $\beta$ -mannanase were significantly reduced from 33.79 $\pm$ 4.17 at no enzyme added to 24.76 $\pm$ 1.46 and 21.36 $\pm$ 0.84 for 0.02 and 0.04% enzyme, respectively (P<0.05). PKM80 also showed a significant  $\beta$ -mannanase effect at both 0.02 and 0.04% (13.59 $\pm$ 1.71 and 14.07 $\pm$ 1.26, respectively). PKM60 showed a significant decrease in %CF of 21.91 $\pm$ 3.36 of no enzyme to 10.24 $\pm$ 1.19 and 4.84 $\pm$ 0.85 for 0.02 and 0.04%, respectively (P<0.05) (Figure 2).

# Soluble sugar content of 6-mannanase treated yeast-premixed PKM

Upon hydrolysis of  $\beta$ -mannan by the mannanase enzymes, the amount of  $\beta$ -mannan fiber is reduced while the soluble sugar is liberated which can be measured by determining of soluble sugar content in the sample. As shown in Figure 3, generally, the soluble sugar content of the yeast-premixed PKM was increased upon incubation time increase from 0 to 24 h. Even though the changes in soluble sugar could be seen without  $\beta$ -mannanase treatment; however, the effect of  $\beta$ -mannanase treatment can be distinguished.

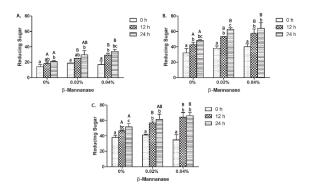


Figure 3. Soluble sugar content (mg/g dwPKM) of  $\beta$ -mannanase treated yeast-premixed PKM at the ratio of PKM100 (A), PKM80 (B.) and PKM60 (C). The different small letter above the bar means significant different among incubation times of each enzyme level in each group of yeast-premixed PKM (P<0.05). The different capital letter above the bar means significant different among enzyme levels of the same incubation time in each group of yeast-premixed PKM (P<0.05).

At 12 and 24 h incubation, 0.02% and 0.04%  $\beta$ -mannanase treatment produced a significantly higher amount of soluble sugar compared to no enzyme (P<0.05) (Figure 3). For PKM100 at 24 h, the amount of soluble sugar of 0.02 and 0.04% were 29.69 $\pm$ 5.31 and 34.08 $\pm$ 2.66 mg/g dwPKM which were significantly higher than no enzyme treatment (21.03 $\pm$ 0.95 mg/g dwPKM). For PKM80 at 24 h, the soluble sugar content of 0.02 and 0.04% were 62.61 $\pm$ 2.16 and 63.99 $\pm$ 7.59 mg/g dwPKM which were significantly higher than 48.35 $\pm$ 1.37 mg/g dwPKM of no enzyme treatment. For PKM60 at 24 h, 0.02 and 0.04%  $\beta$ -mannanase contained soluble sugar of 61.53 $\pm$ 6.67 and 66.08 $\pm$ 4.83 mg/g dwPKM while no enzyme contained lower soluble sugar of 51.65 $\pm$ 4.27 mg/g dwPKM. The significant effect of enzyme treatment found was correlated to the reduction of the crude fiber content of the enzyme-treated PKM.

### Discussion

An abundance of agro-by-products triggers the research invention of making these massive materials more values. PKM which has a moderate profile on proteins, but contains a high content of fiber is of interest for use as a feed ingredient for ruminants; however, it is not suitable enough for monogastric animals. Thus, improving the nutritive values of PKM to increase protein level, but lowering the fiber is one of the promising approaches. In this study, PKM was successfully improved its protein and fiber content by pre-mixed with yeast cake Saccharomyces Content of the promising approaches of the promising approaches.

The results demonstrated that PKM mixed with 40% yeast cake had a high protein content about 32% CP and was found increasing up to 45% when incubated for 24 h. The %CP obtained from an improved PKM is almost comparable to the %CP of soybean meal (SBM) which is about 45 to 50% (Baker and Stein, 2014; Lagos and Stein, 2017). Increasing time of incubation might result in higher protein content. Yeast fermented of poor protein feedstuffs have been showed to improve protein content. Cassava ships fermented with live-cell *Lactobacillus* and *S. cerevisiae* for 3 days showed improve protein content from 8.2% of untreated to 21.5% (Oboh, 2006). Swe *et al.* (2004) reported the higher crude protein in fermented PKM (29.4%) compared to untreated materials (16.9%). However, fermentation may take time and burdensome since it needs a special procedure for bacterial culture conditions. Thus using yeast cake agro-industrial wastes may help reducing time and avoid the troublesome of bacterial culture fermentation to obtain the desired amount of protein content. In addition to level up the protein content, since yeast contains a low level of fiber contents, our yeast cake mixed PKM showed reducing fiber as shown by approximately 50% reduction of crude fiber in PKM60 compared to without yeast, PKM100.

Enzyme treated yeast-mixed PKM significantly reduced the fiber content. The level of the enzyme at 0.04% was more effective than 0.02% in reducing crude fiber even though the soluble sugar of 0.02 and 0.04 is generally not different. Saenphoom *et al.* (2011) reported that pre-treating PKM with exogenous enzyme degraded cellulose and hemicelluloses components by 22.14 and 35.70%, respectively, and increased availability of reducing sugars than the

raw PKM. Enzyme treated PKM enhanced nutrient digestibility, retention of vital nutrients, and metabolizable energy (Iyayi and Davies, 2005; Sekoni *et al.*, 2008; Seanphoom *et al.*, 2013). Several studies also showed that incorporation of enzyme-treated PKM in feed diets provided improve growth performance in poultry production (Esuga *et al.*, 2008). Reports indicated that inclusion of hemicell mannanase improved the performance and health of broilers fed with PKM-based diets (Daskiran *et al.*, 2004; Zou *et al.*, 2006). However, the efficacy of enzyme hydrolysis *in vivo* is difficult to extrapolate since the stability of additive enzyme may vary between different sources of enzymes. Therefore, pretreated of PKM and evaluation of nutritive composition before incorporation in feed diets is advantage.

The results of  $\beta$ -mannanase treated yeast-premixed PKM shown in this study provide the alternative choices for improving PKM nutritive values with easy—to—make procedure avoiding the conventional bacterial fermentation. Evaluation of the nutritive composition of pre-treated PKM could help in the estimation of a suitable level of improved PKM in the formulation of feed diets. this improved yeast-premixed PKM could be further formulated evaluation for its efficacy in replacing of SBM in feed diets on growth performance and quality of meat products.

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Effects of sodium chloride replacement with potassium chloride and flavor enhancers on the physico-chemical and sensorial qualities of low-fat chicken frankfurter sausage

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Abstract: The effects of sodium chloride (NaCl) replacement with potassium chloride (KCl) and flavor enhancer on the physico-chemical and sensorial qualities of low-fat chicken frankfurter sausage was studied to reduce sodium content in the product. Three independent replications of low-fat chicken frankfurters were manufactured to compare seven treatments: control (1.2% NaCl), replacing NaCl by 25% 50% 75% KCl with 1% lysine (25KL, 50KL and 75KL, respectively) and replacing NaCl by 25% 50% 75% KCl with 1% yeast extract (25KY, 50KY and 75KY, respectively). The results showed that NaCl replacement by KCl together with both flavor enhancers did not affect to pH of batter, pH of sausage, and emulsion stability (P>0.05). Replacement of NaCl by KCl with both flavor enhancers in the product resulted in lower values of hardness, gumminess, and chewiness as increased the percentage of KCl (P<0.05). Moreover, the addition of yeast extract in replacing NaCl by KCl showed a lower lightness with higher yellowness than control and lysine groups (P<0.05). The flavor of 50KY and 75KY were lower sensorial liking score than control. Liking scores of taste, texture, and overall acceptability of 75KL, 50KY, and 75KY were lower than control. However, the lysine addition of 25KL and 50KL showed similar flavor, taste, and overall acceptability scores as compared with control. In conclusion, the use of 50% replacement of NaCl by KCl and with 1% lysine (50 KL) was optimum formulation to reduce the sodium content in low-fat chicken frankfurter sausages while maintaining quality characteristics of low-fat frankfurters.

## **Keywords:** Sodium chloride replacement, Potassium chloride, flavor enhancer, low-fat frankfurter

## Introduction

Chronic diseases and high level of sodium intake are related to hypertension and subsequently an increased risk of cardiovascular disease (Vollmer *et al.*, 2001). The World Health Organization (WHO) stated that the consumption of salt in form of sodium chloride (NaCl) more than 6 gram of NaCl /person/day is associated with an increase in hypertension. As reviewed by Weiss *et al.* (2010), the reduced salt intake to 5 gram of NaCl/person/day (accounted for 2 gram sodium content, Na) is recommended to decrease health risks.

NaCl is one of the common ingredients in emulsion-type sausage. It activates the extraction of myofibrillar protein and enhances emulsification. In addition, NaCl has crucial role in texture, taste and shelf-life of frankfurter (Yotsuyanagi *et al.*, 2016).

To reduce the sodium content in meat products, the replacing the NaCl by other salt are one of favorable strategy. Among of altermative salts, potassium chloride (KCl) was widely used to replace NaCl. It can replace NaCl with the equal content, because it has similar chemical properties including molecular composition and the ionic strength (Stanley et al., 2017). However, in some meat procut, high amonts of KCl (30-40%) result in a bitter, metallic taste and astringent taste, leading to decreasing sensory liking score of replacement product. There are some ingredients which can reduce the detrimental impacts caused by KCl. Flavour enhancer are reported as the good masking agent for salt substitutes including lysine and yest extract (Horita et al., 2014).

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Regarding the decreased sodium content in meat product, Campagnol *et al.* (2011) reported that lysine can reduce the sensory defects caused by high KCl levels in low-fat Bologna-type sausages. On the other hand, Campagnol *et al.* (2011) showed that yeast extract can be used as masking agent in fermented sausage when NaCl was replaced by high level of KCl. However, there are no comparative studies among lysine and yeast extract for bitterness-masking agents in sodium replacer by KCl in meat product, especially for low-fat chicken frankfurter sausages. Thus, the objective of this study was to determine the influences of NaCl replacement with KCl and flavor enhancer including lysine and yeast extract on the physico-chemical and sensorial qualities of low-fat chicken frankfurter sausage.

### Materials and Methods

### Formulations and processing of low-fat chicken frankfurter sausage

Skinless boneless breast (SBB) from chicken meat and chicken skin were purchased from modern trade, Ladkrabang, Thailand. Lean materials and chicken skin were ground through an 8 mm plate. Seven formulation frankfurters were shown in Table 1 including 1.2%NaCl (control), replacing NaCl by 25%KCL with lysine (25KL), replacing NaCl by 50%KCL with lysine (50KL), replacing NaCl by 75%KCL with lysine (75KL), replacing NaCl by 25%KCL with yeast extract (25KY), replacing NaCl by 50%KCL with yeast extract (50KY) and replacing NaCl by 75%KCL with yeast extract (75KY). The detail of meat and other ingredients was presented in Table 1. The sausage manufacturing was followed by Sorapukdee *et al.* (2013) with some modification. Briefly, the salts (NaCl, KCl, STPP, sodium nitrite, and sodium erythorbate) and the half of ice were added to the ground meat. The mixture was chopped in 5-liters of stainless steel cutter mixer (Universal Fritter-brane QS-505A, Champ, China) for 120s. Thereafter, ground skin chicken, soy protein, and the rest of ice were added and chopped for 90 s. The frankfurter premix and lysine and/or yeast extract was added and continuously chopped for 90 s. Finally, the meat batter was stuffed into 21-mm diameter collagen casing, handlinked at 6 inch intervals and cooked in a sausage smoke oven (100 BUDGET, Red Arrow Equipment, USA) until a core temperature of 75°C. Thereafter, frankfurters were chilled overnight at 4°C.

Table 1. low-fat chicken frankfurter formulations with various NaCl, KCl, and flavor enhancer (units: g/kg)

Ingredients	Control	25KL	50KL	75KL	25KY	50KY	75KY
Chicken meat	670	670	670	670	670	670	670
Skin chicken	100	100	100	100	100	100	100
Water/Ice	180	180	180	180	180	180	180
Soy protein isolate	14	14	14	14	14	14	14
Sodium nitrite	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium erythorbate	1	1	1	1	1	1	1
Sodium tripolyphosphate	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Frankfurter premix	20	20	20	20	20	20	20
NaCl	12	9	6	3	9	6	3
KCl	-	3	6	9	3	6	9
Lysine	-	10	10	10	-	-	-
Yeast extract	-	-	-	-	10	10	10

## Physical and chemical analysis

## Cooking loss

Cooking loss was evaluated according to the method of Hughes *et al.* (1997). Cooking loss was estimated from weight loss after processing of the uncooked frankfurters until a core temperature of 75°C in a sausage smoke oven and expressed as the percentage (%) of initial sample weight.

### pH values

The pH was evaluated by direct measurement into batter and frankfurter sausage using pH meter after overnight chilling (Mettler Toledo, Greifensee, Switzerland). pH value were masured at triplicate determination for each sample.

## **Emulsion stability**

Emulsion stability was performed according to the method of Colmenero *et al.* (2005). Meat batter was placed in to 50-mL centrifuge tube (six tubes per treatment) and then heated to 70°C for 30 min. After centrifuge for 3 min at 4000 rpm (Sigma2-16KL, Sigma, Germany), samples were opened and left to stand upside-down at room temperature (32±2 °C) for 50 min to release the exudates onto a plate. Total fluid released (TFR) was expressed as percentage (%) of initial sample weight. Water released (WR) was calculated from weight loss after heating the TFR at 105°C in a convection oven (FD115, BINDER, Germany) for 16 h and expressed as percentage (%) of initial sample weight. The difference between TRF and WR was calculated as fat released (FR).

### Texture profile analysis (TPA)

TPA was tested using a texture analyser (Instron Model 3344, Illinois Tool Works Inc., USA) with cylindrical probe. Seven peices of each treatment (21 mm diameter and 20 mm height) were prepared and placed on the instrument's base. TPA parameters as described by Bourne (1978) including hardness (N), cohesiveness (ratio), gumminess (N), chewiness (N) and springiness (ratio) were recoreded from the force-time curves generated by the Bluehill 2 software.

### Color

The central part of the internal surface color of six cylinder-shaped samples (21 mm diameter and 20 mm height) was recorded in the L\*a\*b\* mode of CIE by Hunter Lab colorimeter (Hunter Associates Laboratory Inc., USA). The average values were expressed as L\*, a\*, and b\* for lightness, redness, and yellowness, respectively.

## Sensory evaluation

Sensory evaluation was carried out for cooked low-fat chicken frankfurter sausages with the method as described by Wheeler *et al.* (2016). The sausages were examined for their appearance, flavor, taste, texture, and overall acceptability. The treatments were code with three digits random number. There were 20 semi-trained panelists in all sensory evaluation tests. Sensory attributes were evaluated using a seven points hedonic scale from 1= dislike extremely, 2= dislike very much, 3= dislike, 4= neither like nor dislike, 5=like, 6= like very much and 7= like extremely.

### Statistical analyses

The experiment was conducted as Randomized Complete Block Design. Three independent batches were conducted. Significant effects were performed by One-way ANOVA and mean comparison were compared by the Duncan's multiple range test. Data were analyzed using SPSS (v.19, IBM SPSS Inc.).

### Results

### Cooking loss, pH values, and emulsion stability

Cooking loss of low-fat chicken frankfurter sausage was shown in Table 2. The replacement of NaCl by KCl and flavor enhancer of low-fat chicken frankfurter sausages were not affected on cooking loss (P>0.05). For all treatments, cooking loss varied from 6.05% to 7.94%.

The emulsion stability of low-fat chicken frankfurter sausages was analyzed by TFR, FR, and WR, as shown in Table 2. TFR ranged from 1.24% to 1.68%, FR varied from 0.10 to 0.13 and WR ranged from 1.14 to 1.55. There were not observed significant differences among treatments (P>0.05).

There were no significant differences among treatments for pH of batter and sausage (P>0.05) (Table 2). For all treatments, the batter pH values ranged from 6.17 to 6.23 and the sausage pH values ranged from 6.09 to 6.13.

### TPA

The instrumental TPA was shown in Table 3. It was found that the low-fat chicken frankfurter sausages showed different value of hardness (P<0.05). The treatment 25KL had the highest of hardness, gumminess, and chewiness compared to other treatments. However, the higher addition of KCl, especially for 50KL, 75KL, 50KY, and 75KY seem to be decreased hardness, gumminess, and chewiness as compared with control. Cohesiveness and springiness for each treatment did not shown significant difference (P>0.05).

Table 2. Cooking loss, pH value and emulsion stability of low-fat chicken frankfurter sausage

Cample	Cooking loss	Cooking loss pH values			Emulsion stability				
Sample	(%)	batter	sausage	TFR (%)	FR (%)	WR (%)			
Control	6.73±0.01 <sup>a,1,2</sup>	6.23±0.01 <sup>a</sup>	6.10±0.02 <sup>a</sup>	1.68±0.43 <sup>a</sup>	0.13±0.04 <sup>a</sup>	1.55±0.02°			
25KL	7.48±0.01 <sup>a</sup>	6.22±0.01 <sup>a</sup>	6.13±0.01 <sup>a</sup>	1.39±0.19 <sup>a</sup>	0.13±0.03 <sup>a</sup>	1.26±0.02 <sup>a</sup>			
50KL	6.05±0.01 <sup>a</sup>	6.20±0.01 <sup>a</sup>	6.12±0.01 <sup>a</sup>	1.24±0.16 <sup>a</sup>	$0.10\pm0.01^{a}$	1.14±0.03 <sup>a</sup>			
75KL	7.94±0.01 <sup>a</sup>	6.21±0.01 <sup>a</sup>	6.09±0.01 <sup>a</sup>	1.54±0.70 <sup>a</sup>	$0.13\pm0.07^{a}$	1.41±0.01 <sup>a</sup>			
25KY	6.70±0.01 <sup>a</sup>	6.17±0.01 <sup>a</sup>	6.10±0.02 <sup>a</sup>	1.32±0.70 <sup>a</sup>	$0.11\pm0.06^{a}$	1.21±0.01 <sup>a</sup>			
50KY	7.52±0.01 <sup>a</sup>	6.20±0.01 <sup>a</sup>	6.09±0.01 <sup>a</sup>	1.41±0.27 <sup>a</sup>	0.11±0.03 <sup>a</sup>	1.30±0.02 <sup>a</sup>			
75KY	7.13±0.01 <sup>a</sup>	6.23±0.01 <sup>a</sup>	6.13±0.01 <sup>a</sup>	1.37±0.11 <sup>a</sup>	$0.11 \pm 0.01^{a}$	1.26±0.04 <sup>a</sup>			

 $<sup>^{1}</sup>$  Mean values  $\pm$  SD obtained from three independent batches.

**Table 3.** TPA of low-fat chicken frankfurter sausage

	Texture profile analysis							
Sample	Hardness (N)	Cohesiveness (ratio)	Gumminess (N)	Chewiness (N)	Springiness (ratio)			
Control	22.76±0.27 <sup>ab,1,2</sup>	0.79±0.01 <sup>a</sup>	17.99±0.21 <sup>ab</sup>	15.75±0.56 <sup>b</sup>	0.88±0.02 <sup>a</sup>			
25KL	23.50±0.48 <sup>a</sup>	0.79±0.01 <sup>a</sup>	18.67±0.52 <sup>a</sup>	17.20±0.15 <sup>a</sup>	0.90±0.02 <sup>a</sup>			
50KL	20.15±0.20 <sup>c</sup>	0.79±0.01 <sup>a</sup>	15.93±0.26 <sup>c</sup>	14.05±0.20 <sup>c</sup>	0.88±0.01 <sup>a</sup>			
75KL	18.29±0.22 <sup>d</sup>	0.78±0.01 <sup>a</sup>	14.32±0.10 <sup>d</sup>	12.51±0.15 <sup>d</sup>	0.87±0.01 <sup>a</sup>			
25KY	21.75±1.45 <sup>b</sup>	0.80±0.01 <sup>a</sup>	17.34±1.34 <sup>ab</sup>	15.18±1.38 <sup>bc</sup>	0.88±0.01 <sup>a</sup>			
50KY	21.73±0.52 <sup>b</sup>	0.79±0.01 <sup>a</sup>	17.12±0.63 <sup>bc</sup>	15.31±0.88 <sup>bc</sup>	0.89±0.02 <sup>a</sup>			
75KY	19.93±1.46 <sup>c</sup>	0.80±0.01 <sup>a</sup>	15.89±1.15 <sup>c</sup>	14.20±0.70 <sup>c</sup>	$0.89\pm0.02^{a}$			

 $<sup>^{1}</sup>$  Mean values  $\pm$  SD obtained from three independent batches.

<sup>&</sup>lt;sup>2</sup> Different letters in the same column indicate significant difference (P<0.05).

<sup>&</sup>lt;sup>2</sup> Different letters in the same column indicate significant difference (P<0.05).

#### Color

The partial substitution of NaCl by KCl impacted to color of low-fat chicken frankfurter sausages (Table 4). The replacement of NaCl by KCl together with 1% yeast extract addition (25KY, 50KY and 75KY) decreased lightness with increased yellowness (P<0.05), especially as compared with control. A similar relationship was found in lysine group (25KL, 50KL and 75KL), but smaller impact as compared with yeast extract. However, all treatment of low-fat frankfurters were not significant differences in redness (P>0.05).

### Sensory analysis

The appearance evaluated by panelists demonstrated that there were no significant differences among treatments (P>0.05) (Table 5). The flavor liking scores of 50KY and 75KY were lower than control (P<0.05). The taste and overall acceptability liking scores of 75KL, 50KY and 75KY were also lower than control (P<0.05). Among the sodium chloride replacement formulation, 25KL showed the highest texture score than other treatment and tended to decrease as increased KCl.

**Table 4.** Color (CIE L\*, a\*, b\*) of low-fat chicken frankfurter sausage

Comple		Color parameter	
Sample	Lightness (L*)	Redness (a*)	Yellowness (b*)
Control	68.43±0.32 a,1,2	7.83±0.35 <sup>a</sup>	20.67±0.68°
25KL	67.04±0.49 <sup>b</sup>	7.78±0.17 <sup>a</sup>	20.92±0.30 <sup>bc</sup>
50KL	67.49±0.29 <sup>b</sup>	7.81±0.20 <sup>a</sup>	20.67±0.45 <sup>c</sup>
75KL	66.47±0.44°	7.98±0.17 <sup>a</sup>	21.40±0.15 <sup>b</sup>
25KY	66.03±0.43°	7.80±0.36 <sup>a</sup>	22.25±0.62 <sup>a</sup>
50KY	66.49±0.63°	7.93±0.51 <sup>a</sup>	22.44±0.79 <sup>a</sup>
75KY	66.51±0.36°	7.76±0.22 <sup>a</sup>	22.28±0.34 <sup>a</sup>

 $<sup>^{1}</sup>$  Mean values  $\pm$  SD obtained from three independent batches.

Table 5. Sensory evaluation (7-point hedonic scale) of low-fat chicken frankfurter sausage

Sample	Appearance	Flavor	Taste	Texture	Overall acceptability
Control	6.22±0.28 a,1,2	6.78±0.20 <sup>a</sup>	6.14±0.46 <sup>a</sup>	5.21±0.57 <sup>ab</sup>	5.86±0.29 <sup>a</sup>
25KL	6.43±0.20 <sup>a</sup>	6.28±0.39 <sup>ab</sup>	5.64±0.33 <sup>ab</sup>	5.79±0.36 <sup>a</sup>	5.93±0.32 <sup>a</sup>
50KL	6.65±0.20 <sup>a</sup>	$6.06\pm0.36^{ab}$	5.07±0.20 <sup>ab</sup>	5.50±0.34 <sup>ab</sup>	5.43±0.15 <sup>a</sup>
75KL	6.58±0.40 <sup>a</sup>	6.21±0.59 <sup>ab</sup>	3.50±0.65°	4.29±0.97 <sup>b</sup>	3.86±0.51 <sup>b</sup>
25KY	6.72±0.26 <sup>a</sup>	5.85±0.35 <sup>ab</sup>	4.93±0.07 <sup>b</sup>	5.07±0.43 <sup>ab</sup>	5.00±0.24 <sup>a</sup>
50KY	6.01±0.58 <sup>a</sup>	5.21±0.78 <sup>b</sup>	$3.43\pm0.50^{c}$	4.21±0.71 <sup>b</sup>	3.64±0.39 <sup>b</sup>
75KY	6.43±0.81 <sup>a</sup>	5.21±0.98 <sup>b</sup>	3.36±0.64 <sup>c</sup>	4.14±1.07 <sup>b</sup>	3.50±0.50 <sup>b</sup>

 $<sup>^{1}</sup>$  Mean values  $\pm$  SD obtained from three independent batches.

### Discussion

The reformulation of chicken frankfurter sausage to be the low-sodium product in present study was not influenced on cooking loss, emulsion stability, and pH value. According to Dos Santos Alves *et al.* (2017), the significant differences in cooking loss and emulsion stability between low-fat bolona-type sausages with sodium replacement by KCl and control were not found. Similary, Choi *et al.* (2014) found that there was no significant impact of KCl for sodium replacer on cooking loss of frankfurter sausages when compared with control. These informations suggested that the type of ion not affect to protein extraction capacity as well as moisture retention ability, but depend on ionic strength

<sup>&</sup>lt;sup>2</sup> Different letters in the same column indicate significant difference (P<0.05).

<sup>&</sup>lt;sup>2</sup> Different letters in the same column indicate significant difference (P<0.05).

of the system (Pietrasik and Gaudette, 2015). Furthermore, NaCl and KCl were similarly effective in protein extraction of tumbled hams (Trout and Schmidt, 1986). In present study, it was noted that cooking loss was not difference between treatments might be due to the use of soy protein isolate in our formulation, which could increase the gel forming and water-binding capacity in final product. Concerning pH, Campagnol *et al.* (2012) also reported that pH values of fermented sausage between KCl added product and NaCl group at begin of production were not significantly observed. The other report also found that NaCl content did not influence the pH of frankfurter (Puolanne *et al.*, 2001).

Regarding textural characteristics, hardness, gumminess, and chewiness of products were decreased as increased KCl content. According to Campagnol *et al.* (2012), they also reported that replacing NaCl by KCl in low-sodium fermented sausages decreased hardness value when compared with control. The sodium replacer by using KCl in combination with yeast extract was impacted on color of our low-fat chicken frankfurter sausages. Generally, yeast extract is creamy yellow coloured powder. Therefore, the decreased lightness and increased yellowness could be observed in treatment formulated with yeast extract. Similarly to Campagnol *et al.* (2011), the replacing NaCl by KCl with added yeast extract in fermented sausages also decreased lightness of resulting product.

In present study, the sodium replacer by 50% KCl with added lysine were not contributed the detrimental impact on sensory score as compared with control. Dos Santos Alves *et al.* (2017) reported that consumers did not identify differences in color, aroma, flavor, texture, and overall acceptability among samples between replacing NaCl by 50% KCl with 1% lysine addition and control. In addition, Gou *et al.* (1996) reported that the textural characteristics of ferment sausages between replacement of NaCl by KCl and control groups were similar profiles, but a bitter taste was detected when a 30% substitution level was used.

In conclusion, the replacement of NaCl by 50% KCl with 1 % lysine addition were not negatively impacted on physico-chemical and sensorial properties of low-fat chicken frankfurter sausages. Therefore, this product reformulation could be used to produce a healthier low-fat chicken frankfurter sausage for the consumers who are concerning the high calories and high sodium intake.

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## Sous-vided restructured goat meat: Changes in meat qualities during refrigerated storage

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Abstract The results showed that all microbial counts of sous-vide sample were significantly increased during storage. The aerobic and anaerobic mesophilic bacteria were higher than 5.70 log cfu/g for 28 days which their counts were below limited microbiological quality of cooked food in Food and Container Standard No. 3, Thailand. *Brochothrix thermosphacta* and pathogen counts as *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens* were below 1 log cfu/g. Additionally, *Salmonella* spp., *Listeria* spp., *Listeria monocytogenes* were not detected during storage. For physicochemical quality, pH value of sous-vide sample decreased during storage which due to increased lactic acid bacteria counts. Purge loss had significantly increased (P<0.05) throughout the storage. CIE L\* had significantly increased (P<0.05), whereas CIE a\* and CIE b\* had significantly decreased (P<0.05) with increasing storage time. For texture profile analysis, hardness, gumminess, and chewiness had significantly increased (P<0.05) as storage time was increased. After grilling, there were significantly increased for grilling loss and significant decreases for CIE a\* and CIE b\* values (P<0.05) throughout storage time. Thus, shelf-life of sous-vided restructured goat meat was 28 days during storage at 4°C

Keywords: Shelf-life, Sous-vided meat, Restructured meat, Cold storage

#### Introduction

Products are indicated as 'restructured' or 'reformed' which determined to a huge extent by the size of the composition pieces. Meat restructuring connects the assembly of meat slices into an adhesive product which expects to simulate the texture of high-quality muscles (Sorapukdee and Tangwatcharin, 2018). The restructured meat products regularly contain a mixture of meat and non-meat ingredients in which the meat constituent is contamination of microbial, causing the product to be easily putrid and the short shelf-life (Bolger *et al.*, 2016). But the pre-cooking as sous-vide technique can decrease pathogenic and spoilage bacteria of pasteurizing temperature (Baldwin. 2011).

Sous-vide cooking technique is determined as the method of heated raw meat packed inside a vacuum pouch in the water baths at a stipulated temperature (Roldán *et al.*, 2015). The danger of pasteurized products after cooked does not reduce pathogenic to a safe level (Baldwin. 2011). The safety of sous-vided product depends on the heat and low-temperature storage (New South Wales Government Food Authority. 2015). Coombs *et al.* (2017) reported that the microbial growth, texture analysis and color were factors essential to shelf-life and accordingly to consumer reception of fresh and meat products. The goat meat had higher pH as compared beef and pork but similar to lamb contained an excellent substrate for spoilage bacteria growth.

Although, the sous-vide procedure is used commercially, there has been restricted research work related to sous-vide cooking. It has an effect of quality on meat products and shelf-life (Roldán *et al.*, 2015). However, under cooling conditions, the pre-cooked meat product has limited shelf-life, and the improve meat of preservation methods for meat product is necessary (Hong *et al.*, 2015). The sous-vide product in the package has a low oxygen container prevents the growth of aerobic spoilage microorganism and increased the time that pre-cooked meat can be preserved (Roldán *et al.*, 2015).

New South Wales Government Food Authority (2015) recommended that sous-vided food safety precautions for restaurants meat producers has been required to represent a process adequate to obtain a minimum 6 log cycle reduction of Listeria monocytogenes. The microbiological quality of cooked food in Food and Container Standard No.

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3, Thailand (2017) advised that cooked products were required to found below 5.7 log cfu/g for total plate counts, below 3 log cfu/g for Clostridium perfringens, below 1 log cfu/g for Staphylococcus aureus, Bacillus cereus and Brochothrix thermosphacta not detected pathogens in 25 g of sample such as Salmonella spp., Listeria spp. and Listeria monocytogenes. Therefore, this study was performed in changes of sous-vided restructured goat meat characteristic during refrigerated storage as determination shelf-life of products during refrigerated storage.

#### Materials and methods

## Preparation of sous-vide product

The meat was procured mostly from the neck, hind leg and chump of a carcass of male goats (25% Thai native x 25% Anglo-Nubian x 50% Boer, age 15±3 months, weight 40-45 kg) and purchased from the local slaughterhouse in Krabi, Thailand. The goat meat was carryed on ice to the meat laboratory of the Department of Animal Production Technology and Fisheries, King Mongkut's Institute of Technology Ladkrabang for product processing. Then, goat meat was trimmed connective tissue and fat and cut into strips and minced with the meat grinder (Model NB-MM12SS, Sun Food Co., Ltd, Thailand) using 12 mm plates.

The meat samples were mixed with the ingredients of sodium chloride (0.6%), sodium tripolyphosphate (STPP) (0.5%), sodium nitrite (0.3%), water (15.0%), microbial transglutaminase (MTGase) (1.0%) (ACTIVA TG-B powder, Ajinomoto Co., Ltd., Thailand), sodium caseinate (1.0%) using a jar-lift stand mixer (KitchenAid, Professional 600, USA) (Sorapukdee and Tangwatcharin. 2018). The mixture was set into the block, chilled at 4°C for 4 h, frozen at -18°C for 24 h and cut into 15 mm thick further analysis. The samples were packed in vacuum bags and cooked using sous-vide immersion circulator SV100 (Cuisine Craft Co., LTD., Thailand) at core temperature 60°C for 43 min and stored at 4°C for 49 days. The stored samples were sampling every 7 days for analyses.

### Microbiological analyses

Twenty-five grams of each sample was relocated into 225 ml of 0.85% sodium chloride (NaCl) and homogenized 1 min using stomacher (Inter science Co., France). Ten-fold dilution preparation of the sample was used 0.85% NaCl. It's definited as colony forming units (log cfu/g) with culturing on media; (1) plate count agar and incubated at 35°C for 24 h and 7°C for 10 days for aerobic and anaerobic bacteria counts (BAM. 2001a), (2) MRS with 0.15% agar and incubated at 35°C for 24 h under anaerobic condition for lactic acid bacteria (LAB) (AOAC. 2006), (3) potato dextrose agar and incubated at 30°C for 24 h for yeast and mold (BAM. 2001b), (4) Baird-Parker agar and incubated at 35°C for 48 h for *Staphylococcus aureus* (BAM. 2001c), (5) Mannitol yolk polymyxin (MYP) agar and incubated at 30°C for 24 h for *Bacillus cereus* (BAM. 2007), (6) Tryptone sulphite neomycin agar and incubated at 35°C for 24 h for *Clostridium perfringens* (BAM. 2001d), (7) Streptomycin-thallous acetate-actidione (STAA) plates and incubated at 25 °C for 48 h for *Brochothrix thermosphacta* (Coombs *et al.* 2017). Coliforms and *Escherichia coli* were enriched by the MPN multiple tube method according to BAM (2002); *Salmonella* spp. was enriched according to the method of to ISO-6579 (2002); *Listeria* spp. and *Listeria monocytogenes* were enriched using the method of BAM (2017).

## Physical-chemical analyses

The sous-vided samples before and after storage and grilled sous-vided samples after storage were weighed. The weight of samples was calculated the purge loss (%) and grilling loss (%).

The pH of sous-vide and grilled sous-vide samples were directly measured at three different locations using a standard pH meter (Mettler Toledo 320, Mettler Toledo, Greifensee, Switzerland).

The color was measured on the cut surface of samples after sous-vide and grilling. The CIE L\*, CIE L a\*, CIE L b\* value was determined using by the color meter (Hunterlab Mini Scan EZ LAV, Hunter Associates Laboratory, Inc, USA). Hue angle ( $h^0$ ), chroma ( $C^*$ ) and the ratio was calculated by

$$C^* = \sqrt{b^{*2} + a^{*2}}$$
 ,  $h_0^0 = \tan^{-1}(\frac{b^*}{a^*})$ 

The sous-vide and grilled sous-vide samples were subjected to texture profile analysis using the texture analyzer (Warner-Bratzler, Instron Model 1011, Instron company, Thailand). Texture profile analysis was operated using middle cores of eight piece of each sample (1.5×1.5×1.5 cm). The texture profile analysis and textural parameter measurement was done at room temperature with the following testing conditions; crosshead speed 60 mm/min, 500 N load cell and compression twice to 40% of their original height. The Bluechill 2 software was used to collect and process the data. The following parameters were determined as hardness (N); chewiness (N); gumminess (N); cohesiveness (ratio) and springiness (ratio) (Sorapukdee and Tangwatcharin. 2018).

### Statistical analysis

Data were shown as mean and standard deviations. Significant effects (P<0.05) were performed by one-way ANOVA and mean comparison were compared by the Duncan's multiple range test. Data were analysed using SPSS (v.17. IBM SPSS Inc.).

### Results

The microbial quality of sous-vided restructured goat meat stored at 4°C for 49 days was presented in Table 1. Aerobic mesophilic and psychrotrophilic bacteria counts in sous-vided sample were significantly increased (P<0.05) throughout storage time, ranging from 3.06 to 7.33 log cfu/g of aerobic mesophilic counts and 3.08 to 8.07 log cfu/g of aerobic psychrotrophile counts. The result of aerobic and anaerobic mesophilic bacteria was higher than 5.7 log cfu/g after storage 35 days. The aerobic and anaerobic psychrotrophilic bacteria counts were significantly (P<0.05) as increased storage time and their counts were higher than 5.7 log cfu/g after storage 21 days. In this study was found of spore aerobic and anaerobic mesophilic bacteria counts 1.72 and 1.85 log cfu/g in the sample before storage. However, their counts were below 1 log cfu/g throughout storage at 4°C. LAB counts increased gradually reaching up 2.24 to 8.29 log cfu/g during storage. The molds count was below 1 log cfu/g throughout storage time whereas yeast count recovered and after stored for 28 days. Coliforms could grow after stored for 7 days, ranging from 29 to than 1100 MPN/g. However, *E. coli* was lower than 3 MPN/g throughout storage. The samples were found *S. aureus*, *B. cereus*, *C. perfringens and B. thermosphacta* below 1 log cfu/g enough out storage time. *Listeria* spp., *L. monocytogenes* and *Salmonella* spp. was not detected in fresh meat and products and cooked product during the refrigerated storage.

The results of the purge loss (%), pH and color assessment of the sous-vided and grilled sous-vided sample stored at 4°C for 49 days were displayed in Table 2. For sous-vided samples, the purge loss had significantly increased throughout storage time (P<0.05). Storage time had significant effect (P<0.05) on the pH values of the sample. CIE L\* has a significant increase (P<0.05) in the storage period. CIE a\*, CIE b\* and C\* had significantly decreased during the storage (P<0.05). Storage time had no significant effect (P<0.05) on hº of the sample. For TPA, the values (hardness, gumminess, and chewiness) had significant increases (P<0.05) during storage time (Figure 1A). The springiness and cohesiveness were not significantly changed during storage time (not show data).

After grilling, the grilling loss had significant increases throughout storage time (P<0.05). CIE L\* had a significant increase (P<0.05) in the storage period. CIE a\*, CIE b\*, C\* and hº had significantly decreased during the storage (P<0.05). Hardness, gumminess, and chewiness were significant increases (P<0.05) during storage time (Figure 1B). The springiness and cohesiveness were not changed during storage time (data not shown).

## Discussion

Refrigerated storage affected the microbiological quality and physical-chemical qualities of sous-vided restructured goat meat product in this study. The sous-vide process at 60°C 43 min was good enough for a pasteurized meat due to good microbial quality of the sous-vided sample which was below limited of the microbiological quality of cooked food in Food and Container Standard No. 3, Thailand (Bureau of Quality and Safety of Food. 2017). After

refrigerated storage, the sous-vided sample was found the aerobic mesophilic bacteria were below 5.7 log cfu/g of shelf life in 28 days. Similar to study of Akoğlu *et al.* (2018), the total plate counts of sous-vided turkey cutlet sous-vided at 65°C for 40 min were less than 5.7 log cfu/g until stored at 4°C for 35 days. In this study, LAB counts increased with storage time in anaerobic environments which associated with the spoilage of sous-vided products vacuum packed during storage time (Hong *et al.*, 2015).

The storage at 4°C had a negative effect of purge loss value of sous-vided sample as increased storage time (P<0.05). Hong *et al.* (2015), Coombs *et al.* (2017) and Otremba *et al.* (1999) reported that purge loss values of lamb meat, chicken breast sous-vide and ostrich meat were significantly increased during storage.

The present study, storage time had no significant effect (P<0.05) storage. The present study, storage time had significant effect (P<0.05) on pH values, which may be attributed to lactic acid production of LAB counts an increase in anaerobic environments, results in pH values decrease during storage (Hong *et al.*, 2015). The CIE L\* was significantly increased (P<0.05) in storage period, due to a higher denaturation and aggregation of sarcoplasmic and myofibrillar proteins as increased storage time (Roldán *et al.*, 2015). The increase of CIE a\* depended the less myoglobin degradation and mildest cooking temperature, which CIE a\* was very stable during the whole storage time (Hong *et al.*, 2015, Roldán *et al.*, 2015). According to this study, CIE a\* values of sous-vided and grilled sous-vided samples were found decreased during the storage. Hardness, gumminess, and chewiness were significantly increased (P<0.05) during storage time, which agreed with the results of Wattanachant *et al.* (2008). The sous-vided sample had to the strongest cooking with consistently higher values of TPA during storage.

**Table 1.** Microbial counts of raw meat and sous-vided restructured goat meat<sup>/1</sup> during storage at 4°C for 49 days.

Microbial	Raw meat				Day of stora	ıge at 4ºC <sup>/2</sup>			
	_	0	7	14	21	28	35	42	49
Aerobe (log cfu/g)									
Mesophilic bacteria	4.22	3.06 <sup>g</sup>	4.06 <sup>f</sup>	4.66 <sup>e</sup>	4.98 <sup>d</sup>	5.07 <sup>d</sup>	5.73 <sup>c</sup>	6.97 <sup>b</sup>	7.33ª
Spore Mesophilic bacteria	2.85	1.72	<1	<1	<1	<1	<1	<1	<1
Psychrotrophic bacteria	3.42	3.08 <sup>g</sup>	4.62 <sup>f</sup>	5.49 <sup>e</sup>	6.26 <sup>d</sup>	6.53 <sup>c</sup>	7.54 <sup>b</sup>	7.83 <sup>a</sup>	8.07 <sup>a</sup>
Anaerobe (log cfu/g)									
Mesophilic bacteria	4.11	3.03 <sup>g</sup>	4.70 <sup>f</sup>	4.94 <sup>ef</sup>	5.32 <sup>de</sup>	5.49 <sup>cd</sup>	5.84 <sup>bc</sup>	6.08 <sup>b</sup>	6.63ª
Spore Mesophilic bacteria	3.96	1.85	<1	<1	<1	<1	<1	<1	<1
Psychrotrophic bacteria	2.79	2.54 <sup>e</sup>	4.78 <sup>d</sup>	5.56 <sup>c</sup>	6.44 <sup>b</sup>	6.56 <sup>b</sup>	6.69 <sup>b</sup>	$7.29^{a}$	$7.57^{a}$
Lactic acid bacteria (log cfu/g)	2.37	2.24 <sup>f</sup>	3.71 <sup>e</sup>	4.39 <sup>d</sup>	4.96 <sup>c</sup>	6.55 <sup>b</sup>	6.68 <sup>b</sup>	8.24 <sup>a</sup>	8.29ª
Yeast and mold (log cfu/g)	<1	<1	<1	<1	<1	0.79	0.87	<1	1.19
Staphylococcus aureus (log cfu/g)	<1	<1	<1	<1	<1	<1	<1	<1	<1
Bacillus cereus (log cfu/g)	<1	<1	<1	<1	<1	<1	<1	<1	<1
Clostridium perfringens (log cfu/g)	<1	<1	<1	<1	<1	<1	<1	<1	<1
Brochothrix thermosphacta (log cfu/g)	<1	<1	<1	<1	<1	<1	<1	<1	<1
Coliforms (MPN/g)	240	<3	29	38	43	64	75	240	>1100
Escherichia coli (MPN/g)	<3	<3	<3	<3	<3	<3	<3	<3	<3
Listeria spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND
Listeria monocytogenes	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND

<sup>&</sup>lt;sup>1/</sup> The microbiological quality of cooked food in Food and Container Standard No. 3, Thailand (2017) recommend; aerobic mesophile bacteria < 5.7 log cfu/g, *B. cereus* and *S. aureus* < 2 log cfu/g, *C. perfringens* <3log cfu/g, *B. thermosphacta*, *Listeria* spp., *L. monocytogenes* and *Salmonella* spp. not detected in 25 g.

<sup>&</sup>lt;sup>2/a-f</sup>; values with the same letter within row were significant different for sous-vided samples during storage (P<0.05). ND; not detected.

Table 2. Physical characteristics of sous-vided and grilled sous-vided restructured goat meat during storage at 4 °C for 49 days.

Danamatan				Da	ay of storage at 4º	C <sup>/1,2</sup>		
Parameter	0	7	14	21	28	35	42	49
Sous-vided sample	2S							
Purge loss (%)	2.61±0.02 <sup>f</sup>	2.85±0.16 <sup>ef</sup>	3.83±0.32 <sup>de</sup>	4.45±0.07 <sup>cd</sup>	4.94±0.70 <sup>bcd</sup>	5.46±0.90 <sup>abc</sup>	5.68±0.09 <sup>ab</sup>	6.42±0.56 <sup>a</sup>
рН	6.22±0.01 <sup>ab</sup>	$6.25\pm0.04^{ab}$	6.23±0.04 <sup>abc</sup>	6.29±0.01 <sup>a</sup>	5.99±0.16 <sup>e</sup>	6.02±0.04 <sup>cde</sup>	6.00±0.09 <sup>e</sup>	6.12±0.15 <sup>bcd</sup>
CIE L*	37.01±0.33 <sup>c</sup>	37.97±1.23 <sup>c</sup>	38.25±1.57 <sup>c</sup>	38.82±1.94 <sup>c</sup>	39.68±3.40 <sup>bc</sup>	39.54±2.90 <sup>bc</sup>	42.18±1.02 <sup>ab</sup>	44.74±1.26 <sup>a</sup>
CIE a*	10.68±0.81 <sup>a</sup>	10.03±1.56 <sup>a</sup>	9.93±2.02 <sup>a</sup>	9.26±2.84 <sup>a</sup>	9.07±0.47 <sup>ab</sup>	8.57±0.36 <sup>b</sup>	8.23±1.68 <sup>b</sup>	6.69±0.39 <sup>b</sup>
CIE b*	13.59±1.08 <sup>a</sup>	13.03±0.51 <sup>ab</sup>	13.01±0.19 <sup>ab</sup>	12.04±1.14 <sup>b</sup>	11.89±1.27 <sup>b</sup>	11.79±0.58 <sup>bc</sup>	11.58±1.19 <sup>bc</sup>	10.38±1.02 <sup>c</sup>
Chroma (C*)	17.29±1.12°	16.47±1.09ab	16.43±1.34 <sup>ab</sup>	15.40±1.27 <sup>bc</sup>	14.97±1.04 <sup>bc</sup>	14.57±0.68°	14.23±1.74°	12.36±0.79 <sup>d</sup>
Hue angle (hº)	51.80±2.43	52.56±3.34	52.99±1.15	53.00±1.04	52.50±3.16	53.99±0.29	54.89±1.53	57.09±1.79
Grilled Sous-vided	samples							
Grilling loss (%)	8.91±0.22 <sup>d</sup>	8.39±0.50 <sup>d</sup>	8.97±0.31 <sup>d</sup>	10.47±0.17 <sup>c</sup>	10.95±1.06°	12.83±0.22 <sup>b</sup>	12.89±0.98 <sup>b</sup>	14.47±0.10 <sup>a</sup>
рН	6.22±0.01	6.24±0.04	6.21±0.04	6.24±0.01	6.02±0.03	6.01±0.04	6.01±0.20	6.11±0.10
CIE L*	30.27±0.37 <sup>d</sup>	30.91±0.73 <sup>d</sup>	31.79±0.91 <sup>cd</sup>	32.91±1.93 <sup>bcd</sup>	35.49±2.33 <sup>abc</sup>	35.62±0.54 <sup>abc</sup>	36.08±0.71 <sup>ab</sup>	38.69±0.47 <sup>a</sup>
CIE a*	20.20±0.79 <sup>a</sup>	17.05±0.31 <sup>b</sup>	15.82±0.67 <sup>bc</sup>	14.90±0.06 <sup>bc</sup>	14.36±1.39 <sup>bcd</sup>	13.78±0.21 <sup>cd</sup>	12.13±0.10 <sup>de</sup>	10.88±0.88 <sup>e</sup>
CIE b*	23.05±0.97 <sup>a</sup>	18.69±0.62 <sup>b</sup>	15.34±0.79 <sup>c</sup>	15.09±1.91 <sup>c</sup>	14.13±0.49°	13.56±0.04 <sup>c</sup>	$9.97\pm0.58^{d}$	$9.03\pm0.86^{d}$
Chroma (C*)	30.70±0.66 <sup>a</sup>	25.32±0.70 <sup>b</sup>	22.05±0.67 <sup>bc</sup>	21.23±0.56°	20.16±1.17°	19.38±1.43°	15.75±1.67 <sup>d</sup>	14.16±0.84 <sup>d</sup>
Hue angle (hº)	48.86±0.56 <sup>a</sup>	47.47±1.48 <sup>ab</sup>	44.11±0.04 <sup>b</sup>	45.42±1.19 <sup>ab</sup>	44.63±1.52 <sup>ab</sup>	44.27±1.36 <sup>b</sup>	39.80±0.06 <sup>c</sup>	39.28±0.45 <sup>c</sup>

<sup>1/</sup> Each value was expressed as mean± standard deviation.
2/ a-f; values with the same small letter within row are significantly different (P<0.05)

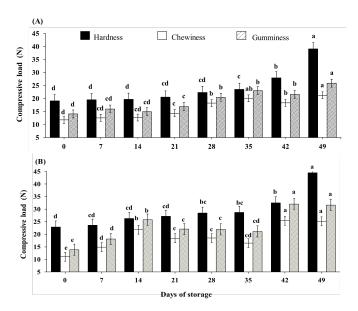


Figure 1. Hardness, gumminess, chewiness values of sous-vided (A) and grilled sous-vided (B) restructured goat meat stored at 4ºC for 49 days. (a-e) values with the same letter within the same parameter were significant different (P<0.05).

After grilling, the grilling loss increased during storage (P<0.05) which according to studies of Jinap *et al.* (2015). Hardness, gumminess, and chewiness were significantly increased (P<0.05). Similar results have been reported by Bainy *et al.* (2015) that burgers cooked up to higher internal temperatures around 80°C presented lower texture values.

This study showed that storage time had the negative effect on microbiological and physical qualities of sous-vided sample. Moreover, the shelf-life of the sous-vided sample was 28 days during storage at 4°C.

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## Effect of sterility value on qualities of Chinese braised culled steer beef in retort pouch

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**Abstract:** The results showed that their cook values were 72.42, 87.05 and 95.04 min, respectively. Microbiological analysis and sterility test proofed that samples produced by all  $F_0$  values were safety for microbiological quality. However, parameters of texture profile (hardness, gumminess, springiness and chewiness) decreased as  $F_0$  value was increased (p<0.05). Similarly, shear force decreased with increasing  $F_0$  value (p<0.05). The CIE L\* value increased and CIE a\* values decreased as  $F_0$  value was increased. For sensory evaluation, semi-trained panelists mostly impressed this product produced by  $F_0$  value of 10 min (p<0.05). Therefore, the development of Chinese braised beef in retort pouch was satisfied with  $F_0$  value of 10 min.

Keywords: Braised beef top round, Toughness beef, Retort pouch, Moist heat sterilization, Fo value

### Introduction

In Thailand, beef cattle have been consumed progressively. The top round muscle of culled steer is low value because it has low marbling, providing tough (Moon, 2006). There have been studies on poor quality of meat. The long time moist heat has been used for poor quality meat due to decreasing toughness (Chumngoen *et al.*, 2018). The fast growing economy of Thailand, people tended to spend less time on preparing food. Over the past few years, food industries have been developed convenience food along with ready for consumption and ready made products to appropriate the life style of clients. The main concerns of sterilization food are food quality and safety. Generally, the extremely problematic microorganism in low acid food (pH>4.5) is *Clostridium botulinum* due to producing botulinum toxin. For sterilization process, the minimal suggested process is to reach 12 D reductions for *C. botulinum* by the temperature of 121°C. This is used in link against the z value for *C. botulinum* to creation of standard lethality table. It's probable to sum the lethalities for a process and estimate the total effects of integrated lethality or F<sub>0</sub> value. Thus, thermal process is one of the most preserving and extending shelf-life effects (Brennan *et al.*, 1990).

Normally, the ready to eat products have associatively long shelf-life due to external regions transportation as the export commodities than other food products. These products have nutritive and sensory values as well as protect them from the attacks of insects and microbes (Gopinath *et al.*, 2007). Retort pouch is the one of most progress in food packaging. The retortable pouches were used to transport easier than steel cans due to making form laminate of flexible plastic and metal foil. Hence, it helps the lower weight and safe storage space (Majumdar *et al.*, 2015). Traditional meat curry of Kashmir in retort pouch produced by F<sub>0</sub> value of 9 min had the highest overall acceptability (Shah *et al.*, 2017). Various researchers demonstrated that thermal processed meat in retort pouches had flavorsome and shelf life more than one year stored at room temperature (Mohan *et al.*, 2006; Bindu *et al.*, 2007; Maheswara *et al.*, 2011; Tribuzi *et al.*, 2015 and Shah *et al.*, 2017).

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However, there have been few studies on the thermal process for added value of poor quality beef. Thus, the aim of this study was carried out the effect of thermal processing on quality of Chinese braised beef in retort pouch at different F<sub>0</sub> values.

### Materials and methods

## Process of Chinese braised culled steer beef in retort pouch

The semimembranosus (top round muscle) was selected on the basis of a culled steer carcass (Chalolais×Brahmn×native). The culled steer had less than 549 kg of weight and 30 months of age from a beef cattle farm in Rayong Province, Thailand. The visible connective tissue and fat were removed. After that, it was stored at  $20 \pm 2^{\circ}$ C before used. The frozen meat was thawed at  $4^{\circ}$ C on the two day before test. It was cut into small cubes of  $4\times3\times2.5$  cm.

The flow chart for production of Chinese braised beef in retort pouch was shown in Figure 1. The recipe of Chinese soup comprising 0.2% golgi berry, 0.2% cinnamon, 0.3% star anise, 0.3% black pepper, 0.1% coraninder seed, 0.2% cadamon, 9.0% soy sauce and 10.0% sugar were boiled in water until seethe. For the prepareation of Chinese braised beef, meat was boiled in the Chinese soup in the ratio of 1:1 (meat:water) for 2 hour. The product was cooled and packed in retort pouches.

Indigenously manufactured retortable pouch (Royal Meiwa Pax Co., Ltd. Samut Sakhon, Thailand) having a four – layer (12  $\mu$ m polyethylene terephthalate/15  $\mu$ m nylon/9  $\mu$ m aluminium foil/80  $\mu$ m cast polypropylene) and size of 15×20 cm were used. About 120±2 g beef and 210±2 g Chinese soup was contained in retort pouch, then the retort pouch was sealed in a vacuum sealing machine. Ninety pouches were distributed evenly into three batches. After that, the pouches were placed on perforated trays and then moved batch wise into retort vessel. The horizontal stationary retorting system (Km Grand Pack Co., Ltd., Samut Prakan, Thailand) was used. The retort pouch was fixed with thermocouple glands and thermocouple probe in coldest point. Thermocouple output was determined using Ellab CTF 9008 data recorder (Ellab A/S, Roedovre, Denmark). The retort temperature was maintained at 121 $^{\circ}$ C and processing time was different to F<sub>0</sub> values of 8, 10 and 12 min. The pouches were cooled to 45 $^{\circ}$ C after retort processing.

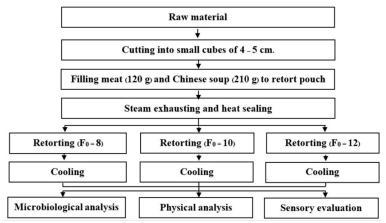
## Cook value

The cook value was determined by measuring the extent of cooking and nutritional loss during processing in a manner similar to the  $F_0$  value, except that the reference temperature is  $100^{\circ}$ C instead of  $121^{\circ}$ C, and the z value of  $33^{\circ}$ C, which is required for the denaturation of thiamine (Ranganna *et al.*, 2002).

### Microbiological analysis and sterility test

Twenty-five gram of sample was aspectically transferred into 225 milliliters of 0.85% sodium chloride and homogenized for 1 minnute in a Stomacher Bag Mixers (400 model VW, France) and cultured on media for microbial enumeration. The mosophilic, thermophilic aerobic and anaerbobic were determined by pour plate technique on plate count agar incubated at 37°C for 48 h. and 55°C for 72 h. under aerobic and anaerobic condition (IS Indian Standard, 1971) respectively. The number of colonies was counted and declared as log cfu/g (BAM, 2001a). Total yeast and mold (BAM, 2001b), *Staphylococcus aureus* (BAM, 2001c), *Clostridium perfringens* (BAM, 2001d), coliforms and *Escherichia coli* (BAM, 2002), *Salmonella* spp. (BAM, 2007) and *C. botulinum* (BAM, 2007) were analyzed.

Sterility test method was analyzed and followed by Tribuzi *et al.* (2015). Twelve samples were divied for two blocks and incubated at  $36 \pm 1^{\circ}$ C for 10 days and at  $55 \pm 1^{\circ}$ C for 5 days. When finished of incubation period, the retort pouches were optically to identify possible swelling. After that, the products were opened of verify the off-odors at room temperature and determined microbiological analysis.



**Figure 1.** Pre – cooking and thermal processing of Chinese braised beef in retort pouch.

## Physical analysis

The color of samples was estimated in the CIE L\*, CIE a\* and CIE b\* mode, using a color measurement Hunter Lab colorimeter (MiniScan EZ, HunterLab, Reston, VA, USA). Three locations of each sample were accomplished. The resulting average was used in data analysis.

The Warner–Bratzler shear force (WBSF) of sample was estimated. A sample was cut into eight rectangular cubes along the longitudinal of muscle fiber for each beef (1×1×2.5 cm). The sample was sheared with a WBSF equipment attached to an Instron universal testing machine (3344, Instron Engineering Corp., Canton, MA, USA). The maximum force (N) was recorded (Sorapukdee and Tangwatcharin, 2018).

Texture profile analysis of sample was performd 5 times in every treatments on the beef using an Instron universal testing machine model 3344 (Illinois Tool Works Inc., USA) with a compression plate surface. A sample was cut into eight cubes along the longitudinal of muscle fiber (1.5×1.5×0.5 cm). TPA parameters were determined at room temperature. The crosshead speed was 60 mm/min and compressed twice to 40 was set for experiment condition. The processing and data recording were used by the Bluehill 2 software (Instron Engineering Corp., USA). The hardness (N), cohesiveness (ratio), gumminess (N), springiness (ratio), and chewiness (N) were estimated from the force-time curves generated for sample (Bourne, 1978).

## Sensory evaluation

Sensory attributes of sample consider to appearance, spice odor, taste, texture and overall acceptability of the sample were evaluated by 30 semi-trained panelists, who are familiar with the stew beef from academic staffs and graduate students of Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang. Sensory attributes were evaluated using a nine-points hedonic scale form 1 = dislike extremely to 9 = like extremely.

## Statistic Analysis

The experiment was conducted as randomized complete block design. Three indepentdent batch replications were conducted. Significant effects (p<0.05) were performed by one-way ANOVA and mean comparison were compared by the Duncan's multiple range test. Data were analysed using SPSS (v.17. IBM SPSS Inc.).

# Results

## Thermal processing and cook value

The retort pouch was processed three different  $F_0$  values of 8, 10 and 12 min. Result showed the heat penetration curve of products during sterilize which could be concerned in three parts including, come up time, heating and cooling (Figure 2). The total process time of three different  $F_0$  values was 17, 21 and 23 min, respectively. All process times were sufficient to obtain commercially sterile products. The cook values of the heat treatment related to nutrient degradation which were 75.42, 87.05 and 95.24 min, respectively.

## Microbiological analysis and sterility test

The numbers of microbe in all samples were counted by standard plate count (SPC) procedures for four ambients, total yeast and mold, *S. aureus* and *C. perfringens* which detected as <1 log cfu/g. *Salmonella* spp. and *C. botulinum* were not detected in sample 25 g. Coliforms and *E. coli* were <3 MPN/g. For sterility test, samples were analyzed by visuality after the incubation. They did not swell and find any microorganisms by SPC procedure.

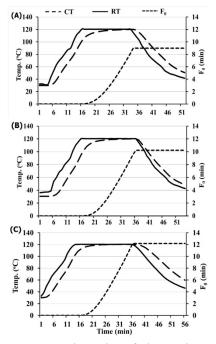


Figure 2. Heat penetration characteristic and  $F_0$  value of Chinese braised beef in retort pouch thermal process at  $121^{\circ}$ C  $F_0 = 8$  (A),  $F_0 = 10$  (B), and  $F_0 = 12$  (C). CT, product core tempareture, RT, retort tempareture,  $F_0$ ,  $F_0$  value.

## Physical quality

The physical parameters of sample thermally processed at different  $F_0$  values were shown in Table 1. The results of CIE L\* indicated that sample processed by  $F_0$  values of 8 min higher than those of  $F_0$  values of 12 min (p<0.05), but it was not different when compared to  $F_0$  values of 10 min. In the case of CIE a\* value, sample processed by  $F_0$  values of 10 min was a higher than those of  $F_0$  values of 8 min (p<0.05), although it was not different when compared to  $F_0$  values of 12 min. No differences were found between treatments for CIE b\* value (p>0.05).

The shear forces value of braised beef in retort pouch at  $F_0$  values of 12 min conditions was a lower than other treatment (p<0.05). For TPA analysis, hardness, gumminess, springiness and chewiness values of sample decreased as  $F_0$  values increased (p<0.05).

**Table 1.** Physical parameters of Chinese brised beef in retort pouch thermally processed at 121°C to F<sub>0</sub> values of 8, 10 and 12 min.

Physical parameters	$F_0 = 8$	$F_0 = 10$	$F_0 = 12$
Color			
CIE L*	$35.14 \pm 0.80^{a/1,2}$	$34.35 \pm 2.03$ ab	33.55 ± 2.88 b
CIE a*	10.95 ± 1.71 <sup>b</sup>	12.66 ± 2.20 a	$11.64 \pm 1.20$ ab
CIE b*	20.20 ± 1.85	19.70 ± 5.84	18.04 ± 2.77
Shear force (N)	$3.44 \pm 1.04^{\circ}$	$1.43 \pm 0.11^{b}$	0.92 ± 0.25 <sup>a</sup>
Total profile analysis			
Hardness (N)	4.64 ± 0.80 <sup>b</sup>	$4.03 \pm 0.68$ b	$2.33 \pm 0.24^{a}$
Cohesiveness (ratio)	$0.51 \pm 0.10$	$0.47 \pm 0.03$	$0.46 \pm 0.09$
Gumminess (N)	2.37 ± 0.54 <sup>b</sup>	$1.87 \pm 0.22$ b	1.11 ± 0.33 <sup>a</sup>
Springiness (ratio)	$0.84 \pm 0.02$ b	$0.79 \pm 0.04^{a}$	$0.74 \pm 0.74$ a
Chewiness (N)	1.75 ± 0.32 <sup>b</sup>	$1.43 \pm 0.11^{b}$	0.92 ± 0.25 <sup>a</sup>

 $<sup>^{1/}</sup>$  Each value was expressed as mean  $\pm$  standard deviation of at least 3 replications.

## Sensory evaluation

The result of 30 semi-trained panelists sensory evaluation is shown in Table 2. The sample processed by  $F_0$  values of 10 min revealed the highest of spice odor, texture, and overall acceptable liking scores compared to other treatments (p<0.05). No differences were found between treatments for appearance and taste (p>0.05).

**Table 2.** Sensory evauation of Chinese braised beef in retort pouch thermally processed at 121°C to F<sub>0</sub> values 8, 10 and 12 min.

Sensory attribute	$F_0 = 8$	$F_0 = 10$	$F_0 = 12$
Appearance	7.13 ± 1.31 <sup>/1</sup>	7.17 ± 1.42	6.90 ± 1.49
Spice odor	$6.53 \pm 1.23^{b/2}$	$7.80 \pm 1.33^{a}$	6.50 ± 1.25 <sup>b</sup>
Taste	6.73 ± 1.37	7.50 ± 1.19	6.36 ± 1.43
Texture	7.13 ± 1.28 <sup>b</sup>	$8.13 \pm 1.32^{a}$	6.37 ± 1.60 °
Overall acceptable	7.10 ± 1.30 <sup>b</sup>	8.13 ± 1.16 a	6.53 ± 0.91 °

 $<sup>^{1/}</sup>$ Each value was expressed as mean  $\pm$  standard deviation of at least 3 replications.

<sup>&</sup>lt;sup>2/a-c</sup> Different letters in the same row were significant difference (P<0.05).

<sup>&</sup>lt;sup>2/a-c</sup> Different letters in the same row were significant difference (P<0.05).

#### Discussion

The microbiological analysis did not detect the microbial growth in all samples. It demonstrated that the microorganism has been entirely destroyed by the thermal process (Shah  $et\ al.$ , 2017). The F<sub>0</sub> value agreed with Brennan  $et\ al.$  (1990). F<sub>0</sub> value of 10 min was perfectly used for meat products on the United Kingdom market.

The present study, the lightness (CIE L\*) value decreased with  $F_0$  value increased. Similar to result of previous studies, CIE L\* value of fish curry and meat curry in retort pouch were decreased with  $F_0$  value increased, due to longer heating time (Majumdar *et al.*, 2015; Shah *et al.*, 2017). In addition, it might be reason from maillard reaction between sugar and amino acid which is changed to brown and protracted heat treatment. It affects the color of the beef which is changed darker (Bindu *et al.*, 2008; Majumdar *et al.*, 2015 and Shah *et al.*, 2017). Whereas, CIE a\* value in this study increased with increased in  $F_0$  value. Similarlly, CIE a\* value of catla in curry medium product was a increasing red with increasing the  $F_0$  value (Majumdar *et al.*, 2015). The processing time had no significant effect on CIE b\* value.

In this study, shear force value decreased with increased in  $F_0$  value. According to study of Shah *et al.* (2017), who reported the shear force value of meat curry, decreased with  $F_0$  value increased. Similar to study of Palka (1999), it might be noted to the the cooking or heating improves tenderness by making collagen soluble, which is the main reason for thoughness. A hight temperature and long heating time result in gelatin formation and tenderness.

In this experiment, TPA values (hardness, gumminess, springiness and chewiness) were decreased with  $F_0$  value increased. Moreover, Chinese braised beef in retort pouch after thermal process was lower value of hardness, gumminess, springiness and chewiness value than these of product before thermal process (data not show). Similar to study of Majumdar *et al.* (2015) the hardness, gumminess, springiness and chewiness of catla in curry medium decreased with  $F_0$  value increased. These decreases of TPA values were mainly due to increase in the heat time. According to study of Bindu *et al.* (2007), it might be noted to the reasons from denaturation of protein content and the destruction of muscle cells during retort processing.

For sensory evaluation, Chinese braised beef at  $F_0$  value of 10 min showed the highest scores for spice odor, texture and overall acceptable. The panelists explained that sample processed by  $F_0$  value of 10 min was the best texture. Whereas, the texture at  $F_0$  value of 8 min was too tough, but it at the  $F_0$  value of 12 min was too soft. Similarly, shear force value decreased with increased in  $F_0$  value.

The results of product indicated that the microorganisms were completely destroyed since  $F_0$  value of 8 min. However, sample processed by  $F_0$  value of 10 min had the best sensory evaluation which were the highest scores of spice odor, texture and overall acceptable. Therefore,  $F_0$  value of 10 min was an optimum thermal processing for commercially sterile of Chinese braised culled steer beef in retort pouch.

## Acknowledegement

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## Identification of Morphology and Pathogenicity of Pyricularia sp. Causing Blast Disease in Grass

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**Abstract** Morphological of 27 *Pyricularia* sp. isolates obtained from grass expressing blast disease symptoms was characterized using RFA (Rice flour agar) media and 8 colony types were grouped. The colony color was greyish green. The conidia shapes were pyriform or pear shape with rounded base and narrow toward the tip which is pointed or blunt. Study of pathogenicity on 40 Thai elite rice varieties showed that only 3 out of 27 isolates could infect different Thai elite rice varieties. Spot lesion to eye shape symptoms were founded on rice leaves. The isolate LPG61102 could infect the most numbers of Thai elite rice varieties with the virulence index of 0.15. While 24 isolates were unable to infect any of 40 tested rice varieties. This first report in Thailand on *Pyricularia* sp. causing blast disease in grass provided preliminary information between the two host species.

Keywords: Blast disease, Blast fungus, Pathogenicity test

### Introduction

Fungal species from the *Pyricularia* genus are associated with blast disease in plants from the Poaceae family, causing losses in economically important crops such as rice, oat, rye, barley, wheat and triticale. *Pyricularia grisea* (Cooke) Sacc. and *P. oryzae* Cav. attacks a wide variety of grasses (Poaceae) family. The species of *Pyricularia* share some morphological traits, while *P. oryzae* and *P. grisea* can be differentiated from other species by morphological traits (Klaubauf *et al.*, 2014). However, no detectable morphological characteristics that are distinct between *P. grisea* and *P. oryzae* have been reported until now. The name *P. grisea* has been widely used since one isolate from crabgrass was first identified in 1880 (Saccardo, 1880), another name, *P. oryzae* has been used since an isolate from rice broad, however, individual strains infect a limited number of grass species (Ou, 1985; Borromeo *et al.*, 1993; Urashima *et al.*, 1993).

The pathogenicity of *P. grisea* have been reported to caused responses in goose grass, weeping love grass, and rice that varied from no visible symptoms, through isolated discrete lesions with or without brown margins, to coalescing lesions that completely killed the inoculated areas of the leaf. The fungus sporulated under conducive conditions, but the degree of sporulation varied considerably, and produced visible symptoms (Heath *et al.*, 1990). It has been reported the pathogenicity of *P. grisea* and *P. oryzae* on green bristlegrass and rice that, pathogenicity of *P. oryzae* isolates from green bristlegrass showed cross-infectivity on green bristlegrass and rice, respectively. The results demonstrated that isolates from green bristlegrass and crabgrass consist of both *P. oryzae* and *P. grisea*, and that *P. oryzae* isolates showed cross-infectivity between rice and green bristlegrass, suggesting host shifts might occur in *P. oryzae* and *P. grisea* (Qi *et al.*, 2019).

In Thailand, the data of grass infected *Pyricularia* sp. was limited. The objective of this study was aimed at identification of the blast isolates collected from area where rice blast disease epidemic was occurred. Their

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morphology was observed, and pathogenicity was assessed using Thai elite rice varieties. Preliminary results obtained in this study will be used for further analysis on the relationships between the 2 hosts of grass infected blast fungi.

#### Materials and methods

### Collection and Isolation of blast pathogen

Samples with typical blast symptoms on grass leaves were collected from different rice growing regions of Thailand during growing season in year 2018. For isolation of single spore, the infected leaves of diseased plants were cut into small pieces and placed on moist filter paper in Petri dishes, then incubated under light for 24 h at 25 °C. Single spore was picked with a fine glass needle under a binocular microscope. Each single spore was transferred onto rice flour agar (RFA) medium. In order to maintain blast isolates for further use, each isolate was grown on RFA where surface was covered with filter paper for 7-14 days. The filter paper was dried and stored at -20 °C.

### Examination of morphological characteristics

Each isolate was cultured on RFA media at 25 °C for 5 days and mycelial disk was transferred to the center of a new RFA medium. The colony morphology and colony color of each isolate on RFA medium were examined daily for 14 days. Sporulation was induced by scraping mycelium in each plate and the plate was let for 48 hr. The spores of each isolate were harvested and mounted in lactophenol cotton blue. The spore morphology was observed, and spore diameter was measured from 100 spores for each isolate using image analyzer under microscope.

#### Plant Material

A total of 40 Thai elite rice varieties were used in pathogenicity test. The standard control varieties, JHN, IR64 and KDML105 were included (Table 1). Germinated seeds were planted in a seedling tray (33  $\times$  11  $\times$  11 cm) and fertilizer was supplied at 7 days after planting by adding ammonium sulphate at 1 g/tray. Seedling were in a greenhouse until use.

### Rice Blast Inoculation

Each blast isolates to be used in pathogenicity test was grown on RFA medium and incubated at  $25^{\circ}$ C. After 8-10 days, sporulation was induced by scraping mycelium in each plate. Counting of spores was performed by using a hemocytometer and concentration of spore suspension was adjusted to  $1\times10^{5}$  spores/ml in 0.5% gelatin for spray inoculation. Inoculation was done by spraying on 14-day-old rice seedlings. The inoculated plants were incubated for 24 h in a humid chamber and then transferred to a glasshouse. The experiment was repeated twice with 4 plants each. Blast symptoms were evaluated 7 days after inoculation using a 0-6 scale based on the type and size of lesions (Roumen *et al.*, 1997). Rice lines that only showed scores from 0 to 2 (no sporulating lesion) were considered as resistant; rice lines were considered susceptible if the majority of the lesions developed were type 3-6. The virulence index (VI) of blast fungus was calculated using formula:

**Table 1.** Forty Thai elite rice varieties used in pathogenicity test.

No	Varieties	Region *	Blast** Resistan ce	No	Varieties	Region *	Blast** Resistanc
1	RD27	С	R	21	Chai Nat 1	All	<u>е</u> В
2	RD41	N	R	22	Pathum Thani 1	C	R
3	RD6	N and NE	R	23	Leb Nok Pattani	S	S
4	RD43	All	R	24	Plai Ngahm Prachin Buri	С	R
5	RD59	N and C	R	25	Prachin Buri 2	С	R
6	RD7	All	S	26	Sang Yod Phattalung	S	S
7	RD61	N and C	R	27	Hahng Yi 71	NE	R
8	Jow Khao Chiangmai	N	R	28	Red Hawn Rice	NE	R
9	Phitsanulok 1	N and C	R	29	Yipun DOA1	N and NE	S
10	Khlong Luang 1	С	R	30	RD8	Ν	-
11	RD31	С	S	31	Homcholasit	С	-
12	Phitsanulok 60_1	All	S	32	RDP3	С	R
13	Suphan Buri 1	NE	R	33	Yipun DOA2	Ν	S
14	Niaw Ubon 1	All	S	34	RD29	N and C	R
15	Suphan Buri 2	С	R	35	Sunpatong 1	Ν	R
16	Suphan Buri 3	All	R	36	Phrae 1	Ν	R
17	Phitsanulok 2	С	R	37	RD10	Ν	S
18	Suphan Buri 60	С	R	38	RD14	Ν	R
19	Suphan Buri 90	С	R	39	KDML105	NE	S
20	Sakon Nakhon	NE	S	40	Jao Hom Nin	NE	R

<sup>\*</sup>C (Central), N (North), NE (Northeast) and S (South)

## Results

# Collection and Isolation of blast pathogen

Blast samples were collected from grass leaves grown around the edge of rice paddy field in 12 provinces of Thailand including Lampang (North), Khon Kaen (North East), Lopburi, Phichit, Phitsanulok, Phetchabun, Sing Buri, Uthai Thani (Central), Ratchaburi, Tak (West), Nakhon Si Thammarat and Phatthalung (South). Using single spore isolation technique, total number of 27 isolates were obtained. (Table 2)

<sup>\*\*</sup>R (Resistances), S (Susceptible) and – (No data)

## Morphological characteristics of grass infected Pyricularia sp.

The 27 isolates of grass infected *Pyricularia* sp. were assigned to 8 morphological groups based on the differences in morphological characteristics (colony color and colony morphology). Various isolates produced circular colonies with rough and smooth margins on RFA media which the color was varied from light yellow, white, to grayish colors. The colony type of Group 1 was greenish gray to white gray in the middle and the edge of colony was circular smooth, Group 2 was black grayish to greenish gray color in the middle and the edge of colony was circular smooth, Group 3 was white colony color and white gray circular smooth at the edge, Group 4 was grayish to white gray color at the middle and the edge of colony was white, Group 5 was white gray to white color at the middle and the edge of colony was white circular smooth, Group 6 was greenish gray at the middle and the edge of colony was circular smooth, Group 7 was grayish white at the middle and the edge of colony was grayish yellow with circular smooth, Group 8 was white and the edge of colony was circular smooth. The conidia shape was pyriform (pear shaped) with rounded base and narrowed towards the tip which is pointed or blunt (Figure 1). The colony diameters of all groups were ranged from 6 to 9 cm (data not shown).

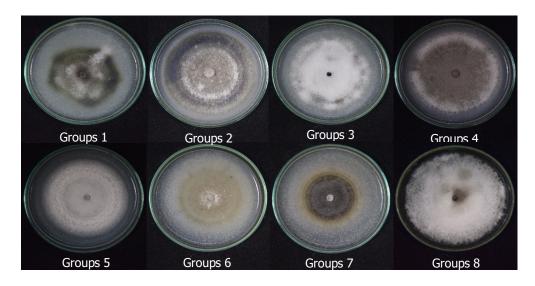
**Table 2.** Location, host, given isolate name, pathogenicity and morphology classification of 27 grass infected *Pyricularia* sp. isolates.

Province	Host	Isolate	Pathogenicity	Morphology
Province	позі	number	group	group
Khon Kaen	grass	KKN61102	1	6
	grass	KKN61103	1	5
Lampang	grass	LPG61102	2	3
	grass	LPG61103	1	1
Lopburi	grass	LRI61104	1	5
Nakhon Si Thammarat	grass	NST61101	1	1
Phichit	grass	PCT61104	1	7
Phatthalung	grass	PL61121	1	6
	grass	PL61122	1	6
Phitsanulok	grass	PLK61106	2	5
	grass	PLK61107	1	1
	grass	PLK61110	1	7
Phetchabun	grass	PNB61105	1	8
	grass	PNB61106	1	6
	grass	PNB61107	1	2
	grass	PNB61110	1	2
Ratchaburi	grass	RBR61109	1	4
	grass	RBR61110	1	5
	grass	RBR61111	1	2
Sing Buri	grass	SBR61101	1	1
	grass	SBR61104	1	1
Tak	grass	TAK61104	1	8
	grass	TAK61105	2	6
Uthai Thani	grass	UTI61102	1	3
	grass	UTI61104	1	6
	grass	UTI61106	1	5
	grass	UTI61107	1	3

## Pathogenicity test

Study of disease reaction on 40 Thai elite rice varieties showed that the pathogenicity of 27 isolates differed in pathogenicity. Blast symptoms were first appeared as small spot and later enlarge up to roundish, slightly elongated to narrow or slightly elliptical lesions more than 3 mm long with a brown. Based on the blast lesions and affected area of leaves, symptoms were scored, and the 27 isolates were designated into 2 groups. The members of the first group were avirulent to tested rice varieties consisting of 24 isolates including KKN61102, KKN61103, LPG61103, LRI61104, NST61101, PCT61104, PL61121, PL61122, PLK61107, PLK61110, PNB61105, PNB61106, PNB61107, PNB61110, RBR61109, RBR61110, RBR61111, SBR61101, SBR61104, TAK61104, UTI61102, UTI61104, UTI61106 and UTI61107. Members of the second group were virulent, consisting of 3 isolates including LPG61102, PLK61106 and TAK61105 (Table 1, Figure 2)

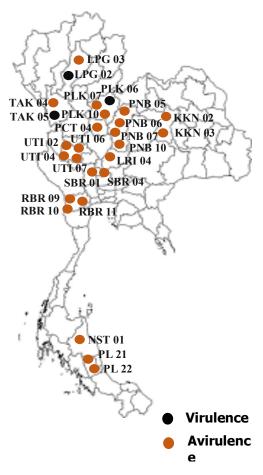
The results showed that the most virulence was the isolate LPG61102. The isolate could infect 6 varieties of Thai elite rice including RD59, Suphan Buri 60, Chai Nat 1, RDP3, RD29 and RD15 with the virulence index scored 0.15. The isolate TAK61105 could infect 5 varieties including RD43, Suphan Buri 1, Niaw Ubon 1, Phitsanulok 2 and Suphan Buri 60 with the virulence index scored 0.13. The isolate PLK61106 could infect 2 varieties of elite Thai rice varieties including Suphan Buri 3 and Sunpatong 1 with the virulence index scored 0.05. While 24 isolates were unable to infect any of 40 Thai elite rice varieties being tested (Table 3).



**Figure 1.** Morphological characteristics of 27 *Pyricularia* sp. isolated from grasses.

**Table 3.** The virulence index of blast fungus tested on Thai elite rice varieties.

Isolate	Virulance Index	Isolate	Virulance Index	Isolate	Virulane Index
KKN61102	0	PLK61106	0.05	RBR61111	0
KKN61103	0	PLK61107	0	SBR61101	0
LPG61102	0.15	PLK61110	0	SBR61104	0
LPG61103	0	PNB61105	0	TAK61104	0
LRI61104	0	PNB61106	0	TAK61105	0.13
NST61101	0	PNB61107	0	UTI61102	0
PCT61104	0	PNB61110	0	UTI61104	0
PL61121	0	RBR61109	0	UTI61106	0
PL61122	0	RBR61110	0	UTI61107	0



**Figure 2.** Distribution of 27 blast isolates collected from 12 provinces of Thailand, virulence isolates were indicated in black and avirulence isolates were in brown.

### Discussion

The result show that 27 isolates of *Pyricularia* sp. collected from grasses were assigned to 8 morphological groups based on the differences in morphological characteristics (colony color, colony and morphology). Blast fungus circular colonies with rough or smooth margin on RFA media. The isolate showed light yellow color, white to grayish color. The colony diameter was ranged from 6-9 cm. The conidia shape was pyriform with rounded base and narrowed towards the tip, which is pointed or blunt. The colony morphology of grass infected *Pyricularia* sp. found in this study was similar to the observation reported in morphological characteristics (colony color, colony morphology and conidia shape) that 10 observed isolates of *P. oryzae* could be assigned to six morphological groups (PG-I to PG-VI). Various isolates produced ring like, circular, irregular colonies with rough and smooth margins on oatmeal agar media having buff color, grayish black to black color. The colony diameters of different groups were ranged from 67.40 to 82.50 mm (Srivastava *et al.*, 2014).

Study of disease reactions on 40 Thai elite rice varieties showed that the pathogenicity of 27 grass infected isolates differed in pathogenicity. Three isolates were found to cause blast symptoms on tested rice varieties. Symptoms on rice leaves were first appeared as small spot and later enlarged up roundish, slightly elongated sporulating spots to narrow or slightly elliptical lesions more than 3 mm long with a brown. The observation in this study accorded by the report of pathogenicity test of *P. grisea* isolated from goose grass, weeping love grass, and rice that varied from no visible symptoms, through isolated discrete lesions with or without brown margins, to coalescing lesions that completely killed the inoculated areas of the leaf (Heath *et al.*, 1990).

The results showed that 3 isolates could infect 6, 5 and 2 rice varieties were LPG61102, TAK61105 and PLK61106 respectively, while others were unable to infect any of 40 tested varieties. It has been reported the pathogenicity tests using artificial inoculation, 6 out of 10 *P. oryzae* isolates from rice and 3 isolates from green bristlegrass showed cross-infectivity on green bristlegrass and rice, respectively. The results demonstrated that isolates from green bristlegrass and crabgrass consist of both *P. oryzae* and *P. grisea*, and that *P. oryzae* isolates showed cross-infectivity between rice and green bristlegrass, suggesting host shifts might occur in *P. oryzae* and *P. grisea* (Qi *et al.*, 2019).

### Acknowledgement

This study was a part of the project "Development of rice blast resistance Khao Dawk Mali 105 variety using *Pi9* resistance gene locus including 4 alleles *Pi2*, *Pi9*, *Piz* and *Piz-t* and study the defense mechanism in rice containing resistance gene *Pik* or *Pi9*", under financial support by The Agricultural Research Development Agency (Public Organization), Thailand.

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## Selection of appropriate of ornamental plant species for outdoor vertical garden

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Abstract The appropriated plant species for the outdoor vertical garden was reported using the hydroponic system. The nutrient solutions were supplied to the plants on felt panel system. The electrical conductivity value of nutrient solutions in the hydroponic system was 0.8 mS/cm. As a result, the light intensity, survival rate and beautifulness score of the plant were known. The seven plant species with more than 80% survival rate and one plant species with 19% survival rate were observed. In addition, the average of beautifulness score of plant species was 1.00-4.00. However, the beautifulness score which was higher than four resulted in four plant species including *Schefflera* sp., *Tradescantia spathacea* Sw., *Tradescantia pallida* (Rose) Hunt, and *Rhipsalis cereuscula*. Therefore, these four ornamental plant species would be considered as an ornamental plant species for outdoor vertical garden in the future.

Keywords: Hydroponic cultivation, nutrient solution, felt panel system

### Introduction

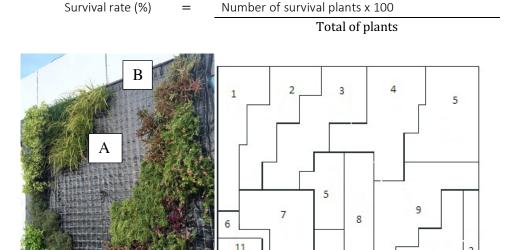
Recently, vertical gardening has become popular in many countries, including Thailand, and people are now increasingly interested in the environment, with fewer vacant areas in the city. The increasing of green space for urban residents and current urban life, living in small houses, townhouses, and condominiums these days are difficult. The space for gardening is limited. Therefore, landscaping of small empty space using the wall would be possibly interested (Sunakorn, 2011). With the advent of the modern industrial city, planners, designers and urban advocates are once again turned to plants-infrastructure as a key strategy to provide cleaner air and water and to improve living environments, human health, and mental well-being. Hydroponics is a way of growing plants in a soilless environment with the use of nutrient solutions. In this method, plants may be grown with their roots in the mineral nutrient solution in an inert or organic medium. The most important benefits of using hydroponics can be practiced even in places where ordinary gardening is impossible; green facades (Salas et al., 2010), and pergolas (Montero et al., 2010). Ecologic terraces can be used as soilless gardens at a relatively low cost. Other advantages of using these methods include faster plant growth, fewer plant infections diseases, lighter cultivation media, and the possibility to recuperate drainage to be recirculated (Salas, 2008; Salas, 2009; Van Os, 1998). The direct delivery of fertilizers through drip irrigation demands the use of soluble fertilizers and pumping and injection systems for introducing the fertilizers directly into the irrigation system. The use of hydroponic techniques with recirculating nutrient solution (Montero et al., 2010; Salas, 2009) to design a new model of gardens that means as a new way to get the profits that traditional gardening offered, as for example natural shade, vegetal covered surface or green urban landscape, with advantages, as they are the high water waste or the need of specifically prepared soil. However, there is a lack of information on vertical gardening in Thailand, especially the selection of plant species to suit the pattern of planting and the environment to maintain the beautifulness and taking care of plant species in the hydroponic system. In addition, the important factors of vertical gardening systems are an automatic irrigation system that provides fertilizer to plants

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together with water systems. Therefore, the objective of this study was investigated in plant species that are suitable for the outdoor vertical garden using hydroponic supplied the nutrient solutions via the felt system.

#### Materials and methods

The location of the study experiment was conducted at mezzanine outside the 5<sup>th</sup>-floor building, Bunnak Building of Faculty of Agricultural Technology, King's Mongkut Institute of Technology Ladkrabang, Thailand. The experiment was a Randomized complete block design with six replications and done from August 2018 to January 2019. The twelve ornamental plant species; Schefflera sp., Rhipsalis simmleri cereuscula aka 'Rice Cactus', Sansevieria trifasciata hort. ex Prain 'Golden Hahnii', Gardenia augusta (L.) Merr., Gardenia jasminoides 'Radicans Variegata', Pandanus stellatus R. Br., Pandanus pygmaeus Thours., Tradescantia spathacea Sw., Xyphidium caeruleum Aubl., Neoregelia fireball donger, Tradescantia pallida (Rose) Hunt. and Rhipsalis cereuscula. were grown under hydroponic nutrient solution in the felt system (Figure 1). The structure of the vertical building was made from the iron which adhered to the wall of the building and left the distance about 10 cm. The size of the plate was 3.0 m  $\times$  3.5 m. The curtain was made by polyvinylchloride and knitted to be plate and dovetailed with the sponge (for keeping the moisture). The plate was stitched to be squares in the size of 10 x 12 cm. Then slit the holes likely the pockets. Nutrient solutions of hydroponic use Hoagland solution formula (Hoagland and Snyder, 1933). The data record was collected as light intensity, plant survival rate, and beautifulness. The light intensity was measured using tasi digital data logging light intensity meter model: TA8133 which divided to the plate into six points and collected data monthly and measured three times at 9 AM, 12 PM and 4 PM. Survival rate was calculated as follows:



2

10

5

Figure 1. Vertical garden structure and the position of ornamental plants on the planting panel. A) planting panels assembled with steel frames attached to the building walls B) the water inlet is sent from the pump pressure to the top of the panel and C) the slurry tank.

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The Beautifulness rate was rated by six peoples every month interval for eight months according to the method of Treenusorn et al. (2013). 0 = No beautifulness (died), 1 = Low beautifulness, 2 = Rather low beautifulness, 3 = Rather low beautifulness, 4 = Rather low beautifulness and 4 = Rather low beautifulness and 4 = Rather low beautifulness.

#### Results

## Light intensity

The optimum light intensity for the plants in the range is around 14,000-16,000 Lux. The results of light intensity values of the plants received in each period are morning (22,547 Lux), afternoon (102,059 Lux), and evening (29,147 Lux), with a total average measured at 51,251 Lux present data record in Table 1.

**Table 1.** light intensity measured three times at 9 AM, 12 PM and 4 PM.

Time		Months after planting							Average
	1	2	3	4	5	6	7	8	-
9 AM	23,747	23,347	24,147	21,647	21,347	21,747	23,447	20,947	22,547
12 PM	103,259	102,859	103,659	101,159	100,859	101,259	102,959	100,459	102,059
4 PM	30,347	29,947	30,747	28,247	27,947	28,347	30,047	27,547	29,147
Total	157,353	156,153	158,553	151,053	150,153	151,353	156,453	148,953	1,230,024
Average	52,451	52,051	52,851	50,351	50,051	50,451	52,151	49,651	51,251

## Survival rates

Survival rates showed all species that gave different survival rates of 95%, and 19% show data records in Table 2. There were eleven species that had survival rates as up to 80%, they were *Schefflera* sp., *Rhipsalis cereuscula*, *Rhipsalis simmleri* cereuscula aka 'Rice Cactus', *Sansevieria trifasciata* hort. ex Prain 'Golden Hahnii', *Gardenia jasminoides* 'Radicans Variegated', *Pandanus stellatus* R. Br., *Pandanus pygmaeus* Thours., *Tradescantia spathacea* Sw., *Xyphidium caeruleum* Aubl., *Neoregelia fireball donger*. and *Tradescantia pallida* (Rose) Hunt. There was one *Gardenia jasminoides* 'Radicans Variegata' species which not appropriated for this gardening system that gave survival rates like 19%.

Table 2. Survival rates of 12 plant species

					Survi	al rates	s (%)		
No.	Plant species	Months after planting							
		1	2	3	4	5	6	7	8
1	Schefflera sp.	100	99	98	98	97	96	95	95
2	Rhipsalis simmleri cereuscula								
	aka 'Rice Cactus'	98	96	95	95	94	92	90	89
3	Sansevieria trifasciata hort.								
	ex Prain 'Golden Hahnii'	100	98	97	96	96	95	94	93
4	Gardenia augusta (L.) Merr.	98	95	89	79	69	49	28	19
5	Gardenia jasminoides								
	'Radicans Variegated'	99	97	93	90	88	86	84	82
6	Pandanus stellatus R. Br.	97	95	93	91	88	86	85	85
7	Pandanus pygmaeus Thours.	98	97	93	90	89	85	48	83
8	Tradescantia spathacea Sw.	100	98	96	95	94	93	92	92
9	Xyphidium caeruleum Aubl.	100	98	97	96	95	94	93	90
10	Neoregelia fireball donger.	95	94	92	90	88	86	85	84
11	Tradescantia pallida (Rose)	100	99	97	96	95	94	92	90
11	Hunt.								
12	Rhipsalis cereuscula.	100	99	98	96	95	94	93	92

# Beautifulness rates

After collecting the data, all species gave beautifulness differently and got different score levels. Four species got more than 4.0 scores, they were *Schefflera* sp., *Tradescantia spathacea* Sw., *Tradescantia pallida* (Rose) Hunt. and *Rhipsalis cereuscula*. Four species got more than 3.0 scores and lower than 4.0 scores, there were *Sansevieria trifasciata* hort. ex Prain 'Golden Hahnii', *Pandanus stellatus* R. Br., *Pandanus pygmaeus* Thours and *Xyphidium caeruleum* Aubl. Three species got more than 2.0 scores and lower than 3.0 scores, there were *Rhipsalis simmleri* cereuscula aka 'Rice Cactus', *Gardenia jasminoides* 'Radicans Variegata' and *Neoregelia fireball* donger. and one species had died, which was *Gardenia augusta* (L.) Merr. (Table 3).

**Table 3.** Beautifulness rate of 12 plant species

				Bea	utifulnes	s rate (sc	ore)		
No.	Plant species			М	onths aft	ter planti	ng		
		1	2	3	4	5	6	7	8
1	Schefflera sp.	4.50	4.50	4.50	5.00	5.00	4.67	5.00	5.00
2	Rhipsalis simmleri cereuscula								
	aka 'Rice Cactus'	2.17	2.17	2.50	3.33	2.33	1.83	2.67	3.16
3	Sansevieria trifasciata hort.								
	ex Prain 'Golden Hahnii'	3.67	3.67	3.67	4.17	3.83	3.83	3.67	3.72
4	Gardenia augusta (L.) Merr.	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5	Gardenia jasminoides								
	'Radicans Variegated'	3.00	3.00	2.50	3.00	2.50	2.33	1.50	1.50
6	Pandanus stellatus R. Br.	3.33	3.67	4.00	4.17	3.17	3.33	4.33	3.00
7	Pandanus pygmaeus Thours.	4.17	4.17	3.83	3.00	2.67	3.17	2.50	2.67
8	Tradescantia spathacea Sw.	4.00	4.00	4.17	4.50	4.67	3.33	4.83	4.83
9	Xyphidium caeruleum Aubl.	2.33	2.50	2.67	3.33	3.83	3.33	3.83	3.50
10	Neoregelia fireball donger.	2.33	2.33	3.00	2.50	2.67	3.00	3.00	2.83
11	Tradescantia pallida (Rose)	117	4 17	2 02	4.50	4 22	4 22	4.00	4 22
11	Hunt.	4.17	4.17	3.83	4.50	4.33	4.33	4.00	4.33
12	Rhipsalis cereuscula.	3.33	3.33	4.50	4.33	4.67	3.33	4.17	4.83
	Mean	3.17	3.21	3.35	3.57	3.39	3.21	3.37	3.39
	CV. (%)	14.44	20.10	16.90	18.80	18.18	20.03	14.24	13.81

# Discussion

In our study, the optimum photosynthetically active radiation (PAR) of the garden includes wavelengths from 450-700 nanometres (Gislerod et al., 2010). Light intensity is one of the main factors for many plants that grow in the vertical garden. The plant different of species required different light intensity The different of plant species require different light intensity some species required dimly light or didn't require directed light but some species required directed light such as Helianthus annuus and Rosa sp. (Joanna, 2009). Phonpho and Saetiew (2017) study on indoor plants were required a different level of light intensity (500–2,000 LUX). Five species (*Monstera delicosa, Scindapsus pictus, Anthurium crystallinum, Caladium lindenii,* and *Philodendron erubescens*) were suitable for growth on 1,7000 LUX – 2,000 LUX. The results of this study in light intensity values of the plants received total average measured at 51,251 Lux

A study of twelve ornamental plant species, it was found that there were eleven plants that could survive and have the plants that passed the selection criteria that were used for vertical gardening outside the building, which has an average beautifulness score with higher than 4.0 and with a survival rate up to

rr 95%. There were eight ornamental plant species did not meet the criteria, and there were three types of plant species because the average beautifulness score was less than 3.0 points and there were one species of survival which are caused by the following; 1) a plant that could survive up to 80% and the beautifulness scores that pass the criteria

that were used for vertical gardening outside the building, which is 3.0 points or more for eight plant species, with two levels of beautifulness, which are, an average of beautifulness rating 4.0 to 5.0 points with four plant species which are Schefflera sp., Tradescantia spathacea Sw., Tradescantia pallida (Rose) Hunt. and Rhipsalis cereuscula Aubl. There has been a very beautiful level due to strong, durable and did not find any problems, that were not found diseases and insects, easy to be handling, grow quickly. Therefore, did not need to trim much, and just removed or discarded old leaves. The average beautifulness rating ranges from 3.0 to 4.0 points. There are four types of plant species: Sansevieria trifasciata hort. Ex Prain 'Golden Hahnii', Pandanus stellatus R. Br., Pandanus pygmaeus Thours and Xyphidium. caeruleum Aubl. The problem was the burning of the leaf tip during hot weather and exposed to sunlight light. The tree could not adapt itself in time, which causes the thinnest part of the leaves. The end of the leaf evaporates too much water until it could dry out. The practice was performed by cutting wilt and severe burns leave. Plants could survive up to 80% and have an average of beautifulness score of less than 3.0, which did not meet the criteria to be used. There are 3 types of plant species, which are Rhipsalis simmleri cereuscula aka 'Rice Cactus', Gardenia jasminoides 'Radicans Variegata' and Neoregelia fireball donger. Because it was found that the plant had abnormal symptoms such as Phytophthora sp. Rot in Rhipsalis simmleri cereuscula aka 'Rice Cactus', yellow leaf in Gardenia jasminoides 'Radicans Variegated' and dry, dry leaf atrophy in the Neoregelia fireball donger. because the root system had a growing system. slow and had few roots due to strong winds, trees are constantly moving, making roots difficult to hold, so plants often come out of their pockets often. Therefore, these plants are not suitable for use and one type of plant that could not grow is Gardenia augusta (L.) Merr., which has been found the insect damage. The insects found are the oleander hawkmoth destroyed the leaves and flowers during the buds of the spotted dwarf plant. Causing the flowers to rot fall away and could not bloom, and it is positioned at the bottom of the planting panel, which has trapped water causing the plant to get too much water.

#### Conclusion

Growing ornamental plants in the vertical outdoor garden system by evaluating the beauty of the growth of plants from all twelve species, the criteria for selecting plants that are suitable for vertical gardening building exterior, which must have a survival rate up to 80% or more and an average beautifulness rating of 3.0 points or more. The eight types of plant species could meet the criteria used in vertical outdoor garden systems as follows: the survival rate of 80% or above and beautifulness between 3.0 and 4.0 score. There are four types of plant species; Sansevieria trifasciata hort. ex Prain 'Golden Hahnii', Pandanus stellatus R. Br., Pandanus pygmaeus Thours and Xyphidium caeruleum Aubl. These plants are easy to handling and no need to trim often. It just removes the wilted leaves or with severe burns out and survival rate of 80% or above and beautifulness scores between 4.0 and 5.0 score. There are four types of plant species: Schefflera sp., Tradescantia spathacea Sw., Tradescantia pallida (Rose) Hunt. and Rhipsalis cereuscula. These plants had the characteristics of easy handling, plant growth quickly, so it does not require much trimming, only picking old leaves or discarded leaves, except the purple heart tree with rapid growth, therefore having to take care by cutting once a month

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Fine particles of fungal active metabolites constructed from Emericella sp to control rice blast disease in China

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**Abstract** The biological control of rice blast in China was investigated by using the fine particles of active metabolites constructed from *Emericella* sp. The metabolite fine particles were inhibited *Magnaporthe oryzae* causing rice blast disease by using poisonous food method. Fine particles-EN inhibited the blast pathogen of 62 % in 12 days. *In vivo* biological activity of fine particles from active metabolites were proved to control blast disease of rice var. Co39 in tested tube and pot experiments compared with chemical fungicide, tebuconazole. Test tube experiment resulted that fine particles -EN reduced the blast disease incidence of 49 % which higher than tebuconazole of 33 %n when compared to the inoculated with *M. oryzae*. Pot experiment showed that tebuconazole treatment reduced the blast incidence of 63 % and fine particles-EN reduced the blast incidence of 44 % when compared to the inoculated control. The further research findings are being investigated for rice immunity to blast disease.

Keywords: rice blast, Magnaporthe oryzae, Emericella sp., fine particles

#### Introduction

The chemical pesticides in crop production has become deleterious effect to living organisms and agroecosystem over 100 years. Those toxic agrochemicals are residue in the soil, surrounding environment and agricultural products that harmful to human being and short life. Biological control strategy become one of the potential method to use for plant disease control instead of chemical fungicides.

Rice blast disease caused by *Magnaporthe oryzae*, is one of the most important rice disease. China is the world's top producer and consumer of rice, about 200 million tons a year. Rice blast is harmful disease that affects China's rice crop. Interspersing two varieties of rice have doubled yields in Yunnan and occurrence of rice blast decreased by 93 percent (Rice Agriculture in China, 2018).

Emericdella sp is reported as a biological control agent against Fusarium wilt of tomato (Sibounnavong, P., 2012). Emericella rugulosa is reported to produce five new prenylxanthones, ruguloxanthones A-C (1-3), 14-methoxytajixanthone (4), and tajixanthone ethanoate (5), a new bicyclo[3.3.1]nona-2,6-diene derivative, rugulosone (6), and seven known compounds, shamixanthone, tajixanthone, 14-methoxytajixanthone-25-acetate, tajixanthone hydrate, tajixanthone methanoate, isoemericellin, and ergosterol., The structures of 1-6 were established using spectroscopic techniques. Compound 6 exhibited antimalarial and antimycobacterial activities, as well as cytotoxicity against three cancer cell lines (Moosophon et al., 2009).

The construction of fine particles from natural products have been investigated to control plant pathogens and induce plant immunity. The objective of research finding was to investigate the fine particles derived from *Emericella* against *Magnaporthe oryzae* causing rice blast disease.

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#### Materials and Methods

The isolate *Magnaporthe oryzae* is provided by Key Laboratory of Biotechnology, Zhejiang University, Hangzhou, P.R. China. It was cultured on completed medium (CM) which consisted of NaNO3 6 g., KCl 0.52 g, KH2PO4 1.52 g, MgSO4.7H $_2$ O 0.52 g, D-glucose 10 g, peptone 2 g, yeast extract 1 g, casaminoacid 1 g and agae 15 g in 1 L, and adjusted pH to 6.5 and incubated at 27°C for 10 days. The morphology was observed under binocular compound microscope.

Pathogenicity tests were made in two methods by detached leaf and directly inoculated to seedling plants. The spore suspension of  $Magnaporthe\ oryzae$  at the concentration of  $1x10^5$  spores/ml was inoculated by high pressure inoculation machine onto 20 days of rice seedlings var Co 39 or onto detached leaves. The inoculated seedlings and detached leaves were kept in 90% relative humidity in dark condition for 2 days in moist chambers. Control treatment was done by spraying sterilize distilled water. The experiments were designed as a Completely Randomized Design (CRD) with four replications. Disease incidence was assigned a disease index (DI) at 5 days of post-inoculation using a 0 to 9 scales ( $modified\ from\ Xia\ et\ al.$ , 1993) where 0 =no infection observed;1 = small brown specks and small pinpoint infection < 1 mm and 9 = lesions with expanding open centers on > 90% of the leaf area evaluated.

Crude extract of natural product derived from *Emericella* sp was constructed to be fine-particles which followed the method of Dar and Soytong (2014) to yield fine particles-EN (FP-EN). The fine particles from natural products were kept in capped bottles after operated in electrospinning.

Fine particles were evaluated to control *M. oryzae* using poisonous food method. Treatments were non-treated control, fine particle-EN (FP-EN). FP-EN was mixed into media at the concentration of 100 ppm, autoclaved for 30 min. *M. oryzae* was cultured for 10 days under 25°C. The culture agar plug of 0.3 mm dia. was transferred to the middle poisonous plate, and incubated at 25°C. Data were collected as colony diameter and number of spores after 15 days. Experiment was designed by CRD with 4 replications. Data were subjected to analysis of variance.

Tube experimental test: Rice seedlings in big tube with 60 ml 1/5 Murashige & Skoog Medium (1/5 MS media) were prepared. The sterilized tubes were filled with 60 ml of sterilized 1/5 MS media, then transferred 3 rice seedlings into one tube and cultured for 14 days under 25 °C. Experiment was designed by CRD with 4 replications. Treatments were T1 = non-inoculated control, T2 = inoculated control, T3 = FP-EN and T4 = Tebuconazole (chemical fungicide). Each treatment was sprayed 0.5 ml of 300 ppm FP-EN and 0.5 ml of Tebuconazole. Thereafter, spore suspension of the tested pathogen at concentration  $1 \times 10^5$  spores/ml was inoculated after 12 hours treatments. All treatments were maintained at 22 °C for 3 days. Data was gathered as chlorophyll, disease index, plant and root weights and subjected to analysis of variance and compared treatment mean using Duncan's Multiple Range Test (DMRT).

Pot experiment: Rice seeds were sterilized with 1% sodium hypochlorite for 20 min and sterilized water several times, then placed on tissue paper with sterilized water in petri dish at 37  $^{\circ}$ C for 3 days germination. The plastic pots (6 cm diameter) were prepared and put substrate: vermiculite at the ratio of 3:1, then planted 10 seeds per pot for 20 days before treatments. Experiment was designed by RCBD with 4 replications. Treatments were T1 = non-inoculated control, T2 = inoculated control, T3 = FP-EN and T4 = Tebuconazole at recommended rate. Each treatment was sprayed 2 ml at the rate of 1,000 ppm FP-EN and 2 ml Tebuconazole, then inoculated spore suspension of *M. oryzae* at concentration  $1 \times 10^5$  spores/ml, 2ml/pot. All treatment were maintained at 22  $^{\circ}$ C. Data was gathered as chlorophyll, disease index, plant fresh weight, plant high, plant dry weight and root fresh weight, root dry weight, root length. Data were subjected to analysis of variance.

#### Results

The isolate is offered from Key laboratotory of Biotechnology, Zhejiang University, Hangzhou, PR China. It was cultured on CM media for 15 days, incubated at 25 C. and observed under compound microscope. Cuture on CM

medium appeared creamy white colony at first stage of growing and turned to yellowish brown. It showed septate mycelia, produced branched conidiophores and borne conidia. Conidia hyaline, 3-celled, 2 septa. The pathogenicity test in detached leaf method showed typical lesions after inoculation at averaged DI level 7 (lesions with expanding open centers on 51 to 75% of the leaf area evaluated) in rice. The pathogenicity test which directly inoculated the blast pathogen to seedling plants averaged DI level 6 (lesions with expanding open centers on 26 to 50% of the leaf area evaluated) in the same rice variety. The natural product *Emericella* sp was constructed to be fine particles yielded fine particle-EN(FP-EN).

Result showed that FP-EN Na at the concentration of 100 ppm gave the inhibition of 78 % when comparted to the inoculated control, FP-EN treatment showed abnormal conidia leading to pathogenicity loss. It was observed that the conidia were deformed shape and being protoplast plugs inside the pathogen cells.

Rice seedings in tested tubes after treatment showed tebuconazole gave the lowest blast disease incidence of 3.7 % and highest disease reduction of 93% when comparted to inoculated with *M. oryzae*. FP-EN showed blast disease reduction of 73% when comparted to inoculated one.

The rice seedlings tested in pot experiment which inoculated with *M. oryzae* and treated with FP-EN compared to Tebuconazole (chemical fungicide). Result showed FP-EN reduced the blast incidence of 44n % and Tebuconazole reduced the blast incidence of 63 % when compared to the inoculated control.

The treated FP-EN and Tebuconazole in rice var Co39 were significantly higher chlorophyll content, plant height, fresh and dry weight of stems, root length, fresh and dry weight of roots than the inoculated with *M. oryzae* in 7 days after inoculation (Table 1).

**Table 1.** The effect of fine particles derived from Emericella sp. in comparison with to Tebuconazole to control blast incidence in pot experiment

Treatments	Index	Disease		Plant			Root	
	Chlorophyll	Reduction	Fresh	Dry	Height	Fresh	Dry	Length
	content1	(%)¹	Weight	Weight	$(cm)^1$	Weight	Weight	$(cm)^1$
			$(g)^{1}$	$(g)^{1}$		(g)	(g)	
inoculation	20.43*	-	2.22	0.60 <sup>d</sup>	34.37 <sup>bc</sup>	1.08 <sup>b</sup>	0.17 <sup>b</sup>	13.00 <sup>a</sup>
FP-EN	25.42	44.44	2.71	0.79 <sup>abcd</sup>	38.30 <sup>a</sup>	1.31 <sup>ab</sup>	0.19 <sup>ab</sup>	13.38 <sup>a</sup>
Tebuconazole	27.10	63.88	2.37	0.61 <sup>cd</sup>	36.00 <sup>ab</sup>	1.36 <sup>ab</sup>	0.21 <sup>ab</sup>	12.75 <sup>b</sup>

<sup>\*</sup>P < 0.01

#### Discussion

The research finding that *M. oryzae* which supported by Key Laboratory of Biotechnology, Zhejiang University, Hangzhou, P.R. China was confirmed to be pathogenic to cause rice blast disease.

The natural product *E. nidulans* namely FP-EN inhibited the tested pathogen of 78 % which similar to the report of *Emericella rogulosa* inbihited some plant pathogenic fungi eg. E. rugulosa ER01 could inhibit the tested plant pathogen, *F. oxysporum* f. sp. lycopersici (Sibounnavong and Soytong, 2011). The treated FP-EN expressed abnormal spores of *M. oryzae* leading to lost of pathogenicity that also similar reports of Tann and Soytong (2017).

 $<sup>^{1}</sup>$ /Disease reduction (%) = R1-R2/R1x100 where R1 is the disease incidence in the control rice plant and R2 is the disease incidence in treated rice plant.

As result rice seedlings in testing tubes, tebuconazole gave the lower blast disease incidence than FP-EN when comparted to inoculated one. It is explained that the concentration of FP-EN may possible to test in various concentrations and tebuconazole treatment used as successfully recommendation rate. It was similar result which explained by Soytong (2014), who stated that bioproducts from *Chaetomium* sp. to control rice leaf spot caused by *Curvularia lunata*, but at a high application rate.

The pot experiment resulted that the treated with FP-EN and Tebuconazole decreased the blast incidence as compared to the inoculated control, and also higher chlorophyll content, plant height, fresh and dry weight of stems, root length, fresh and dry weight of roots than the inoculated with *M. oryzae*. These results confirmed successfully application either fine particles from natural product of *Emericella* sp or Tebuconazole chemical fungicide that reduce the disease incidence and increase plant strands. Similar results are reported that nanoparticles derived from *Chaetomium* sp reduced blast incidence, and increased plant growth parameters (Soytong, 1989). The future research findings are being evaluated induction immunity to blast disease of rice through phytoalexin production.

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## Natural products of fine particles derived from Chaetomium globosum to inhibit Fusarium wilt of tomato

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**Abstract** *Fusarium solani* was proved to cause wilt of tomato var Sida. It is confirmed by morphology and molecular phylogenic identification on the basis of ITS1-5.8S-ITS2 ribosomal gene sequence acquisition and analyses. It is pathogenic confirmed by pathogenicity test. Thease antagonists expressed ability to inhibit the growth and spore production of *F. solani* causing wilt of tomato var sida. The Hexane crude extract of *Ch. globosum* inhibited the spore production of *F. solani* at the  $ED_{50}$  of 200 ppm, and followed by crude EtOAc crude extract and methanol extract which the  $ED_{50}$  values of 314 and 378 ppm, respectively. The nautral products of fine particles constructed from the tested *Chaetomium* gave significantly inhibited *F. solani* at all tested crude extracts. Fine particle CGM actively expressed antifungal activity of *F. solani* which the  $ED_{50}$  of 1.48 ppm, and followed by fine particle -CGH and fine particle-CGE which the  $ED_{50}$  values were 3.41 and 3.48 ppm, respectively.

**Keywords:** Fusarium solani, Chaetomium globosum, Fine particle

#### Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important crop in the world due to the high value of its fruits for fresh market consumption (Arici *et.al*, 2013). Tomato is a products rich in health-related food components as they are good sources of carotenoids (in particular, lycopene), ascorbic acid (vitamin C), vitamin E, folate, flavonoids and potassium (Beecher, 1998; Leonardi *et al.*, 2000). Regular consumption of tomatoes has been correlated with a reduced risk of various types of cancer (Gerster, 1997) and heart diseases (Lavelli *et al.*, 2000; Pandey *et al.*, 1995). Tomato is an economically important vegetable crop, suffering from many fungal diseases (Ketelaar and Kumar, 2002). Fusarium wilt is one of the most serious disease in tomato throughout the world, especially in upland. This disease is caused by *Fusarium solani* leading to serious economic losses (Snyder and Hansen, 1940). *Fusarium solani* is an abundant saprophyte in soil and organic matter and occurs worldwide in the rhizosphere of many plant species. Plants infected by this soil-dwelling fungus show leaf yellowing and wilting that progress upward from the base of the stem. Initially, only one side of a leaf midrib, one branch, or one side of a plant would be affected. The symptoms soon spread to the remainder of the plant. Wilted leaves usually drop prematurely to minimize losses from *Fusarium* wilt, it is advisable to plant resistant varieties, and many resistant varieties are available. It is reported tomato wilt and pathogenic to other economic plants (Ajilogba *et al.*, 2013)

Recently, there have been many reports that antagonistic fungi can be used to control Fusarium wilt in tomato plant such as *Trichoderma asperellum, Chaetomium elatum* ChEO, *Chaetomium globosum* N0802, *Chaetomium lucknowense* CLT, *Trichoderma harzianum* PCO1, *Emericella rugulosa* ERO1, *Chaetomium cupreum* (Mahmoud *et.al.*, 2015; Soytong,2015; Sibounnavong *et.al.*, 2011). Moreover, the use of bioactive compound extracted from difference species of antagonistic fungi were reported to inhibit the growth of the Fusarium wilt disease such as Trichotoxin A50 extracted from *Trichoderma harzianum* PCO1 and Chaetoglobosin C extracted from

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*Chaetomium globosum*. These compounds have been reported to elicit resistance or immunity in plants by inducing oxidative burst in plant cells for plant immunity (Soytong *et al.*, 2001).

#### Material and Methods

#### Isolation, identification and pathogenicity test

Fusarium solani causing wilt disease was isolated form tomato root by tissue transplanting technique. Roots of tomato were properly cleaned with running tap water and after air-dried for a few minutes and cut it in small pieces and soaked in sterilized water, followed by 1% sodium hypochlorite for 3 min and then sterilized water again. All of the small piece roots were transferred onto water agar (WA) medium for firstly observation of appearing colonies and sub-cultured to PDA until get pure culture. Morphological identification was done by observation fungal characteristic under binocular compound microscope.

#### Morphology study of the *Fusarium* spp.

Isolate of *Fusarium* spp was morphological identified by culturing in potato dextrose agar (PDA) and incubated at room temperature for 14 day observation. The characters of *Fusarium* spp were determined under binocular compound microscope and the details of fungal morphology were recorded as mycelia structure, shape and size of macroconidia, microconidia, conidiophores and chamydospores.

#### Pathogenicity test

The experiment was designed by using Completely Randomized Design (CRD) with four replications. The mycelia of F. solani were removed into sterilized distilled water and conidia suspension which adjust to  $1\times10^6$  conidia/ml by using haemacytometer. Seedling of tomato var. sida was grown in mix soil for 15 days. The root dip method was used to inoculate which followed the method of Bao et~al. (2002), gently removed dirt and excess soil from roots of tomato var. sida seedling by using tap water. Root tips of seedlings were cut by sterilized scissors for 5 mm and dipped into conidia suspension for 30 seconds. Seedling roots in control were cut root tips and dipped into sterilized distilled water without inoculum. Before transfer to plastic pots that contained the sterilized soil which autoclaved at  $121^{\circ}$ C, 15lbs/inch² for 1 hour. Disease severity index (DSI) was scored by followed the modified method of Sibounnavong et~al. (2012), as follows: 1= no symptom; 2= yellowing leaves and root rot 1-20%, 3= yellowing leaves and root rot 21-40%, 4= yellowing leaves and root rot 41-60%, 5= yellowing leaves and root rot 61-80%, and 6= yellowing leaves and root rot 81-100% or die. Disease severity index (DSI) was analysed by using analysis of variance (ANOVA) and mean comparison was computed Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05.

The antagonistic fungi, *Chaetomium globosum* were tested by using method of Soytong (1992). The experiment was designed as Completely Randomized Design (CRD) with four replications. *Fusarium solani* and *Chaetomium* sp were cut with 0.5 mm sterilized cork borer and one agar plug of each fungus were transferred to PDA plate at one side 4 cm from center of plate. For control treatment either agar plug of *F. solani* or *Ch. globosum* was placed on PDA plate at 4 cm from center of the medium. The tested plates were incubated at room temperature. The data were collected as colony diameter, number of conidia of pathogenic fungus. Percentage of growth and conidia inhibition of pathogen was calculated using formula below:

Inhibition (%) = 
$$\underline{A-B}$$
 ×100 — (1)

A = colony diameter or conidia number of pathogen in control

B = colony diameter or conidia number of pathogen in control in dual culture plate

The data were statistically computed for analysis of variance (ANOVA) and mean comparison was computed by using Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05. The effective dose (ED<sub>50</sub>) was computed by using probit analysis

#### Extraction antagonistic crude extracts and preparation of fine particle from Ch. globosum

The fungal antagonistic *Ch. globosum* were separately cultured in potato dextrose broth (PDB) and incubated at room temperature for 30 days. Biomass of each antagonistic fungus was removed from PDB by filtering thorough cheesecloth and air-dried at room temperature. Biomass was collected and weighted as fresh and dried. Dried biomass were ground with electric blender. Dried biomass of each antagonist was extracted by the method described by Kanokmedhakul *et.al* (2003) Each dried biomass was extracted with hexane (1:1 v/v) in 1000 ml of flask and incubation at room temperature. The ground marc was separated from the solvent by filtering through filter paper (Whatman No.4). The hexane filtrate was evaporated thought rotary vacuum evaporator to yield crude hexane extract. Further, the marc was then extracted with ethyl acetate (EtOAc) and methanol (MeOH), respectively using the same method as hexane. Finally, it yielded crude hexane, crude ethyl acetate and crude methanol of each antagonist. Preparation of nano particles was done using the method of Dar and Soytong (2013) to get fine particles of each *Chaetomium* sp.

## Bioactivity test of crude extracts from Chaetomium sp against F. solani

The crude extracts of *Ch. globosum* were tested ability to inhibit the growth of mycelia and spore production of *F. solani*. The experiments were designed as 2 factors factorial in Completely Randomized Design (CRD) with four replications. Factors A represented crude extracts which included of crude extracts from hexane, ethyl acetate (EtOAc) and methanol (MeOH). Factors B represented different concentrations of crude extracts as follows 0, 10, 50, 100, 500, 1,000 µg/ml. Each crude extracts were dissolved by 2% dimethyl sulfoxide (DMSO) and mixed with PDA before autoclaved at 121°C, 15lbs/inch² for 30 min. *F. solani* was cultured on PDA and incubated at room temperature for 5 days, then colony margin was cut by 0.5 mm sterilized cork borer. The agar plugs of *F. solani* was transferred into the middle of 5 cm of petri dish in different concentrations and incubated at room temperature for 5-7 days. The normal and abnormal spores were observed and compared under compound microscope.

## Testing fine particles form *Chaetomium* sp against *F. solani*

The experiments were designed as 2 factors factorial in Completely Randomized Design (CRD) with four replications. Factors A represented different kinds of nano particles. Nano particles derived *Ch. globosum* namely fine particles- CGH, fine particles- CGE and fine particles- CGM.

Factor B represented concentrations of 0, 3, 5, 10, 15  $\mu$ g/ml. Each nano particle was dissolved by 2% dimethyl sulfoxide and mixed with PDA before autoclaved at 121°C, 15lbs/inch² for 30 min. The pathogen was cultured on PDA and incubated at room temperature for 5 days, then colony margin was cut by 0.5 mm sterilized cork borer. The agar plugs of *F. solani* was transferred into the middle of 5 cm of petri dishes in deferent concentrations and incubated at room temperature for 5-7 days. Data were collected as colony diameter, number of conidia. Percentage of inhibition of mycelial growth and number of conidia was calculated using formula (1) above and data were statistically computed for analysis of variance (ANOVA) and mean comparison was calculated by Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05. The effective dose (ED<sub>50</sub>) was computed by using probit analysis. The comparison of normal and abnormal spores was observed under compound microscope.

#### Results

#### Morphological identification

Fusarium solani was isolated from diseased plant parts, especially from roots of tomato var sida. The characteristics of colony were fast growing with aerial mycelium floccose, white or cream colour, reaching 9 cm diameter in 5-7 day at 29-32 °C. Conidiophores formed singly, slender and cylindrical shape. Macroconidia found abundant, moderately curved, blunt apical and pedicellate basal cell. Microconidia usually were abundant with 1 septate. Chlamydospores were singly or in paired in terminal, lateral or more rarely intercalary positions, smooth wall.

#### Pathogenicity test

Pathogenicity test was conducted by dipping cut root into spore suspension of F. solani at the concentration of  $1 \times 10^6$  conodia/ml for 30 seconds which resulted tomato seedlings showed yellowing leaves and root rot 41-60 %. It was significantly differed when compared to the non-inoculated control.

**Table 1**. Percent disease index of *F. solani* in tomato.

Treatments	Disease index
T1= Control	1
T2= Fusarium solani	4

Disease index are as follows: 1= no symptom; 2= yellowing leaves and root rot 1-20%, 3= yellowing leaves and root rot 21- 40%, 4= yellowing leaves and root rot 41-60%, 5= yellowing leaves and root rot 61-80%, and 6= yellowing leaves and root rot 81-100% or die.

## Morphological of antagonistic fungi

The culture of *Ch. globosum* was slow growing with olivaceous colour. Ascospore dark brown colour when mature with an apical germ pore. Colonies of *Ch. globosum* was slow growing with little superficial mycelium and a dense olivaceous layer on ascomata. Ascomata was dark brown or black colors, globose to subglobose. lateral hairs dark brown with paler tips, minutely roughened, terminal hairs dark olive brown with paler tips, wavy or loosely coiled and interwined. Ascopores was pale greenish to dark olive-brown, flattened lemon-shaped.

#### **Dual-culture test**

*Ch. globosum* were proved their abilities to inhibit plant pathogen *F. solani* causing disease of tomato by using dual-culture tests. The results showed that *Ch. globosum* gave significantly inhibition of *F. solani* which were 5.00 cm in colony diameter when compared to the control plate. After 3 month *Ch. globosum* showed ability to grow over the colony of *F. solani* 

**Table 2.** Colony growth on dual-culture antagonistic tests.

Antagonist fungi	Fusarium solani				
Antagonist fungi	Colony(cm)	% inhibition of colony			
Control	9.00 <sup>a 1/</sup>	-			
Chaetomium globosum	5.00 <sup>b</sup>	44.35			
CV%	1.02	1.37			

1/: Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.05

## Bioactivity test of crude extracts from Ch. globosum against F. solani

Crude hexane extract from *Ch. globosum* gave significantly highest inhibition of 64.75% for the colony growth of *F. solani*, followed by crude methanol extract which inhibited 52.00% (Table 3). Crude hexane extract from *Ch. globosum* gave significantly highest inhibition for the spore production of *F. solani* as 93.29% with  $ED_{50}$  of 200.05 ppm. Crude methanol extract gave 79.75% inhibition with the  $ED_{50}$  was 378.47 ppm. Crude ethyl acetate extract showed 72.08% inhibition with  $ED_{50}$  was 314.16 ppm

Table 3 Crude extracts of Ch. globosum testing for growth and spore inhibition of F. solani at 7 days

Crude extracts	Concen- tration (ppm)	Colony diameter (cm) <sup>/1</sup>	Growth inhibition (%) <sup>/2,3</sup>	Number of spores <sup>/1</sup> (10°)	Inhibition (%) <sup>/2,3</sup>	ED <sub>50</sub> (ppm)
	0	5ª	-	6.52ª	-	
	10	5 <sup>a</sup>	-	5.81 <sup>bc</sup>	11.01 <sup>ij</sup>	
Hexane	50	5ª	-	4.88 <sup>ef</sup>	25.13 <sup>fg</sup>	200.05
	100	5 <sup>a</sup>	-	4.75 <sup>f</sup>	27.23 <sup>f</sup>	
	500	3.82 <sup>c</sup>	23.50 <sup>e</sup>	2.52 <sup>h</sup>	61.27 <sup>d</sup>	
	1,000	1.76 <sup>g</sup>	64.75°	0.44 <sup>k</sup>	93.29ª	
	0	5ª	-	6.52 <sup>a</sup>	-	
	10	5 <sup>a</sup>	-	5.58 <sup>bc</sup>	12.85 <sup>ij</sup>	
EtOAc	50	5 <sup>a</sup>	-	5.10 <sup>def</sup>	21.84 <sup>fgh</sup>	314.16
	100	5 <sup>a</sup>	-	4.74 <sup>f</sup>	27.34 <sup>f</sup>	
	500	3.72 <sup>d</sup>	25.50 <sup>d</sup>	2.91 <sup>gh</sup>	55.40 <sup>de</sup>	
	1,000	3.40 <sup>e</sup>	32.00 <sup>c</sup>	1.82 <sup>i</sup>	72.08 <sup>c</sup>	
	0	5ª	-	6.52 <sup>a</sup>	-	
	10	5 <sup>a</sup>	-	6.04 <sup>b</sup>	7.28 <sup>j</sup>	
MeOH	50	5 <sup>a</sup>	-	5.43 <sup>cd</sup>	16.59 <sup>hi</sup>	
	100	5 <sup>a</sup>	-	5.23 <sup>de</sup>	19.83 <sup>gh</sup>	378.47
	500	3.92 <sup>b</sup>	21.00 <sup>f</sup>	3.08 <sup>g</sup>	52.65 <sup>e</sup>	
	1,000	2.39 <sup>f</sup>	52.00 <sup>b</sup>	1.32 <sup>j</sup>	79.75 <sup>b</sup>	
C.V. (%)		4.39	11.46	4.41	11.66	

<sup>&</sup>lt;sup>1</sup>/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. <sup>2</sup>/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. <sup>3</sup>/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

# Testing fine particles form Ch. globosum against F. solani

The fine particle from *Ch. globosum* was showed that fine particles-CGH inhibited colony growth of *F. solani* as 90.00%. fine particles -CGE inhibited colony growth of *F. solani* as 90.00% and nano-CGM inhibited colony growth of *F. solani* as 90.00% (Table 4). The resulted showed that fine-CGH, fine particles -CGM and fine particles -CGM gave the highest ability to inhibit spore production of *F. solani* as 99.39%, 99.54% and 99.69% respectively and ED $_{50}$  were 3.41, 3.48 and 1.48 ppm respectively

Table 4 Growth inhibition and ED<sub>50</sub> of fine-particles against *F. solani* at 7 days

Fine particles	Concen- tration (ppm)	Colony diameter (cm) <sup>/1</sup>	Growth inhibition (%) <sup>/2,3</sup>	Number of spores <sup>/1</sup> (10 <sup>6</sup> )	Inhibition (%) <sup>/2,3</sup>	ED₅o (ppm)
fine	0	5 <sup>a</sup>		82.37ª	_	
particles-	3	4.71 <sup>b</sup>	5.75 <sup>h</sup>	50.87 <sup>b</sup>	38.24 <sup>g</sup>	
CGH*	5	3.56°	28.75 <sup>g</sup>	17.75 <sup>d</sup>	78.48 <sup>e</sup>	3.41
3311	10	2.02 <sup>f</sup>	59.50 <sup>d</sup>	6.12 <sup>e</sup>	92.56°	3.11
	15	0.50 <sup>i</sup>	90.00ª	0.50 <sup>g</sup>	99.39ª	
fine	0	5ª	-	82.37ª	-	
particles-	3	4.69 <sup>b</sup>	6.00 <sup>h</sup>	52.62 <sup>b</sup>	36.13 <sup>h</sup>	
CGE	5	3.67°	26.50 <sup>g</sup>	17.25 <sup>d</sup>	79.07 <sup>e</sup>	3.48
	10	2.73 <sup>e</sup>	45.25 <sup>e</sup>	7.50 <sup>e</sup>	90.91 <sup>d</sup>	
	15	0.50 <sup>i</sup>	90.00ª	0.37 <sup>g</sup>	99.54ª	
fine	0	5ª	-	82.37ª	-	
particles-	3	3.23 <sup>d</sup>	35.25 <sup>f</sup>	19.87°	75.88 <sup>f</sup>	
CGM	5	1.73 <sup>g</sup>	65.25 <sup>c</sup>	7.50 <sup>e</sup>	90.90 <sup>d</sup>	1.48
	10	0.77 <sup>h</sup>	84.50 <sup>b</sup>	3.12 <sup>f</sup>	96.21 <sup>b</sup>	
	15	0.50 <sup>i</sup>	90.00ª	0.25 <sup>g</sup>	99.69ª	
C.V. (%)		3.08	4.29	4.51	1.61	

<sup>&</sup>lt;sup>1</sup>/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

#### Discussion

Fusarium solani was isolated from diseased plant tissues of tomato and ensured by pathogenicity test which also reported by Sibounnavong et al. (2011). The morphological identification of pathogen was done by culture into potato dextrose agar (PDA) and observed under microscope. The colony of Fusarium solani are fast growing, white or cream colour, reaching 9 cm diameter in 5-7 day at 29-32 °C. Macroconidia found abundant, moderately curved, blunt apical and pedicellate basal cells 3-5. Microconidia usually are abundant with 1 septate. Chlamydospores are singly or in paired in terminal, lateral or more rarely intercalary positions, smooth wall. This is similar to the reported of Domsch and Gams (1993) who stated that colony of F. solani are fast growing, cream colour. Macroconidia produced in variable quantities on shorter, branched conidiophores which soon form sporodochia, usually moderately

<sup>&</sup>lt;sup>2</sup>/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

<sup>&</sup>lt;sup>3/</sup>Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

curved, blunt apical and indistinctly pedicellate basal cells, mostly indistinctly 3 septate. Microconidia usually abundant, produced on elongate, sometime verticillate. Chlamydospores are produced singly or in pairs, in terminal, lateral, or more rarely positions, hyaline, smooth or rough walled.

Dual culture test exhibited that *Ch. globosum* could control *F. solani* causing tomato crown and root rot. Based on the result *Ch. globosum* had ability to inhibit the growth of *F. solani* and the result was similar to the report of Tongon and Soytong (2015). Tongon and Soytong (2016) stated that *Ch. globosum* showed efficacies to inhibit colony growth of *F. solani* and *Curvularia lunata* causing leaf spot disease in rice and also *Ch. globosum* showed efficacies to inhibit conidial production of *F. solani* over 50% and this resulted similar to the report of Moya *et al.* (2016) who stated that *Chaetomium* spp showed high potential to inhibit *Drechslera teres* and *Bipolaris sorokiniana* causing foliar diseases of barley.

The result showed that crude hexane from *Ch. globosum* gave significant highest inhibit colony of *F. solani*. *Ch. globosum* were shown highest inhibition for the spore production of *F. solani* by treated with crude ethyl acetate, crude ethyl acetate and crude hexane. *Ch. globosum* also gave highest significantly inhibition for the spore production of *F. solani*. Soytong (2014) reported that crude hexane of *Ch. cochliodes* inhibited spore production of *Drechslera sorokiniana* causing spot blotch of wheat at concentration of 1,000  $\mu$ g/ml (93.85 %) which ED<sub>50</sub> was 66.45 ppm (Biswas *et al.* 2002) and similar to Sibounnavong (2012) who reported that *Ch. brasilense* CB01 and *Ch. cupreum* CC03 inhibited the spore production of *F. oxysporum* f.sp. *lycopersici* NKSC02 between 63-77%. The fine particles from *Ch. globosum* were used to test their abilities to inhibit the growth of *F. solani* the result similar with Hung *et al.* (2015) stated that methanol extract of *Ch. globosum* CG05 expressed strongest inhibitory effects on mycelial growth and sporangium formation of *P. palmivora* PHY02 with effective dose ED<sub>50</sub> values of 26.5  $\mu$ g/mL and 2.3  $\mu$ g/mL, respectively.

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Natural products of fine particles derived from *Neosartorya hiratsukae* against brown spot of rice cause by *Drechslera oryzae* 

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Abstract Efficacy of *Neosartorya hiratsukae* was confirmed to control brown leaf spot of rice caused by *Drechslera oryzae*. In bi-culture antagonistic test, *Neosartorya hiratsukae* had highest significantly inhibited the colony growth and spore production which were 51.10% and 55.65% respectively. Crude methanol extract metabolites of *N. hiratsukae* gave significantly highest inhibition of colony growth and spore production of *D. oryzae* which ED<sub>50</sub> was 168  $\mu$ g/ml. Testing fine particles constructed from metabolites of *N. hiratsukae* showed that fine particles from methanol crude extact of *N. hiratsukae* gave significantly highest inhibition of colony growth and spore production of *D. oryzae* which ED<sub>50</sub> of 4.11  $\mu$ g/ml.

**Keywords:** *Neosartorya hiratsukae*, brown spot, rice disease.

#### Introduction

Rice is one of the most important staple food for increasing world population, especially in Asia. Asian farmers still account for 87% of the world's total rice production. Rice is the main export of Thailand. Disease damage to rice can greatly reduce yield. They are mainly caused by bacteria, viruses, or fungi. Brown spot is one of the important rice diseases in the world. It can be a serious disease causing a considerable yield loss. Brown spot causes both quantity and quality losses. On average, the disease causes 5% yield loss across all lowland rice production in South and Southeast Asia. Severely infected field can have as high as 45% yield loss (IRRI, 2019). The traditional chemical fungicides have been used for years and some case the pathogens become resistance to those chemical fungicides. However, there are many researcher were reported to use the biocontrol agents to control those disease.

This objective was to investgate the morphology of *Neosartorya hiratsukae* and *Drechslera oryzae*, pathogenicity test. Tesing crude extraxts and fine particles from *Neosartorya hiratsukae* to control brown leaf spot of rice var Chai Nat 1 were also conducted.

#### Materials and methods

#### Isolation of pathogen and Pathogenicity test

Drechslera oryzae causing by brown leaf spot of rice will be isolated form leaf rice var. Chai Nat 1 by tissue transplanting technique leaf of rice were soaked in sterilized water and followed by 1% sodium hypochlorite (NaClO) for 3 min and then sterilized water again. All seeds were transferred onto water agar (WA) medium for firstly observation of appearing mycelia and sub-cultured to PDA until get pure culture. Morphological identification was done by observation fungal characteristic under binocular compound microscope.

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#### Pathogenicity test

The experiment will be designed in Completely Randomized Design (CRD) with four replications. The isolates of pathogen will be tested for pathogenicity using detached leaf method in pot followed Koch's Postulate. The mycelia of *Drechslera oryzae* will be removed into sterilized distilled water and conidia suspension which adjust to  $1\times10^5$  conidia/ml. by using haemacytometer. Seedling of rice will be grown in pot for 21 days. And then sprayed on leaves. The inoculated leaves will be covered with plastic sheet and maintained to observe the infected leaves. The inoculated leaves with only spraying sterilize distilled water will be done to serve as controls.

# Bi-culture antagonistic test

#### Strain of antagonist used for experiments

Antagonists Neosartorya hiratsukae (EU06) which offered from Assoc. Prof. Dr. Kasem Soytong.

The assay for antagonism fungi will be performed on PDA on Petri dishes by the dual culture method (Fokkema, 1978). A 0.5 cm diameter sterilized cork borer will be used to remove agar plugs from the actively growing edge of cultures of the pathogenic fungus and of the antagonistic fungi and will be used to inoculate 9 cm diameter PDA plates: an agar plug of the pathogen will be placed on one the opposite site an agar plug of an antagonistic fungus. Plates will be inoculated with a single plug of an antagonistic fungus or of the pathogen acted as the controls. The plates will be incubated at room temperature for 30 days. The experiment is one with four replications of each treatment. The zone of inhibition will be recorded as the distance between the fungal pathogen and the area of antagonist growth after 7 days.

Observation of abnormal spores and nornal spore of pathogen from each treatment will be observed under compound microscope and take phoyograph for comparison.

The data were collected as colony diameter, number of conidia of pathogenic fungus. Percentage of growth and conidia inhibition of pathogen will be calculated using formula below:

Growth inhibition;  $GI = R1-R2 \times 100$ 

R1

R1 = colony diameter or conidia number of pathogen in control

R2 = colony diameter or conidia number of pathogen in control in dual culture plate

The data will be statistically computed for analysis of variance (ANOVA) and mean comparison was compared by using Duncan's New Multiple Range Test (DMRT) at P=0.01 and P=0.05.

## In vitro test of crude extracts from antagonist against *Drechslera oryzae* causing brown spot of rice.

#### Crude extraction of antagonists

Extraction method

The effective isolates of antagonistic fungi from Bi-culture test will be used for bioactive substances testing. The crude extracts from antagonistic will be assayed for their biological mechanism against pathogen.

The antagonist *Neosartorya hiratsukae* will be cultured in potato dextrose broth (PDB) at room temperature for 30 days. The fungal biomass of endophytics will be removed from PDB, filtered through cheesecloth and air-dried overnight. Fresh weight and dry weight of fungal biomass will be weighted. The fungal biomass will be ground with electrical blender, and placed in triangular flask. And then will be dissolved with equal volum hexane 3-5 days at room temperature, and then, the biomass will be separated by filtration through Whatman filter paper. The solvents will be then evaporated in vacuo to yield crude hexane. The marc will be further extracted with ethyl acetate and methanol

respectively using the same procedure as hexane and yield crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracts.

Testing biological activity of crude extracts from antagonist against *Drechslera oryzae* causing brown spot of rice.

The crude extract substances will be assayed for inhibition of pathogen. The experiment will be conducted by using factorial experiment in Completely Randomized Design (CRD) with four replications. Factor A= different solvents, A1= hexane, A2= ethyl acetate and A3= methanol solvents. Factor B= different concentration of crude extract substances  $\mu$ g/ml which is consisted of B1 = 0, B2 = 10, B3 = 50, B4 = 100, B5 = 500 and B6 = 1000 ppm. Each crude extract will be dissolved in 2% of dimethyl sulfoxide (DMSO), and then mixed with PDA before autoclaved at 121°C, 15lbs/inch2 for 30 minutes. The assay will be cut the colony margin on PDA by cork borer with 5 mm diameter and transferred on the centre of PDA Preti dishes (5 cm in diameter) which mixed with crude extract substance in each concentration and incubated at room temperature approximately 27-30 °C until the pathogen in control grown full plate. Abnormal and normal spores of pathogen from each treatment will be observed under binocular compound microscope and taken photograph for comparison.

Data will be collected as the colony diameter (cm), number of spore. The pathogen cell will be separately examined from each concentration under compound microscope. Percentage of inhibition will be computed as described in Bi-culture test. The data will be statistically computed analysis of variance. The comparison among treatment mean will be computed with DMRT at P=0.01 and 0.05. The effective dose ( $ED_{50}$ ) will be computed by using Probit analysis. The effect of crude extracts to pathogen cells will be observed under microscopic compound. The experiment will be repeated in two times.

# Test efficacy of fine particles from antagonist against Drechslera oryzae causing brown spot of rice.

Preparation of fine particles;-fine paticle will be done using the method of Dar and Soytong (2014) to get fine particle hexane, fine particle ethyl aectate and fine particle methanol. Experiment will be designed by using two factors factorial experiment in CRD with four replications. Factor A will be represented fine particle hexane, fine particle ethyl aectate and fine particle methanol and factor B will be represented concentrations at 0, 1, 3, 5, 7 and 10 ppm. Each fine particle dissolved in one drop 2% dimethyl sulfoxide (DMSO), and then mix into 30 ml PDA medium before autoclaving at 121°C, 15 lbs/inch 2 for 30 min. The culture of *Drechslera oryzae* will be cut at the edge of colony with sterilized cock borer (5 mm). Agar plug of pathogen will be transferred to the middle of PDA media in plate (5.0 mm diameter) incorporated with each fine particles. The transferred plates will be incubated at room temperature until the pathogen in control plates growing full. Abnormal and normal spores of pathogen from each treatment will be observed under binocular compound microscope and taken photograph for comparison.

Data will be collected as colony diameter (cm) and the number of spores that counted by using haemacytometer. Percentage of inhibition will be computed as described in Bi-culture test. The data will be statistically computed analysis of variance. The comparison among treatment mean will be computed with DMRT at P=0.01 and 0.05. The effective dose (ED<sub>50</sub>) will be computed by using Probit analysis. The effect of crude extracts to pathogen cells will be observed under microscopic compound.

#### Results

#### Isolation of pathogen and Pathogenicity test

*D. oryzae* was isolated from leaf spot of rice var. Chai Nat 1 in this study and demonstrated to be pathogenic on this host. (Figure 1)



**Figure 1.** Pathogenicity test of *Drechslera oryzae* on rice. The inoculated control (A) and inoculated leaves (B) after 5 days.

## Bi-culture antagonistic test

Result showed that *N. hiratsukae* could inhibit mycelial growth of *D. oryzae* which averaged colony of 4.40 cm when compared to control plate of 9.00 cm. It could inhibit mycelia 51.10 percent in 10 days. However, *N. hiratsukae* significantly inhibited spore production of *D. oryzae* 55.56 percent (Figure 1, Table 1).

Table 1. N. hiratsukae against Drechslera oryzae in bi-culture tests

	Colonydiameter (cm.) <sup>1/</sup>	Growth inhibition (%)	Spore number (×10 <sup>5</sup> /ml) <sup>1/</sup>	Spore inhibition (%)
Control	9.00a	0.00	6.37a	0.00
Bi-culture	4.40b	51.10	2.75b	55.65
CV (%)	2.85		25.99	

<sup>1/:</sup> Means four repeated experiments and followed by the same letter are not significantly differed by DMRT at P=0.05.

# In vitro test of crude extracts from antagonist against Drechslera oryzae causing brown spot of rice.

Result showed that methanol crude extract from *N. hiratsukae* gave significantly highest inhibition of 37.50 % for the colony growth of *D. oryzae* at the concentration of 1,000 ppm when compared to the control (Tables 2). Moreover, methanol crude extract from *N. hiratsukae* gave significantly highest inhibition of 79.47 % for the spore production of *D oryzae* at the concentration of 1,000 ppm when compared to the controlwhich the ED<sub>50</sub> was 168 (Table 2).

**Table 2**. Crude extracts of *N. hiratsukae* testing to inhibit *Drechslera oryzae* 

Crude extracts	Concen-	Colony-	Growth	Number of	Spore	ED <sub>50</sub>
	tration	diameter	inhibition	spore	inhibition	(µg/ml)
	(ppm)	(cm) <sup>1/</sup>	(%) <sup>1/</sup>	$(\times 10^5)^{1/}$	$(\%)^{1/}$	
Hexane	0	5.00a	0.00h	3.01a	0.00h	
	10	4.73abc	5.50fgh	1.94c	34.89f	
	50	4.50abc	10.00fgh	1.86c	36.73f	
	100	4.32bcd	13.50efg	1.78cd	38.78f	-
	500	4.22bcde	15.50def	1.68cde	43.49def	
	1000	3.52fgh	29.50abc	1.53cdef	48.72cdef	
Ethyl acetate	0	5.00a	0.00h	3.01a	0.00h	
	10	4.77ab	4.50gh	2.66ab	12.34gh	
	50	4.30bcd	14.00efg	2.50b	16.25g	
	100	3.92def	21.50cde	1.78cd	40.04ef	544.44
	500	3.55fgh	29.00abc	1.44cdef	50.83cdef	
	1000	3.35gh	33.00ab	1.28defg	55.88cde	
Methanol	0	5.00a	0.00h	3.01a	0.00h	
	10	3.75efg	25.00bcd	1.5cdef	50.25cdef	
	50	3.57fgh	28.50abc	1.2efg	58.55bcd	
	100	3.32gh	33.50ab	1.1fg	62.42bc	168
	500	3.17h	36.50a	0.8gh	72.76ab	
	1000	3.13h	37.50a	0.6h	79.47a	
	C.V. (%)	8.05	20.12	17.49	26.34	

<sup>1/:</sup> Means four repeated experiments and followed by the same letter are not significantly differed by DMRT at P=0.05.

# Test efficacy of fine particles from antagonist against Drechslera oryzae causing brown spot of rice.

Result showed that fine particle methanol from *N. hiratsukae* gave significantly highest inhibition of 64.00 % for the colony growth of *D. oryzae* at the concentration of 10 ppm when compared to the control (Tables 3). The fine particle methanol, fine particle ethyl aectate and fine particle hexane at the concentration of 10 ppm inhibited spore production by 94.87%, 93.32%, and 88.74%, respectively. These nanoparticles expressed antifungal activity against *Drechslera oryzae* with ED<sub>50</sub> values of 4.11, 4.60, and 4.63  $\mu$ g/ml, respectively (Table 3).

**Table 3**. Fine particles of *N. hiratsukae* testing to inhibit *Drechslera oryzae* 

Fine particle	Concen- tration	Colony- diameter	Growth inhibition	ED <sub>50</sub> (μg/ml)	Number of spore	Spore inhibition	ED <sub>50</sub> (μg/ml)
	( ppm )	(cm) <sup>1/</sup>	$(\%)^{1/}$		$(\times 10^5)^{1/}$	$(\%)^{1/}$	
Hexane	0	5.00a	0.00i		4.23a	0.00g	
	1	3.57b	28.50g		3.56b	15.71f	
	3	3.42c	31.50g	8.70	3.06c	27.12de	4.63
	5	2.97d	40.50f		2.80d	33.79d	
	7	2.52f	49.50d		1.30f	68.89b	
	10	2.27g	54.50c		0.47g	88.74a	
Ethyl	0	5.00a	0.00i		4.23a	0.00g	
acetate	1	2.97d	40.50f		3.35b	20.31ef	
	3	2.92de	41.50ef	5.28	3.34b	20.50ef	4.60
	5	2.92de	41.50ef		2.32e	44.84c	
	7	2.22g	55.50c		1.24f	70.66b	
	10	1.99h	60.00b		0.28g	93.32a	
Methanol	0	5.00a	0.00i		4.23a	0.00g	
	1	3.00d	40.00f		3.32b	20.95ef	
	3	2.97d	40.50f	4.73	3.00cd	28.57de	4.11
	5	2.80e	44.00e		2.40e	42.96c	
	7	2.29g	54.00c		1.43f	65.69b	
	10	1.79i	64.00a		0.22g	94.87a	
	C.V. (%)	3.09	5.02		7.85	14.14	

1/: Means four repeated experiments and followed by the same letter are not significantly differed by DMRT at P=0.05.

## Discussion

The brown spot of rice caused by *Drechslera oryzae* was isolteds and proved pathogenicity as similar report of Chaijuckam *et al.* (2019).

In this study, *N. hiratsukae* could inhibit mycelial growth of *D. oryzae* as 51.10% similar report confirmed *Neosartorya* sp. (KUFC 6301) could inhibit 30% mycelium growth of Bipolaris maydis, *Collectrichum capsici* and *C. gloeosporioides* (Eamvijarn *et al.*, 2009). Methanol crude extract from *N. hiratsukae* gave significantly highest inhibition of 37.50 % for the colony growth of *D. oryzae* at the concentration of 1,000 ppm this similar to the report of *N. pseudofischeri* KUFA0060 and *N. quadricincta* KUFA0064 crude extracts inhibited the mycelial growth of *P. palmivora* and *C. capsici* at a concentration of 100 ppm (Boonsang *et al.*, 2014). Jantasorn *et al.* (2016) also reported that *N. fischeri* Bodhi004 crude extract also recorded antifungal activity, 100% growth inhibition against *P. palmivora*, *P. grisea*, *Alternaria* sp. and *R. solani* at a concentration of 10,000 ppm. Fine particle methanol from *N. hiratsukae* gave significantly highest inhibition of 64.00 % for the colony growth of *D. oryzae* and 94.87% for inhibited spore production at the concentration of 10 ppm. This study was similar to the report *Chaetomium brasiliense* effectively against *Drechslera oryzae* at the concentration of 10 ppm (Vareeket, *et al.* 2014).

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## Bioactivity test of Chaetomium isolate CNC1 to control Pestalotia spp causing leaf spot of Orchid

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**Abstract** The active strain of *Chaetomium* CNC1 isolated from forestry soil in China. It was confirmed to inhibit *Pestalotia* spp causing leaf spot of Orchid from Thailand by dual agar culture test. It revealed that the colonies of *Chaetomium* grew over the pathogren's colony within 30 days incubation. The tested *Chaetomium* proved to be inhibited the colony growth and spore of *Pestalotia* sp. Crude hexane, ethyl acetate and methanol extracts resulted to inhibit colony growth and spore of tested pathogen.

Keywords: biocontrol, antagonist, Chaetomium, Orchid, leaf spot

#### Introduction

Orchid is the most popular plant for decorate and expensive in many country (Pares and Whitecross, 1982) because there are beautiful plant many people always plant orchid to decorate garden or restaurant. But when orchid was inoculated by pathogen the price of orchid will decrease because when they got lesion on leaves that make them cant decorate anymore and the most important pathogen of orchid is *Pestalotia* spp. that will inoculate on leaves of orchid and make a leaves go brown so in this case called "Leaf spot" (Maharachchikumbura, 2014) to many prople always use chemical fungicide to control but in a long term that will made the pathogen mutation (Pasche, 2015) so bicontrol is a good choice to control *Pestalotia* spp. for avoid mutations of pathogen.

# Materials and methods

## Isolation of the pathogen from leaf spot of orchid

Disease samples were taken from orchid leaves showing leaf spot disease

Symptom. The pathogen was isolated by tissue transplanting technique. The surface of the diseased orchid leaves were washed by sterile distilled water, then sterilized by rinsing them in alcohol 70% for 5 seconds, and then washed again by sterile distilled water. After that, the diseased orchid leaf samples were put into sterilized tissue papers until no more water drop on the surface of the orchid leaves. The advance margin of between healthy tissue and diseased tissue was then cut into small pieces of 2x2 mm in size, then transferred into water agar, then followed by potato dextrose agar (PDA) until get pure culture. The pure cultures of *Pestalotia* spp. were identified by morphological characteristic (Chen *et al.*, 2011) under a compound microscope at the Bio-control Research Unit and Mycology Section, Faculty of Agricultural Technology, KMITL, Bangkok, Thailand.

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#### Pathogenicity tests

The isolates were proved for their pathogenicity by using the detached leaf technique in the laboratory. A sterilized filter paper was placed in a sterilized 9 cm-diameter Petri dish. The leaves were wounded by a sterilized needle before placed on the filter paper in the Petri dish. A 0.5 cm diameter sterilized cork borer was used to remove agar plugs from the actively growing edge of the cultures of the *Pestalotia* spp. and placed onto the wounded position of the leaf surface. The filter paper in the Petri dish was moistened by sterilized distilled water. The non-inoculated leaves were treated with 0.5 cm sterilized agar plug served as control. All petri dishes were incubated at room temperature (27-30 $^{\circ}$ C) for 10 days before data collection. symptom was rate as Disease severity (DS) 0 = no disease, 1 = 1 - 10% leaf spot, 2 = >10 - 25% leaf spot , 3 = >25 - 50% leaf spot , 4 = >50 - 75% leaf spot , 5 = >75% leaf spot (Wheeler, 1969)

#### Bi-culture test

A mycelial disc of *Pestalotia* spp. (5 mm diameter) was placed singly (as controls) or oppositely to a mycelial disc of each above antagonist on 9-cm-diameter Petri dishes, which contained PDA. After incubation at 25°C for 30 days, data were collected as colony diameter and number of sporangia produced by *Pestalotia* spp. Numbers of sporangia were counted by using haemacytometer.

#### In vitro effects of antagonistic crude extracts on the growth of Pestalotia spp.

Chaetomium spp. were offered from Assoc. Prof. Dr. Kasem Soytong, from Faculty of Agricultural Technology, KMITL, Bangkok, Thailand. Crude extracts Chaetomium spp. were done by following the method of Kanokmedhakul et al. (2006). The antagonists were cultured in PDA and incubated at room temperature for 35 days before fresh fungal biomass were collected. The dried fungal biomass of the antagonists were serial extracted by soaked in hexane, ethyl acetate (EtOAc) and methanol (MeOH), 866 respectively to get filtrates before subjected to a rotary vacuum evaporator then yielded Hexane, EtOAc and MeOH crude extract.

The crude extracts of two antagonists were tested for inhibition of *Pestalotia* spp. The experiment was conducted by using the two factorial experiment in CRD with four replications.

#### Factor A represented crude extracts:

A1 = crude hexane extract

A2 = crude ethyl acetate extract

A3 = crude methanol extract

#### Factor B represented the concentrations:

 $B1 = 0 \mu g/ml$  (control)

 $B2 = 50 \,\mu g/ml$ 

 $B3 = 100 \,\mu g/ml$ 

 $B4 = 500 \, \mu g/ml$ 

 $B5 = 1,000 \mu g/ml$ 

The crude extracts at different concentration were separately dissolved in 2% dimethyl sulfoxide and added to separate PDA, then autoclaved at 121°C (15 psi) for 20 minutes. A sterilized 5-mm diameter cork borer was used to transfer agar plugs from the actively growing edge of the culture of *Pestalotia* spp. An agar plug of the pathogen was transferred to the center of a 5 cm-diameter Petri dish containing the prepared media, then incubated at room temperature until seeing colony of the pathogen on the control plates reach to the rim of the Petri dish.

Data were collected regarding colony diameter and number of spores

produced by *Pestalotia* spp. The number of observed spores and the colony diameter was then used to calculate percentage of spore producing inhibition and percentage of colony growth inhibition. The effective dose (ED50) was also calculated using the Probit analysis software.

#### Results

## Isolation, Identification and Pathogenicity Test of pestalotia spp.

Pestalotia spp. were isolated and identified was confirmed is Pestalotia spp. from leaf spot of orchid with obvious pure culture and coloni indentified (Fig.1). The isolate was confirmed pathogenic isolate from pathogenecity test. The result showed that isolate could infected in the leaves of orchid (Table 1). Pestalotia spp. Was isolated from orchid leaves exhibiting leaf spot symptom the pathogenicity of isolate was confirmed. The isolate produced typical leaf spot on orchid leaves.

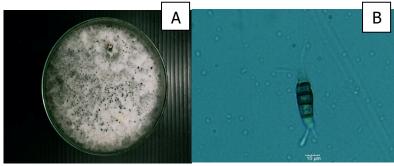


Figure 1. Pestalotia spp.A: Pure culture in PDA; B: Conidia

**Table 1**: Pathogenicity test on orchid leaves

Treatmants	Disease severity (DS)
Control	0
Pestalotia spp.	3

Table 1. Pathogenic test of leaf spot caused *Pestalotia* spp. 0 = no disease, 1 = 1 - 10% leaf spot, 2 = >10 - 25% leaf spot, 3 = >25 - 50% leaf spot, 4 = >50 - 75% leaf spot, 5 = >75% leaf spot (Wheeler, 1969)

## Bi-culture antagonistic tests

Chaetomium spp. isolate CNC-1 was proved its abilities to inhibit the growth of *Pestalotia* spp. by using bi-culture tests. The result showed that CNC-1 gave significantly colony inhibition of *Pestalotia* spp. when compared to the control. The number of spores that producing by the pathogen *Pestalotia* spp. was counted by using Hemacytometer. The results showed that CNC-1 significantly inhibited pathogen spores when compared to the control plate. The results showed that CNC - 1 significantly inhibited number of pathogen spores of 40 % when compared to the control

# Bioactivity test of crude extracts from Chaetomium spp. isolate CNC-1 against Pestalotia spp. causing leaf spot of orchid

Antagonistic *Chaetomium* spp. isolate CNC-1 was yielded metabolites as crude extracts and examined to control the growth of plant pathogen *Pestalotia* spp. The results showed that crude ethyl acetate from CNC-1 gave significantly highest inhibition of the colony growth of *Pestalotia* spp. when compared to the control, followed by

crude methanol. All tested crude extracts, ethyl acetate, hexane and methanol crude extracts from CNC-1 gave significantly inhibition of the colony growth and spore production of *Pestalotia* spp. It showed that antagonistic fungus *Chaetomium* isolate CNC-1

Table 2. Effect of crude extracts from Chaetomium spp. Isolated CNC1 on colony growth of Pestalotia spp.

Crude extract	Concentration (ppm)	Colony diameter (cm)	Growth inhibition (%)	Number o sporangium (×10 <sup>6</sup> )	f Sporangium inhibition (%)	ED <sub>50</sub> (μg/ml)
	0	5.00a	-	20.47a	-	10.53
	10	4.47bc	10.50l	9.69e	52.61e	
Hexane	50	4.14def	17.50k	8.60f	57.98e	
	100	4.12def	17.00k	5.29h	74.10b	
	500	3.94f	21.00j	4.09j	79.97bc	
	1000	3.5g	30.00i	2.23jk	89.06c	
	0	5.00a	-	20.47a	-	4.43
	10	4.50bc	10.00	8.35f	59.20de	
EtOAc	50	4.34cd	13.0l	6.86g	66.29d	
	100	4.07ef	18.50k	4.60i	77.52c	
	500	4.05f	19.00k	2.50j	87.78b	
	1000	3.44g	31.00i	1.67k	91.81a	
	0	5.00a	-	20.47a	-	91.29
	10	4.60bc	8.001	13.82b	32.46g	
MeOH	50	4.52b	9.501	11.62c	43.20f	
	100	4.47bc	10.50l	10.34d	49.43ef	
	500	4.42bc	11.50k	7.16g	65.00d	
	1000	4.30cde	14.00k	5.84h	71.42c	
C.V.(%)		3.48	•		4.78	

The result from *Chaetomium* spp. Isolate CNC1 that hexane crude extract at the concentrations of 50, 100, 500 and 1000  $\mu$ g/ml gave significant difference in the percentage of sporangium inhibition of *Pestalotia* spp. Which were 52.61 , 57.98 , 74.10 , 79.97 and 89.06 respectively. Followed growth inhibition which were 10.50 , 17.50 , 17.00 , 21.00 and 30.00 respectively. EtOAc crude extract at concentrations of 50, 100, 500 and 1000  $\mu$ g/ml significant difference in the percentage of sporangium inhibition of *Pestalotia* spp. Which were 59.20 , 66.29 , 77.52 , 87.78 and 91.81 respectively. Followed growth inhibition which were 10.00 , 13.00 , 18.50, 19.00 and 31.00 respectively. MeOH crude extract at concentrations of 50, 100, 500 and 1000  $\mu$ g/ml significant difference in the percentage of sporangium inhibition of *Pestalotia* spp. Which were 32.46 , 43.20 , 49.43 , 65.00 and 71.42 respectively. Followed growth inhibition which as 8.00 , 9.50 , 10.50 , 11.50 , 14.00 respectively. (Table.2) In this case *Chaetomium* spp. isolate CNC1 with EtOAc significant inhibition to *Pestalotia* spp.

# Discussion

As the results, *Chaetomium* isolate CNC-1 is proved to act as biological activity against *Pestalotia* spp. causing leaf spot of orchid from crude extracts of CNC-1 was successful to control *Pestalotia* spp. (Tathan *et al.*, 2012). at low concentration. It is useful resource as nature product to inhibit the pathogen which causing leaf spot of orchid. It is

not only reduced the production loss of growers but also reduced to pollute the environment as compared the traditional method. Some report showed in the same result that *Pestalotia* spp. can control by biocontrol. For instance grey blight of tea caused *Pestalotia* spp. (Phong *et al.*, 2017) *Trichoderma viride* can control *Pestalotia* spp. in post – harvest (Bhuvaneswari and Subba, 2001) and *Trichoderma harzianum* control *Pestalotia* spp on lettuce (Hutchinson, 1972). *Chaetomium* also control many disease such as *Chaetomium globosum* in biocontrol of *Pythium ultimum* (Di Pietro, 1992), *Chaetomium cupreum* for biocontrol of *Colletotrichum gloeosporioides* Causing Coffee Anthracnose on Arabica (Vilavong and Soytong, 2017).

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# Factors Affecting Okra Productivity in Suphan Buri Province, Thailand

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**Abstract** The study has been conducted to identify the factors affecting okra productivity by using multiple regression analysis in Suphan Buri Province, Thailand. Structure questionnaires were conducted to gather data from 82 okra growers in Suphan Buri Province. Descriptive statistics were use to describe socio-economic variables of the respondents as presented. Besides, multiple regression analysis was employed in determining the factors influencing farmers' productivity. The results revealed that the variables found to be highly significant and which influenced farmers' productivity include gender (p<.05), age (p<.01), experience in okra production (p<.01), okra farm size (p<.01), and quantity of chemical fertilizer (p<.01), respectively, with the adjusted R<sup>2</sup> was 89.80.

**Keywords:** Okra, okra productivity, okra growers, regression model.

#### Introduction

Okra (*Abelmoschus esculentus* L. Moench) is believed to have originated from Africa and is being grown in most sub-tropical and tropical regions of the world (CBI Market Intelligence, 2016), which has been one of the important export vegetables cultivated in Thailand for decade, especially in the central region of Thailand. Almost all okra farmers in Thailand are contract farmers for the export companies (FAO Vegetable IPM 2004), which is more than 95 percent of fresh pod was exported to Japan in 2002.

Thailand's export of fresh okra to Japan in 2007 decreased sharply from 2006 (Thai Custom, 2019). Therefore, the objectives of this study are 1) to investigate the socio-economic characteristics okra growers who are the members of the okra farming network for export in Suphan Buri Province, Thailand, and 2) identify factors that lead to okra productivity

#### Literature Review

Regression analysis is a related technique to assess the relationship between an outcome variable and one or more risk factors or confounding variables which the dependent variable is denoted "y" and the independent variables are denoted by "x", which is widely used for prediction and forecasting, also used to understand which among the independent variables are related to the dependent variable, and to explore the forms of these relationships. In

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restricted circumstances, regression analysis can be used to infer causal relationships between the independent and dependent variables.

A review of previous research found that several studies have used regression analysis to determine plant productivity such as Nosiru *et al.* (2012) used regression analysis to improved Productivity of Okra (Abelmoschus esculentus) by Farmers in Lowland Areas of Ogun State, Nigeria. Jelena *et al.* (2011) used regression analysis to analyze external and internal factors influencing the growth and biomass production of short rotation woods genus Salix and perennial grass Miscanthus.

#### Materials and methods

#### 1. The study area

U Thong and Song Phi Nong District of Suphan Buri Province located in Central Thailand (Fig.1), and is the most productive area of okra production for export (DOAE, 2016).



Fig.1 Map of Suphan Buri Province, Thailand

#### 2. Data collection procedure

A total of 440 okra growers were cultivating okra commercially by referred the data from Department of Agricultural Extension: DOAE (2016) for January-December, 2016; a sample of 82 okra growers were selected randomly by referred a list of farmers in the okra sell center by using probability proportionality size following a simplified formula provided by Yamane (1967).

The required sample size at 90% confidence level with degree of variability of 10% will be used to obtain a sample size required which represents a true population as follow:

$$n = \frac{N}{1 + N(e^2)} \tag{1}$$

Where; n is Sample size, N is Population size and e is Allowable error (e = 0.10).

An estimated of n as follows equation (1) based on the intensity of okra production in Suphan Buri Province is equal 82.

Primary data was collected by using a structured questionnaire adapted from a baseline survey Export Okra Production in Thailand conducted by the FAO Vegetable IPM. (2004)

#### 3. Method of data analysis

The study was based on primary data and was confined to Suphan Buri Province. Farm size in okra production in rai (1,600 square meters) was used to standardize of the inputs in terms of the quantities per rai.

The data included information on okra production such as: amount of work hours in production, quantity of inputs (seed, chemical fertilizer), number of plowing, and okra farm size.

For estimating the impact of various factors on okra productivity, regression analysis was carried out. Various inputs and agricultural practices were considered as independent variables and the okra productivity as dependent variable, following the multiple regression equation was used and presented below to identify factors affecting okra productivity in Suphan Buri Province, Thailand.

$$Y_{i} = \theta_{0} + \theta_{1}X_{1} + \theta_{2}X_{2} + \theta_{3}X_{3} + \theta_{4}X_{4} + \theta_{5}X_{5} + \theta_{6}X_{6} + \theta_{7}X_{7} + \theta_{8}X_{8} + \theta_{9}X_{9} + \theta_{10}X_{10} + \theta_{11}X_{11} + \theta_{12}X_{12}$$
(3)

#### Where:

Y = Okra productivity of each okra growers in Suphan Buri (KG./Rai)

 $\theta_0$  = Intercept

 $\theta_{1}$ – $\theta_{8}$  = Regression coefficients of the respective input variables

 $X_1$  = Gender of respondent (Male = 1, Female = 0)

 $X_2$  = Age of respondent (Years)

 $X_3$  = No. of schooling year (Years)

 $X_4$  = Marital status (Married = 1, single = 0)

 $X_5$  = Experience in okra production (Year)

 $X_6$  = Family members for okra production (Person/household)

 $X_7$  = Okra farm size (Rai/household)

 $X_8$  = Quantity of okra seed (KG./Rai)

 $X_9$  = Quantity of chemical fertilizer (KG./Rai)

 $X_{10}$  = Farm Type (garden grove = 1, ridge tillage = 0)

 $X_{11}$  = Number of plowing (Time/Rai/Household)

 $X_{12}$  = Sum total of work hours in okra production (Hour/Rai)

#### Results

#### Socio-economic characteristics of okra growers

The socio-economic characteristics of okra growers in the study area consist gender, age, years of schooling, marital status, number of family member, and experience in okra production were analyzed by using descriptive statistics which the result is presented in Table 1.

 Table 1. Socio-economic Characteristics of okra growers in Suphan Buri Province

Characteristics	%	Mean	Minimum	Maximum
Gender				
Male	48.80	n.a.	n.a.	n.a.
Female	51.20	n.a.	n.a.	n.a.
	100.00%	0	0	0
Age (Year)				
30 - 39	34.10	n.a.	n.a.	n.a.
40 - 49	30.50	n.a.	n.a.	n.a.
50 - 59	22.00	n.a.	n.a.	n.a.
> 60	13.40	n.a.	n.a.	n.a.
	100.00%	0	0	0
No. of schooling year (Year)				
0 - 6	72.00	n.a.	n.a.	n.a.
7 - 9	13.40	n.a.	n.a.	n.a.
10 - 12	12.20	n.a.	n.a.	n.a.
14	2.40	n.a.	n.a.	n.a.
	100.00%	0	0	0
Marital status				
Single	17.10	n.a.	n.a.	n.a.
Married	82.90	n.a.	n.a.	n.a.
	100.00%	0	0	0
Experience in okra production (Year)				
1-5	57.30	n.a.	n.a.	n.a.
6 - 10	19.50	n.a.	n.a.	n.a.
11 - 15	17.10	n.a.	n.a.	n.a.
> 15	6.10	n.a.	n.a.	n.a.
	100.00%	0	0	0
Farm Type				
Garden grove	34.10	n.a.	n.a.	n.a.
Ridge tillage	65.90	n.a.	n.a.	n.a.
	100.00%	0	0	0
Family members (Person/household)	n.a.	3.98	2.00	7.00
Okra farm size (Rai/household)	n.a.	1.52	0.25	8.00
Quantity of okra seed (KG./Rai)	n.a.	0.76	0.13	4.00
Quantity of chemical fertilizer (KG./Rai)	n.a.	137.03	22.50	720.00
Number of plowing (Time/Rai)	n.a.	2.05	1.00	3.00
Sum total of work hours (Hour/Rai)	n.a.	13.55	10.38	17.16
Sum total of okra product (KG. /Rai)	n.a.	2,708.78	970.00	4,500.00

From gender perspective, about 48.80% were found to be male for okra production in Suphan Buri Province while 51.20% were found to be female.

On age classification, 64.60% were found to be within the age bracket of 40 – 49 years. While 13.40% of the respondents is older than 60 years. Contrary to findings of past studies which reported the farming population to be ageing (Idowu, 1989), the present study shows a young farming population.

Number of schooling years of okra growers, from this study reveals that 72.00% of the respondents have 6-year schooling while about 12.20% are okra grower who have 12-year schooling. Thus, over than 60% of the respondents have had one form of formal education. Evidence indicates that the okra growers in Suphan Buri Province is an educated one similarly with Gabriel *et al.* (2006).

On experience in okra production, 57.30% were revealed to be the respondents have 1-5 years experience in okra production while 6.10% were revealed to be respondents have more than 15 years experience in okra production.

The total productivity of each okra grower household as revealed in the Table 1, an average productivity is 2,807.93 KG./Rai/Crop. While the minimum productivity and maximum productivity is 1,350.00 KG./Rai/Crop and 4,500.00 KG./Rai/Crop respectively. It is clear that okra production in Suphan Buri Province is highly productivity.

# Estimation of factors affecting okra productivity in Suphan Buri Province.

In table 2, the coefficient of determination ( $R^2$ ) was 89.80 which states that given independent variables; explain 89.80 percent variation in the okra productivity. The Durbin Watson statistics (1.887) was also normal showing absence of autocorrelation in the data series.

**Table 2.** Enter Regression Analysis Results of Factors Affecting<sup>1</sup> on the Okra productivity in Suphan Buri Province, Thailand.

Variables	В	Std. Error	Beta	t	P-Value
(Constant)	4,722.329	840.526		5.618	0.000
Gender	119.309	174.037	0.079	0.686	0.034*
Age	-22.511	106.561	-0.031	-0.211	0.003**
No. of schooling year	177.478	113.264	0.187	1.567	0.122
Marital status	-288.785	253.340	-0.144	-1.140	0.258
Experience in okra production	-20.585	110.350	-0.026	-0.187	0.000**
Family members	107.117	72.321	-0.163	-1.481	0.143
Okra farm size	-0.565	0.179	0.156	0.106	0.002**
Quantity of okra seed	-172.411	182.938	-0.110	-0.942	0.349
Quantity of chemical fertilizer	0.219	0.048	0.238	0.073	.000**
Farm type	-355.411	213.794	-0.223	-1.662	0.101
Number of plowing	-60.993	91.018	-0.081	-0.670	0.505
Sum total of work hours in production	-88.759	46.160	-0.221	-1.923	0.059
Multiple $R^2 = 0.912$	F = 24.954				
Adjusted $R^2 = 0.898$	Durbin – Watson = 1.887				

Note:  $^{1} * p \le 0.05$ ,  $^{**} p \le 0.01$ 

Table 2 shows that there is 5 out of 12 independent variables in the regression equation were significant. That is gender(t = 0.686), age(t = -0.211), okra farm size(t = 0.106), experience in okra production(t = -0.187), quantity of

**chemical fertilizer** (t = 0.073), which all the coefficients were statistically significant at 5%, 1% and 0.1% level, respectively.

**Gender** ( $X_1$ ) had a positive sign at the 5% level of probability. By increasing in male okra growers by a unit, the output level will increase by 119.31%. Due to 51.20% were found to be female.

Age  $(X_2)$  had a negative sign at the 1% level of probability. By increasing in age by a unit, the output level will decrease by 22.51%. Due to 64.60% were found to be within the age bracket of 40 – 49 years.

**Marital status** ( $X_4$ ) had a negative sign. By increasing in married status by a unit, the output level will decrease by 288.79%. According to 82.90% were found to be married.

**Experience in okra production (** $X_5$ **)** had a negative sign at the 1% level of probability. By increasing in experience in okra production by a unit, the output level will decrease by 20.59%. Because 57.30% had 1-5 years' experience in okra production.

Okra farm size  $(X_7)$  had a negative sign at the 1% level of probability. By increasing in okra farm size by a unit, the output level will decrease by 56.50%. This is because okra plants demand much attention from grower, especially while maintaining and harvesting. If okra farmers can't attend adequately, the productivity will decrease.

Quantity of okra seed ( $X_8$ ) had a negative sign. By increasing in quantity of okra seed by a unit, the output level will decrease by 172.41%. This is because using more seed will reduce space of okra plants, especially fruit length, which is accordance with Singh (1990)

Quantity of chemical fertilizer ( $X_9$ ) had a positive sign at the 1% level of probability. By increasing quantity of chemical fertilizer by a unit, the output level will increase by 21.90%. This indicated that the yields can be explained by increasing in chemical fertilizer use. This result is in line with the findings of Rahman *et al.* (2012); Anyiro *et al.* (2013).

Farm type ( $X_{10}$ ) had a negative sign. By increasing in garden grove farm type by a unit, the output level will decrease by 355.41%. This is because planting by garden grove method has space between okra plant less than ridge tillage method.

Number of plowing  $(X_{11})$  had a negative sign. By increasing in number of plowing by a unit, the output level will decrease by 60.99%. This is because more plowing will kill soil organisms, which is affected by okra productivity.

Sum total of work hours in okra production ( $X_{12}$ ) had a negative sign. By increasing in sum total of okra operating hour by a unit, the output level will decrease by 88.76%, a finding is similar to Baree *et al.* (2011); Rahman *et al.* (2012), who showed the importance of labor in farming.

Base on all details above, the final equation of multiple regression for determining okra productivity in Suphan Buri Province will be:

```
Y_i = 4,722.329 + 119.309X_1 - 22.511X_2 + 177.478X_3 - 288.785X_4 - 20.585X_5 (5.618 (0.686) (-0.211) (1.567) (-1.140) (-0.187) + 107.117X_6 - 0.565X_7 - 172.411X_8 + 0.219X_9 - 355.411X_{10} - 60.993X_{11} (-1.481) (0.106) (-0.942) (0.073) (-1.662) (-0.670) - 88.759X_{12} (-1.923)
```

#### Conclusion

The regression analysis result was used to identify the determinant factors of okra productivity, which the result showed that independent variable such as gender, age, okra farm size, experience in okra production, and quantity of chemical were statistically significant variables that affected the okra productivity, especially experience and quantity of chemical fertilizer these parameters could be observed to be most significantly contributing to improving productivity of okra growers.

Moreover, this study found that okra growers in Suphan Buri Province should be encouraged to increase quantity of chemical fertilizer. While should decrease quantity of okra seed, number of plowing and sum total of okra operating hour in okra production for increasing their productivity.

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# Trends of Precipitation and Temperature in Morogoro Region in Tanzania

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Abstract Climate changes can seriously affect the environment and rural communities, water availability, health, food security, biodiversity, and agricultural production. Farmers in Africa who depend on rain to grow crops are already recognizing the effects of changing climate. Weather is essential to farmers' decision-making. The present study analysed spatial and temporal trends of precipitation and temperature over the Morogoro region by using available climate data from the local meteorological agencies. The data were analysed during the period of 1991–2015 for the mean monthly precipitation and temperature and predicted the monthly precipitation and temperature by using the time series analysis. The result showed that the climate is highly variable from arid to humid. At the same time, the trend line of mean monthly showed a slightly decreasing linear trend in precipitation and an increase in temperature. The forecast values of the rain and temperature until 2025 by the decomposition method with the multiplicative model showed that precipitation in May — October will be low. Moreover, the seasonal indices predicted that precipitation in those months will be lower than previously. The future trends should be carefully considered to help farmers in selecting proper cultivation strategies.

Keywords: Precipitation, Temperature, Time series analysis, Morogoro region, Tanzania

#### Introduction

Over the last few decades, most parts of Tanzania have experienced incidences of extreme climatic events, particularly floods and droughts that have been associated with severe socio-economic and ecological implications. The most recent severe floods include those of 2006, 2009, 2010, 2011, 2012, 2014, 2016 and 2017 that caused in many parts of the country loss of many lives and infrastructural destructions. (Kijazi and Reason, 2009) (Viste, Korecha and Sorteberg, 2013).

The climate in Africa is semi-desert (semi-arid), and the rainfalls vary over the area and time. Morogoro region lies between the seasonal rainfall patterns of Northern and Southern Tanzania. Northern Tanzania has bimodal rainfall. The short Vuli rains start between mid-September and mid-October and continue until December. The long Masika rains start in mid-March and last until late May. Southern Tanzania has unimodal Msimu rains, which start in November and end in April or May. (Paavola, 2008).

General climatic conditions and climate changes are well described by mean monthly values, while the extremes are better reflected by the daily data. During the last decades, daily temperature and precipitation observations have been used for computation of the indices. (Zhang et al., 2011).

Annual rainfall in Morogoro region varies from 4000 mm in the high altitudes of the Eastern slopes of the Uluguru mountains to about 600 mm in the low altitude plains (Kangalawe *et al.*, 2014). Families live in farms which in many places are smaller than 2 hectares (Rapsomanikis, 2015). More than 95% of sub-Saharan African agriculture is rainfed, and rainfall changes are felt particularly by those who directly depend on reliable weather patterns. (Simelton *et al.*, 2013) (Mkonda and He, 1980). Weather is a multiple parameter made up of air temperature, atmospheric pressure, humidity, precipitation, solar radiation, and wind. In the dry and semi-dry areas climate naturally plays very

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crucial role in the agriculture and livelihood of the population. As a result of the climate change and variability, climate indicators have become unstable. The change in the quantity and distribution of rain has major long-term effects on the natural systems, such as surface waters and rivers and especially on those people who rely on agriculture. Rainfall fluctuations are the main cause of crop changes in many areas of Tanzania. (Dayoub *et al.*, 2018), although other factors such as soil type, temperature, and low agricultural inputs may play a role in the productivity. Therefore, the study of characteristics of mean monthly precipitation and temperature including monthly precipitation and temperature in Tanzania using time series analysis is of importance. (Hamisi, 2013).

#### Materials and methods

### Description of the study area

The United Republic of Tanzania locates to the South of the equator between 1 - 12°S and 29 - 41°E. The country has a total area of 945,087 km² of which 883,749 km² is covered by land area, and 59,050 km² is covered by inland waters and part of the Indian Ocean. The country is characterized by diverse and complex topographical features extending from a narrow coastal belt of the western Indian Ocean with sandy beaches to an extensive plateau with altitude ranging from 1000 to 2000 m above mean sea level. The plateau is fringed by narrow belts of highlands, including Mount Meru with an altitude of (4566 m), and the highest mountain in Africa and the second highest mountain in the world: Mount Kilimanjaro with an altitude of (5895 m). Tanzania has several fresh water bodies, including the largest lake in Africa; Lake Victoria, the longest and deepest lake in Africa; Lake Tanganyika and Lake Nyasa (Chang'a et al., 2017) (Kijazi and Reason, 2009).

Morogoro is a town with a population of 315,866 (2012 census) in the Eastern part of Tanzania, 196 km west of Dar es Salaam, the country's largest city and commercial center, and 260 kilometres east of Dodoma, the country's capital city. Morogoro is the capital of the Morogoro region. It is also known informally as "Mji kasoro bahari". The annual temperature averages here 24.6 °C and rainfall 935 mm.

#### Data collection

Weather data for precipitation and temperature in Morogoro region, Tanzania were collected from WeatherOnline Ltd. - Meteorological Services. ('www.weatheronline.co.uk', 2018).

The precipitation data set was consecutive monthly from January of 1991 to December of 2015 and January of 2004 to August of 2019 for temperature data set.

#### Trend analysis

Trend analysis of mean monthly precipitation and temperature were analysed with Microsoft Excel 2013 to determine the trend line of mean monthly precipitation and temperature in Morogoro region. The model is Y = a + bt, where Y is precipitation /temperature, a is intercept, b is slope and t is time.

Time series analysis

The decomposition method was used with the multiplicative model to identified trend cycle, and the seasonal and irregular analysis for mean monthly precipitation and temperature.

Multiplicative model is  $Y = T \times C \times S \times I$ , where Y = precipitation/temperature, T = the trend component, C = the cycle component, S = the seasonal component and I = the irregular component. The analysis was carried out using Microsoft Excel 2013. Measures of forecast accuracy consist of Mean Absolute Deviation (MAD), Mean Squared Deviation (MSD) and Mean Absolute Percent Error (MAPE).

# **Results and Discussion**

# 1. Descriptive Statistics of mean monthly precipitation and temperature

Temperature and precipitation are fundamental measures for describing the climate and can have wide-ranging effects on farming. Table 1 shows the average monthly rainfall from January to December (1-12) during the 25-year period. The standard deviation (SD) represents variation in the values for the temperature and precipitation, the highest relative SDs of rainfalls occurs during the dry season (6-8). The SDs of temperatures are annually rather small.

Table 1.	Mean $\pm$ SD of mean monthly precipitation and temperature in Morogoro, Tanzania.
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Month	1	2	3	4	5	6	7	8	9	10	11	12
Precipi tation	150.3± 41.8	129.9 ±36.4	174.5 ±36.3	147.8 ±29.5	56.8 ± 17.8	12.7 ± 6.1	5.3± 2.9	7.9± 3.7	17.8 ±0.0 3	33.1 ± 0.5	89.4 ± 7.1	148.6 ±53.1
Tempe rature	27.29± 0.8	27.20 ±0.8										

### 2. Trend Analysis of mean monthly precipitation and temperature

The trend line of mean monthly precipitation and temperature in Morogoro region were presented in figures 1 and 2, respectively. The trend lines showed slightly deceasing linear trend following the equation: y=83.5-0.015t. It shows that every 83.5 months in Morogoro precipitation would decrease by 0.015 mm. Furthermore, it was observed that there was season component but there was no cycle and irregular component.

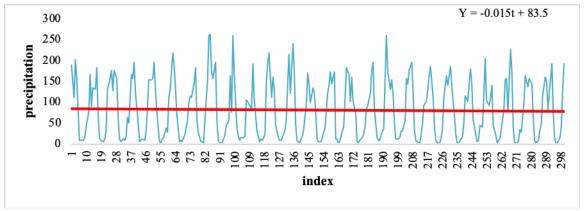


Figure 1. Trend line of mean monthly precipitation in Morogoro, Tanzania (1991–2015).

The trend line of mean monthly temperature (Fig.2), shows a slightly increasing linear trend described by equation: y = 25.1 + 0.0002t. The linear equation shows that for every 25.1 months the temperature would increase by 0.0002 C.

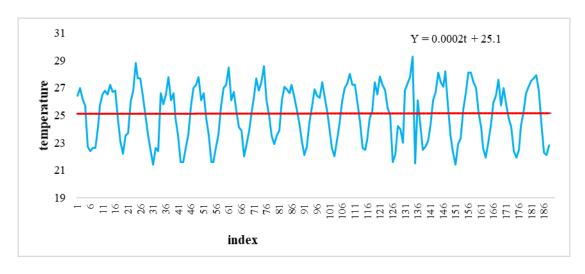


Figure 2. Trend line of mean monthly temperature in Morogoro, Tanzania (2004–2019).

# 3. Time series Analysis of mean monthly precipitation

The result of time series analysis of mean monthly precipitation by using the decomposition method with multiplicative model indicated that the Mean Absolute Deviation (MAD) was 18.7. Mean Square Deviation (MSD) was 810.4. Mean Absolute Percentage Error (MAPE) was 38.4. Forecast values mean monthly precipitation in the future (2016 - 2025) was presented in Table 2 and Figure 3.

**Table 2.** Forecast values of monthly precipitation in the future (2016 – 2025).

Voor	Month												
Year	1	2	3	4	5	6	7	8	9	10	11	12	
2016	149.4	129.9	174.2	143.8	60.6	12.7	4.7	7.78	16.9	31.7	93.3	152.2	
2017	149.2	129.8	174.0	143.6	60.6	12.7	4.7	7.77	16.9	31.7	93.2	152.1	
2018	149.1	129.7	173.8	143.5	60.5	12.7	4.7	7.76	16.9	31.6	93.1	151.9	
2019	148.9	129.5	173.6	143.3	60.4	12.7	4.7	7.76	16.9	31.6	93.0	151.7	
2020	148.7	129.4	173.4	143.2	60.4	12.7	4.7	7.75	16.8	31.6	92.9	151.6	
2021	148.6	129.2	173.3	143.0	60.3	12.6	4.7	7.74	16.8	31.5	92.8	151.4	
2022	148.5	129.1	173.1	142.9	60.3	12.6	4.6	7.73	16.8	31.5	92.7	151.3	
2023	148.3	129.0	172.9	142.7	60.2	12.6	4.6	7.72	16.8	31.5	92.6	151.1	
2024	148.2	128.9	172.7	142.6	60.1	12.6	4.6	7.72	16.8	31.4	92.5	151.0	
2025	148.0	128.7	172.6	142.4	60.1	12.6	4.6	7.71	16.8	31.4	92.4	150.8	

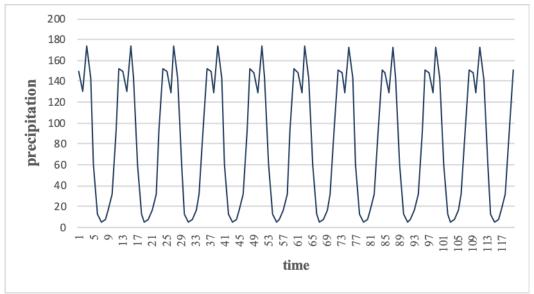


Figure 3. Forecast values of monthly precipitation in the future (2016–2025).

The result of seasonal indices is presented in Table 3. It shows that precipitation in July is 94.3% lower than the normal level. Secondary was August (90.4%), June (84.4%), September (79.2%), October (61.1%) and May (25.6%), respectively. For the higher than normal level was March at 113.8%. Secondary was December (87%), January (83.4%), April (76.5%), February (59.5) and November (14.6%), respectively.

Table 3.Seasonal indices of precipitation

	Seasonal Period (month: January-December)											
	1	2	3	4	5	6	7	8	9	10	11	12
Index	183.4	159.5	213.8	176.5	74.5	15.6	5.7	9.6	20.8	38.9	114.6	187

# 4. Time series analysis of mean monthly temperature

The result of time series analysis of mean monthly temperature by using the decomposition method with multiplicative model indicated that Mean Absolute Deviation (MAD) was 0.484. Mean Square Deviation (MSD) was

0.6. Mean Absolute Percentage Error (MAPE) was 1.9. Forecast values mean monthly temperature in the future (September of 2019 – December of 2025) were presented in Table 3 and Figure 4.

Table 3. Forecast values of monthly temperature in the future (2016 – 2025).

Vaan		Month													
Year 	1	2	3	4	5	6	7	8	9	10	11	12			
2019									24.2	25.8	26.7	27.4			
2020	27.7	27.2	26.7	25.5	24.3	22.7	22.1	23.1	24.2	25.8	26.8	27.4			
2021	27.7	27.2	26.7	25.5	24.3	22.7	22.1	23.0	24.2	25.8	26.8	27.4			
2022	27.7	27.2	26.8	25.5	25	22.7	22.1	23.1	24.2	25.8	26.8	27.4			
2023	27.7	27.2	26.8	25.5	24.3	22.7	22.1	23.1	24.2	25.9	26.8	27.4			
2024	27.7	27.2	26.8	25.5	24.3	22.7	22.1	23.1	24.2	25.9	26.8	27.4			
2025	27.7	27.2	26.8	25.5	24.3	22.7	22.2	23.1	24.2	25.9	26.8	27.5			

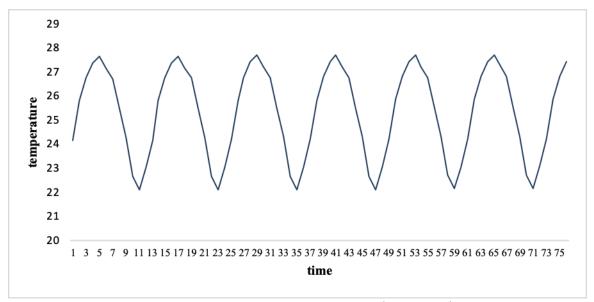


Figure 4. Forecast values of monthly temperature in the future (2019–2025).

The result of seasonal indices is presented in Table 4. It shows that temperature in January was 9.6% higher than normal level. Secondary was December (8.4%), February (7.5%), November (5.9%, March (5.8 %), October (2.2%) and April (0.8%. For the lower than normal level was July (12.5%). Secondary was June (10.4%), August (8.9%), September (4.3%) and May (3.9%).

Table 4.Seasonal indices.

	Seasonal Period (month)											
	1	2	3	4	5	6	7	8	9	10	11	12
Index	109.5	107.5	105.8	100.8	96.1	89.6	87.5	91.1	95.7	102.2	105.9	108.4

# Conclusions

The trend line of the mean monthly precipitation in the period of 1991-2015 showed a slight decreasing linear trend. The forecast values in the future (2016-2025) by the decomposition method with the multiplicative model showed that precipitation in May – October is low (these months are very critical for crops). Moreover, the result of seasonal indices showed that precipitation in that month is lower than the normal level, as well. The present study suggests that Morogoro gets a higher rainfall in December. The study demonstrates the existence of trends in the yearly rainfall. However, in Morogoro, there is a decreasing trend.

Temperature showed no significant warming during the period of study even some very small linear warming component was noticed.

The strategy for farmers and policymakers may be advised to pay attention to improve new crop varieties which are more tolerant for drought and required less water. At the same time, there is a need for further studies with daily and monthly data for longer periods. To get more accurate data, more of the meteorological stations and their services would be needed available which data should be also available to farmers in village level.

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