"Research and Innovation towards Sustainable Food System"

THE 4th POSTGRADUATE SYMPOSIUM

9 December 2021

Faculty of Agro-Based Industry,
Universiti Malaysia Kelantan,
Jeli Campus, Kelantan,
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The 4th Postgraduate Symposium
"Research and Innovation towards Sustainable Food System"
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Organized by
Faculty of Agro-based Industry (FIAT)
Universiti Malaysia Kelantan

in collaboration with
Guru Ghasidas University, University Gadjah Mada, Vikrama Simhapuri University, Princess of Naradhiwas University & Yogi Vemana University

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Preface

The Postgraduate Symposium is organized by Faculty of Agro-based Industry, Universiti Malaysia Kelantan to provide a platform for research postgraduate students to present and share their exciting ideas, and progress in research. The year 2021 marks the 4th Postgraduate Symposium rendering the theme "Research and Innovation towards Sustainable Food System", aims to promote academic interaction and intellectual advancements among the participants. It is hoped that the postgraduate students will benefit from the sharing sessions among presenters, invited speakers, supervisors and fellow students. The faculty welcomes anyone that shares the same interest to participate in this symposium either from the local or international universities. The organizing committee sincerely hopes that this symposium provides interesting and multidimensional perspectives and relevant information which is useful for participants' professional and personal use.

Message from

DEAN OF FACULTY OF AGRO-BASED INDUSTRY

Associate Professor Dr. Seri Intan Binti Mokhtar

Assalamualaikum Wr Wb.,

Salam Sejahtera, and very good morning. In the name of Allah, the Most Gracious and the Most Merciful.

On behalf of the Faculty of Agro-based Industry, Universiti Malaysia Kelantan, I welcome you to the 4th Postgraduate Symposium 2021. This year, our central theme is "Research and Innovation towards Sustainable Food System". I would like to extend our greetings to all distinguished speakers, participants and guests from Malaysia, India, Indonesia, and Thailand who are here with us today. High appreciation is also extended to the committee members of this symposium for organizing it and ensuring that it proceeds smoothly.

Research and innovation are crucial in ensuring a sustainable food system for our ever-expanding global population, reaching 10 billion people in 2050. At the same time, the agriculture sector faces the critical challenge of producing and distributing sufficient food in climate change conditions and scarce natural resources. Because of that, we must share knowledge in the face of such challenges; a challenge shared is a challenge divided, but knowledge shared is knowledge multiplied.

Building sustainable food systems requires work in all three dimensions of sustainable development – social, economic, and environmental. More efficient food systems are critical for alleviating poverty, meeting the world's food needs, and shrinking agriculture's ecological impact.

Today we will be witnessing, discussing and listening to progress made in the area of sustainable food systems from distinguished speakers and excellent participants. I wish the participants a very fruitful and productive symposium. I am looking forward to hearing the outcome and constructive conclusions of this meeting.

Herewith I officially open this symposium.

Thank you very much.

Message from

CHAIRMAN OF
THE 4TH POSTGRADUATE SYMPOSIUM

Ts. Dr. Nor Dini Binti Rusli

Dear Participants,

It is my great pleasure to welcome you to the 4th Postgraduate Symposium, organised by the Faculty of Agro-based Industry, Universiti Malaysia Kelantan. We are honoured to host this symposium virtually in the midst of the pandemic Covid-19. The postgraduate symposium has become an innovative platform for participants, particularly the postgraduate students to contribute and exchange ideas with like-minded peers at international level in order to improve their oral, writing, and presentation skills.

We, at the Faculty of Agro-based Industry are committed to taking a leadership role in addressing global sustainability challenges, which is food system sustainability through inter-disciplinary research and engagement, as well as through its campus operations. Hence, "Research and Innovation towards Sustainable Food System" as the theme of our symposium is much justified. The symposium covers five scopes which include agro-technology, animal science, food security, agribusiness and product development.

I would like to take this golden opportunity to thank our international collaborators; Guru Ghasidas University, India, Vikrama Simhampuri University, India, Yogi Vemana University, India, Universitas Gadjah Mada, Indonesia and Princess of Naradhiwas University, Thailand for your commitments and contributions. My sincere thanks also for all keynote speakers, plenary speakers and all participants for their contribution to make this symposium a great success and fruitfulness.

As a chairperson of the conference, I wish to express my sincere thanks to committee members for their devotion and efforts to make this postgraduate symposium a resounding success.

Thank you and I look forward to having you again in other conference and symposium, organised by the Faculty of Agro-based Industry.

Yours sincerely;

NOR DINI RUSLI

Symposium Committee

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Chairman	Ts. Dr. Nor Dini Rusli
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Tentative Programme

The 4th Postgraduate Symposium
Faculty of Agro-Based Industry (FIAT)

Date: 9 December 2021 (Thursday)
Time: 9.00 am - 5.30 pm

Time		Programme
9.00	a.m	Opening ceremony Welcoming remarks by: **Ts. Dr Nor Dini Rusli**, Chairperson for the 4th Postgraduate Symposium, Faculty of Agro-based Industry, UMK Officiating speech by: **Assc. Prof. Dr. Seri Intan Mokhtar**, the Dean, Faculty of Agro-based Industry, UMK
9.15	a.m	Keynote speech Speaker: **Prof. Dr Abdul Rashid bin Mohamed Shariff**, Faculty of Engineering, UPM Title: Opening New Frontiers with Smart Agriculture
10.00	a.m	Invited speaker 1: **Assc. Prof. Dr Nitty Hirawaty Kamarulzaman**, (Department of Agribusiness and Bioresource Economics, Fac. of Agriculture, UPM) Title: Rethinking Supply Chain for Sustainable Food System
10.20	a.m	Invited speaker 2: **Dr Chan Chian Wen** (Prebio-Tech/Pan-Gaea TripleVs Venture Sdn. Bhd.) Title: What does it take to become a Top-3 innovator in UN FAO?
10.40	a.m	Invited speaker 3: **Ir. Cuk Tri Noviandi**, **(**S. Pt., M. Anim. St., PhD, IPM., ASEAN Eng. Faculty of Animal Science Universitas Gadjah Mada) Title: Achieving balanced ruminant ration by utilizing local feedstuffs in Indonesia.
11.00	a.m	Invited speaker 4: **Assoc. Prof. Dr Ashwini Kumar Dixit** (Department of Botany, Guru Ghasidas Vishwavidyalaya, Koni India) Title: Immunomodulatory herbal medicine and nutraceuticals.
11.20	a.m	Tea break
11.30	a.m	Parallel sessions (breakout rooms)
12.30	p.m	Lunch break
2.30	p.m	Parallel sessions (breakout rooms)
4.30	p.m	Closing ceremony Closing speech by: **Prof. Dr Mohd Rosli Mohamad**, the Dean, Center for Postgraduate Studies, UMK Award giving ceremony for Best Presenters
5.00	p.m	End of session

Breakout Rooms

Code	Name	Category	Room	Presentation
		MORNING-AFTERNOON		
AB01	Robi Agustiar	(AB) Agribusiness	1	11.30 am - 11.45 am
AB02	Endah Satiti	(AB) Agribusiness	1	11.45 am - 12.00 pm
AB03	Erlina Astuti	(AB) Agribusiness	1	12.00 pm - 12.15 pm
AB04	Muhamad Hanis Bin Abd Razak	(AB) Agribusiness	1	12.15 pm - 12.30 pm
FS01	Romalee Chedoloh	(FS) Food Security	2	11.30 am - 11.45 am
FS02	Syeda Anjum Mobeen	(FS) Food Security	2	11.45 am - 12.00 pm
FS04	Nurul Athirah Binti Mohd Zuki @ Rosli	(FS) Food Security	2	12.00 pm - 12.15 pm
FS10	Muhammad Hanif Bin Rosdi	(FS) Food Security	2	12.15 pm - 12.30 pm
AG01	Nifareesa Chealoh	(AG) Agrotechnology	3	11.30 am - 11.45 am
AG02	Muhammad Akmal Bin Mohd Zawawi	(AG) Agrotechnology	3	11.45 am - 12.00 pm
AG03	Muhammad Khairulanam Zakaria1	(AG) Agrotechnology	3	12.00 pm - 12.15 pm
*AG05	Basiri Bristone	(AG) Agrotechnology	3	12.15 pm - 12.30 pm
*AS01	Muhammad Amiruddin Bin Wahab	(AS) Animal Science	4	11.30 am - 11.45 am
*AS02	Nor Syairah Atiqah Binti Mohamad Hanafiah	(AS) Animal Science	4	11.45 am - 12.00 pm
*AS03	Muhammad Aiman Bin Adam	(AS) Animal Science	4	12.00 pm - 12.15 pm
AS04	Nur Nazhiifah Amiirah Binti Mohd Suhaimi	(AS) Animal Science	4	12.15 pm - 12.30 pm
PD01	Chan Ke Xin	(PD) Product Development	5	11.30 am - 11.45 am
*PD02	Nurul Fadzlin Binti Ab Llah	(PD) Product Development	5	11.45 am - 12.00 pm

PD03	Shahirah Binti Ahamad	(PD) Product Development	5	12.00 pm - 12.15 pm
PD04	Nur Solehin Bin Sulaiman	(PD) Product Development	5	12.15 pm - 12.30 pm
		EVENING		
AG06	Ferid Abdulhafiz Kemal	(AG) Agrotechnology	1	2.30 pm - 2.45 pm
AG07	Rivitra A/P Vintisen	(AG) Agrotechnology	1	2.45 pm - 3.00 pm
AG09	Gunavathy Selvarajh	(AG) Agrotechnology	1	3.00 pm - 3.15 pm
AG12	Siti Maryam Salamah Binti Ab Rhaman	(AG) Agrotechnology	1	3.15 pm - 3.30 pm
AG13	Nurihan Chehwaesulong	(AG) Agrotechnology	1	3.30 pm - 3.45 pm
AG14	Miss Nusanisa Chedao	(AG) Agrotechnology	1	3.45 pm - 4.00 pm
AG15	Fadzlin Qistina Binti Fauzan	(AG) Agrotechnology	2	2.30 pm - 2.45 pm
AG16	Suzie Haryanti Bt Husain	(AG) Agrotechnology	2	2.45 pm - 3.00 pm
AG18	Munirah Binti Mokhtar	(AG) Agrotechnology	2	3.00 pm - 3.15 pm
AG19	Arigela Chandrasekhar	(AG) Agrotechnology	2	3.15 pm - 3.30 pm
AG20	Yusniza Binti Muhamad Che Ya	(AG) Agrotechnology	2	3.30 pm - 3.45 pm
AG21	Harika Katepogu	(AG) Agrotechnology	2	3.45 pm - 4.00 pm
AS05	Zaenab Nurul Jannah	(AS) Animal Science	3	2.30 pm - 2.45 pm
AS06	Diana Rahmawati	(AS) Animal Science	3	2.45 pm - 3.00 pm
AS07	Sareena Semae	(AS) Animal Science	3	3.00 pm - 3.15 pm
*AS08	Ismah Ulfiyah Azis	(AS) Animal Science	3	3.15 pm - 3.30 pm
*AS09	Kholifatus Sholiha	(AS) Animal Science	3	3.30 pm - 3.45 pm
AS10	Hafis Datumada, Muhammad-Charif Ma and Thanaset Thongsaiklaing	(AS) Animal Science	3	3.45 pm - 4.00 pm
AS13	Jennielyn Anak Jendy	(AS) Animal Science	4	2.30 pm - 2.45 pm
AS14	Moh Ihsan Zain	(AS) Animal Science	4	2.45 pm - 3.00 pm
PD05	Nurul Amira Binti Zainurin	(PD) Product Development	4	3.00 pm - 3.15 pm
*PD06	Nagarjuna Reddy Vendidandala	(PD) Product Development	4	3.15 pm - 3.30 pm
PD07	Huda Binti Awang	(PD) Product Development	4	3.30 pm - 3.45 pm
PD08	Nor Asfaliza Abdullah	(PD) Product Development	4	3.45 pm - 4.00 pm

PD09	Erman Shah Jaios	(PD) Product Development	5	2.30 pm - 2.45 pm
*PD10	Muhammad Irfan Bin Jalalludin	(PD) Product Development	5	2.45 pm - 3.00 pm
PD11	Nursyafiqah Binti Shafi'E	(PD) Product Development	5	3.00 pm - 3.15 pm
PD12	Noramalina Binti Abdullah	(PD) Product Development	5	3.15 pm - 3.30 pm
PD13	Arni Binti Mansor	(PD) Product Development	5	3.30 pm - 3.45 pm

The extended abstract for each participant can be found and coded according to the theme, except those with an asterisk*.

List Of Participants

No.	Code	Name	Abstract Title	Institution
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2.	AB02	Endah Satiti	Income Analysis of Dairy Cattle in Berdaya and Aura Women's Livestock Farmer Groups (A Case of Samiran Village, Selo Subdistrict, Boyolali Regency)	Universitas Gadjah Mada
3.	AB03	Erlina Astuti	Motivation of Cattle Farmers in Collective Action through Farmer Group in Bantul District	Universitas Gadjah Mada
4.	AB04	Muhamad Hanis Bin Abd Razak	Pineapple Supply Chain during Covid-19 in Malaysia: A Preliminary Study	Malaysian Pineapple Industry Board
5.	AG01	Nifareesa Chealoh	Identification of AFLP Marker Related to Low Salinity Tolerance in Postlarval-15 Black Tiger Shrimp (*Penaeus monodon*)	Princess of Naradhiwas University
6.	AG02	Muhammad Akmal Bin Mohd Zawawi	Internet of Things (IoT) Approach to Detect and Modelling Fusarium Wilt Disease on Banana	Universiti Malaysia Kelantan
7.	AG03	Muhammad Khairulanam Zakaria	Optimisation of pH and Temperature for Protein Degradation by *Lactobacillus* sp.	Universiti Malaysia Kelantan
8.	*AG05	Basiri Bristone	Herbicidal Effect of Isolated Compounds from Parthenium (*Parthenium hysterophorus*) on Jungle Rice (*Echinochloa colona*) in Aerobic Rice Systems	Universiti Malaysia Kelantan
9.	AG06	Ferid Abdulhafiz Kemal	Tissue Culture Technique for Rapid Clonal Propagation of Keladi Candik (*Alocasia longiloba* Miq.)	Universiti Malaysia Kelantan
10.	AG07	Rivitra A/P Vintisen	Effects of Combined Fertilizer Sources on Pest Population and Plant Growth Performance of Chilli	Universiti Malaysia Kelantan
11.	AG09	Gunavathy Selvarajh	Rice Straw Biochar Application Enhances Soil Nitrogen Availability for Efficient Rice Plant Growth	Universiti Malaysia Kelantan
12.	AG12	Siti Maryam Salamah Binti Ab Rhaman	Evaluating the Suitability of Heavy Metal Content in Sawdust, Paddy Straw and Oil Palm frond For Mushroom Cultivation	Universiti Malaysia Kelantan
13.	AG13	Nurihan Chehwaesulong	Colchicine-Induced Variation in Xanadu (*Philodendron xanadu*) as Commercial Feature Aspects	Princess of Naradhiwas University

14.	AG14	Miss Nusanisa Chedao	The Macronutrient Evaluation of the Recycling and the Modifying of Sajor-caju Mushroom Waste	Princess of Naradhiwas University
15.	AG15	Fadzlin Qistina Binti Fauzan	Dihaploidization of Anther and Ovary Cultures of Rock Melon (*Cucumis Melo* L.) as the Basis for Production of Hybrid Cultivars	Universiti Malaysia Kelantan
16.	AG16	Suzie Haryanti Binti Husain	Calcium Release Pattern of Calcium Amendments Applied to Strongly Acid Soils	Universiti Malaysia Kelantan
17.	AG18	Munirah Binti Mokhtar	The Effect of *Blaptica dubia* on *Oreochromis* spp. Growth	Universiti Malaysia Kelantan
18.	*AG19	Arigela Chandrasekhar	Antidiabetic Properties of Bitter Gourd Honey in Streptozotocin- Nicotinamide Induced Diabetic Rats	Universiti Malaysia Kelantan
19.	AG20	Yusniza Binti Muhamad Che Ya	Response on Vegetative Growth and Yield of Butternut Squash (*Cucurbita moschata*) Cultivar Waltham under Different Bokashi Dosage	Universiti Malaysia Kelantan
20.	AG21	Harika Katepogu	Isolation and Characterization of *Pediococcus pentosaceus* HLV1 from Idly Batter, a Fermented Food of South India	Yogi Vemana University
21.	*AS01	Muhammad Amiruddin Bin Wahab	Colour Evaluation and Physical Properties of Different Egg Custard Formulation using *Moringa oleifera* and *Curcuma longa* as a Feed for Giant Freshwater Prawn, *Macrobrachium rosenbergii* Larvae	Universiti Malaysia Kelantan
22.	*AS02	Nor Syairah Atiqah Binti Mohamad Hanafiah	Effect of Age and Parity in PSPB Concentration of Kedah Kelantan (KK) Cattle in Early Pregnancy	Universiti Malaysia Kelantan
23.	*AS03	Muhammad Aiman Bin Adam	Partial Purification and Molecular Weight Determination of Pregnancy-Specific Protein B (PSPB) from Kedah-Kelantan Cattle	Universiti Malaysia Kelantan
24.	AS04	Nur Nazhiifah Amiirah Binti Mohd Suhaimi	High-Tech Herbal Medicine: Sustainable Ruminant Formula for the Future	Universiti Malaysia Kelantan
25.	AS05	Zaenab Nurul Jannah	Strategy Analysis on Small Ruminant Livestock Business in Pandemic Covid-19 (Case Study of HPDKI Farmers, Banyumas, Indonesia)	Universitas Gadjah Mada
26.	AS06	Diana Rahmawati	Effect of Guanidinoacetic Acid with Different Protein Levels on Growth Performance of Broiler Chicken	Universitas Gadjah Mada
27.	AS07	Sareena Semae	*In Vitro* Ruminal Fermentation of Ration Supplemented with Calcium Soap	Princess of Naradhiwas University

28.	*AS08	Ismah Ulfiyah Azis	Effects of Nutrition Improvement and Premix Mineral Supplementation to Blood Metabolite Profile of Repeat Breeder Cattle	Universitas Gadjah Mada
29.	*AS09	Kholifatus Sholiha	Effects of Coriandrum Essential Oil Nanoemulsion in Drinking Water on Growth Performance of Broiler Chickens	Universitas Gadjah Mada
30.	AS10	Hafis Datumada, Muhammad-Charif Ma and Thanaset Thongsaiklaing	Single Nucleotide Polymorphism (SNP) in 5' franking region of Dopamine Receptor D2 (DRD2) Gene in Thai Native Chicken	Princess of Naradhiwas University
31.	AS13	Jennielyn Anak Jendy	Antibacterial Activity of Ivory Snail (*Babylonia areolate*) Extractions against *Aeromonas hydrophila* and *Streptococcus agalactiae*	Universiti Malaysia Kelantan
32.	AS14	Moh Ihsan Zain	Evaluation of Coconut Leaf Silage as an Alternative Feedstuff Forage for Ruminants	Universitas Gadjah Mada
33.	FS01	Romalee Chedoloh	Types and Source of Velvet Tamarind on Physical and Chemical Properties	Universiti Malaysia Kelantan
34.	FS02	Syeda Anjum Mobeen	The Challenges of Food Security: A Comprehensive Study on Safety, Sustainability, Transforming Food Systems and Machine Learning Based Approaches	Yogi Vemana University
35.	FS04	Nurul Athirah Binti Mohd Zuki @ Rosli	Effect of Salts, and Protease in Protein Hydrolysis of Black Soldier Fly Larvae (*Hermetia illucens*)	Universiti Malaysia Kelantan
36.	FS10	Muhammad Hanif Bin Rosdi	Texture of High-Pressure Cooking Toli Shad (*Tenualosa toli*) Fish Marinated with Velvet Tamarind Paste	Universiti Malaysia Kelantan
37.	PD01	Chan Ke Xin	Total Phenolic Content of Stingless Bee Honey with Treated Cornsilk Extract	Universiti Malaysia Kelantan
38.	*PD02	Nurul Fadzlin Binti Ab Llah	Antimicrobial Activities and Total Yield of Potential Natural Preservatives Extracted using Different Solvents	Universiti Malaysia Kelantan
39.	PD03	Shahirah Binti Ahamad	Optimization of Watermelon Rind Extraction Conditions by Sonication Extraction	Universiti Malaysia Kelantan
40.	PD04	Nur Solehin Bin Sulaiman	Optimization of Extraction Conditions of Ultrasonic-Assisted Extraction (UAE) Technique for the Analysis of Antioxidant in *Solanum lycopersicum*	Universiti Malaysia Kelantan
41.	PD05	Nurul Amira Binti Zainurin	Total Phenolic and Flavonoid Content and Chemical Profiling using GCMS of *Cocos nucifera* Sap (CNS) and its Extract	Universiti Malaysia Kelantan

42.	*PD06	Nagarjuna Reddy Vendidandala	Effect of Gallocatechin-Silver Nanoparticles Impregnated Cotton Gauze Dressing on Normal and Diabetic Wound Healing Rat Model	Universiti Malaysia Kelantan
43.	PD07	Huda Binti Awang	Optimization of Turbidity Removal from Groundwater using Nanomagnetic Adsorbent Composite	Universiti Malaysia Kelantan
44.	PD08	Nor Asfaliza Abdullah	The Efficiency of Dry Leaf-Based Biocarbon for Ammonia Removal in Aquaculture Wastewater	Universiti Malaysia Kelantan
45.	PD09	Erman Shah Jaios	Inhibition of Pro-Inflammatory Mediators by Methanolic Extract of *Opuntia monacantha* Haw. (Cactaceae) in Raw 264.7 Macrophages Cells	Universiti Malaysia Kelantan
46.	*PD10	Muhammad Irfan Bin Jalalludin	Utilization of Spent Mushroom Blocks and Vegetables Waste as Fish Feed to Reduce Agriculture Wastage in Kelantan Community	Universiti Malaysia Kelantan
47.	PD11	Nursyafiqah Binti Shafie	Production of Gelable Exopolysaccharides from *Agrobacteria* sp.	Universiti Malaysia Kelantan
48.	PD12	Noramalina Binti Abdullah	Phytochemical Analysis of *Piper sarmentosum* Leaves Extract	Universiti Malaysia Kelantan
49.	PD13	Arni Binti Mansor	Isolation and Morphological Characteristics of Acetic Acid Bacteria (AAB) from Dokong (*Lansium domesticum*) and Rambutan (*Nephelium lappaceum*) Vinegars	Universiti Malaysia Kelantan

The extended abstract for each participant can be found and coded according to the theme, except those with an asterisk*.


Scope
Agribusiness (AB)

AB01

The Study of Import Cattle Supply Chain Processes through Feedlots from Traditional to Modern Markets

Robi Agustiar[1], Budi Guntoro[1,] *, I Gede Suparta Budisatria[2]

[1] Department of Livestock Socio-Economics, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2] Department of Livestock Production, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

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ABSTRACT

Increased income and lifestyles encourage consumers to switch from buying beef in traditional markets to supermarkets. It has led to a change in feedlot local beef supply from traditional markets to modern retail. This research is expected to provide an overview of the environmental conditions of the industry and supply chain of imported local beef cattle and provide recommendations for changes in the value chain of imported beef from traditional markets to modern retail markets. The research method was a survey, and a purposive sampling technique was used to select key informants. A structured questionnaire was designed to collect the data supplemented with focus group discussion. Data were analyzed using descriptive quantitative statistics. The descriptive statistics revealed a supply chain. There is a change in imported cattle fattening companies which initially only made the traditional market the main market, to be transferred to the modern retail market to obtain more profitable economic value when compared to selling live cattle only. The study's implication is developing a business model for modern retail or butchering-based meat shops that increases the added value of local meat and benchmarking a long beef chain efficiency at post-cutting (downstream). The development of a modern retail business model is expected to drive the shifting system sales of beef from a commodity to a product basis.

Keywords: Feedlot, Supply Chain, Beef, Market, Import Cattle

INTRODUCTION

Indonesia's per capita consumption is below the average of its peer countries. Compared to peer countries in the ASEAN region, Muslim majority countries, and Brazil, Russia, India, China, and South Africa (BRICS), Indonesia has a per capita consumption below the average. Based on data from Indonesian Livestock Service and Animal health (2019), The national demand for beef is supplied by local cattle as much as 59%, 15% from imported buffalo, 12% frozen meat, and imported feeder beef equivalent to 14%.

Linkage of 120 economic sectors (upstream-downstream), more than 4.5 trillion investments in feedlots for local meat, 19,625 HKP (Hari Kerja Pria /Mens Weekday Work) in rural areas, and the added value of the cattle business 26.85 trillion (Asikin et al., 2020). Participation 16.16% middle and above, and in urban areas. Percentage of national poultry consumption is 56.98%-69% in West Java, East Java, Central Java, and 17% is Jakarta. The difference between beef and chicken prices in Indonesia is 3x, which is more extreme than the global difference of only 13% (Putra & Triatmojo, 2018). There is a gap between supply and demand for beef, according to Agus and Widi (2018) domestic meat production can only meet about

45 % of the national needs. High demand for beef will have an impact on increasing meat prices, and the price of meat from 2012-2017 has increased and is expected to continue to rise.This study aims to analyze the supply chain of imported cattle from the traditional market to the modern retail market.

MATERIALS AND METHODS

The research design in this study uses quantitative research. The research method was a survey, and a purposive sampling technique was used to select key informants. A structured questionnaire was designed to collect the data supplemented with focus group discussion. The research location was in Feedlot Company in West Java, including 3 companies namely; PT Citra Agro Buana Sejahtera, PT Widodo Makmur Perkasa, PT Kadila Lestari Jaya in March – April 2021. Data were analyzed using descriptive quantitative statistics to reveal a beef supply chain.

RESULTS AND DISCUSSION

The supply chain pattern for local meat production from imported cattle in the post-slaughter process chain has two compliance standards that are part of the standards set by the government, namely halal and NKV (veterinary control number) for hygienic products. With a complex supply chain pattern, as illustrated in Figure 1, it will not be easy to apply these standards consistently. Trade chain actors in modern retail who generally have halal product certification and NKV have the consequence that the products supplied come from actors who also have the same compliance system certification.

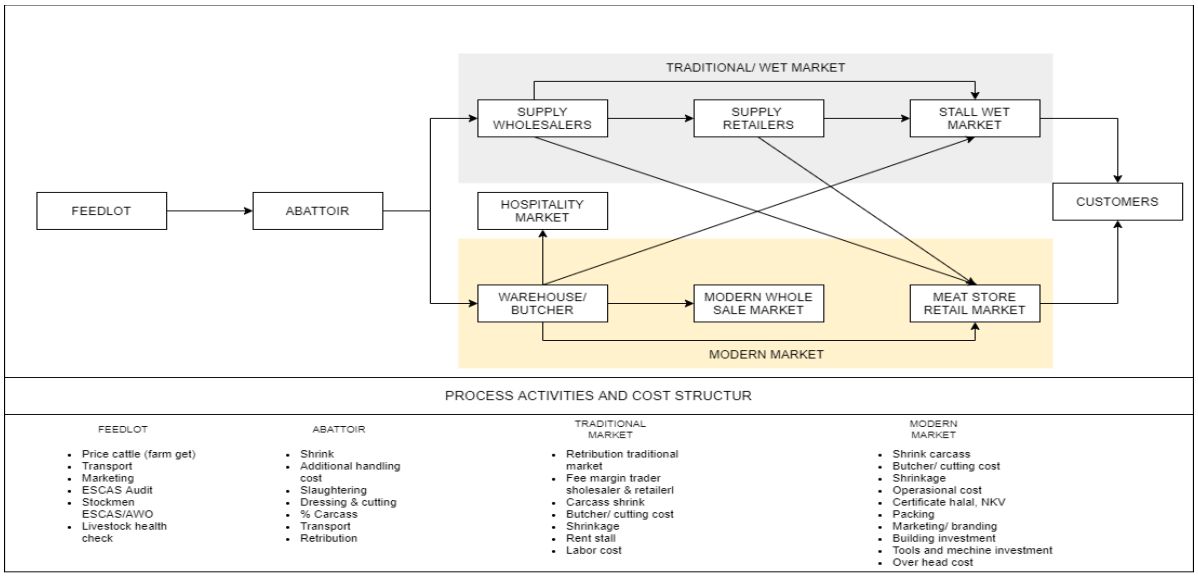

Figure 1. Local Beef Supply Chain Patterns from Ex-Imported Beef. (Research Result)

On the other hand, the trade chain actors in the traditional market segment do not fully share the same procedure system as the modern market, considering the complex and dynamic nature of the trade chain actors involved. In the end, the implementation of certified halal and NKV supply chain standards is partial (Waldron at al., 2013). It does not form a supply chain pattern for industrialized trade chain actors. As is the case with the application of the Exporter Supply Chain Assurance System (ESCAS) standard in the upstream production system, its application not only forms trade chain actors to maintain the quality of goods but also minimizes opportunities for trade chain actors who do not contribute to increasing the added value of beef products produced. The implementation of supply chain standards impacts supply chain efficiency and added value from the production process so that the existence of the involved trading chain actors is maintained and developed. The users' needs determine the post-slaughter or off-farm supply chain pattern for each available meat cut. At least 24 pieces of beef can be grouped into seven pieces from

one beef that is slaughtered. The pattern of goods sold from each actor in the traditional market segment and modern retail market is depicted in table 1.

Table 1. Beef supply chain pattern based on cuts from each traditional market segment and modern market.

No	Meat variety	% Live Weight	Traditional market			Modern market		
			Wholesalers	Retailers	Stall	Super Market	Hospitality	Retail Shop
1	Prime Cut	3,30%		x		x	x	x
2	Knuckle	12,16%	x	x	x	x	x	x
3	Chuck	15,89%	x		x		x	x
4	Bone in	5,91%		x	x		x	x
5	Offal	10,01%			x			
6	Variety Meat	22,23%			x			
7	Bone	5,14%			x			
	TOTAL	74,64%						

Based on the data in table 1, the total volume of the part of one cow that can be value for money is 74.64% of the live weight of the slaughtered cow. In general, the pattern of supply chain separation from the traditional and modern market segments can be seen based on selecting the products sold. The combination matrix above appears to form 3 segmented patterns: (1) the first pattern, the prime cut, hamstrings, and bone in the meat tend to be concentrated in the modern market segment, although the supply chain process also involves trading chain actors in traditional markets. The final consumer segment of the discount section is relatively more preferred for household and hospitality consumers. (2) the second pattern, in 3 types of cut parts, namely offal, variety meat, and bones, is generally concentrated in the traditional market segment and are not found in the modern market, both the actors in the trading chain and the final consumers of the product. (3) the third pattern, the quads, are nodes part of a slow-moving product with a higher selling risk and lower price.

The final main absorber in the product section is the processed industry, where local beef products will be juxtaposed with the price of imported beef. The pattern of the selling system does not distinguish the value of the part from the type of piece. Usually, hamstrings will go into the processed industry in some parts of the product that is not sold. The speed of selling these products is the key to getting better-added value. The longer the product is held, the lower the price and quality of the product will be.

CONCLUSION

There is a change in imported cattle fattening companies which initially only made the traditional market the main market, to be transferred to the modern retail market to obtain more profitable economic value when compared to selling live cattle only. The changing supply chain structure requires companies to invest in infrastructure along the changing supply chain. The business model for modern retail or butchering-based meat shops that increases the added value of local meat will be benchmarking a long beef chain efficiency at post-cutting (downstream). The development of a modern retail business model is expected to drive the shifting system sales of beef from a commodity to a product basis.

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AB02

Income Analysis of Dairy Cattle in Berdaya and Aura Livestock Farmer Groups: A Case of Samiran Village, Selo Subdistricts, Boyolali Regency

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ABSTRACT

Women contribute a lot to the agricultural and livestock sectors, especially in rural areas. This study aims to identify the income analysis of dairy farms. This study was conducted in Samiran Village, Selo Subdistrict, Boyolali Regency, Central Java as it is one of the centers of milk production in Boyolali. Berdaya and Aura livestock farmer groups were selected as the sample of respondents. The samples were collected from the census of all members (49 people). The data analysis was carried out in a quantitative descriptive manner to identify revenue and production costs for raising livestock and their products. The results showed that the marketing of dairy products in the Berdaya livestock farmer group and the Aura livestock farmer group was limited to the domestic market, Boyolali and Solo. Breeders have not dared to market their products to the modern market or retail. The results showed that the income of farmers from the sale of fresh milk, processed milk, and livestock manure in the Berdaya Cattle Farmers Group was Rp. 1,300,000/person/month, which is higher than the Aura livestock farmer group, which is Rp 1.289.000/person/month. This is due to the declining market demand due to the implementation of Level 4 Community Activity Restrictions. The Regional Minimum Wage value in Central Java of Rp 1.942.500.000 is still above the income of the Livestock Farmer Group. Overall, smallholder dairy cattle businesses in farmer groups can play a role in dairy cattle rearing activities with average ownership of 3-4 lactating broods and contribute income for economic improvement.

Keywords: Female livestock farmer groups, revenue, cost, income

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INTRODUCTION

The income of each livestock farmer group is different from one another. Cattle that generate direct income for farmers are productive cows or lactating cows. Besides being influenced by the number of livestock as a whole, the price of milk also affects the income of farmers. This price is set based on the quality of the milk. Milk quality checks are carried out every day, each milk is deposited to the Dairy Cooperative, thus allowing for differences in prices for each group of livestock farmers. In addition to milk quality, dairy cows or lactating cows produce different volumes of milk for each individual. This is also influenced by the quality of care for the dairy cow and the growing period of the dairy cow. Dairy cows that are well cared for produce lots of milk that are of good quality.

The livestock sector has great potential to be developed because it plays a very important role in increasing milk production, increasing job opportunities, and increasing farmers' income for self-sufficiency. Animal foods are high in protein, energy, vitamins, and minerals, which increases the need for animal foods. Public awareness of nutrition and health, especially the importance of animal protein, makes this food widely available in the community. Indonesia's protein needs to be increased from 2016 to 2017, with an average consumption of 56.67 grams and 62.20 grams/person/day (Central Bureau of Statistics, 2017). Therefore, the high demand can be a business opportunity. Human-managed dairy farms still face many obstacles, including the size of small businesses due to weak capital, unskilled breeders, and inadequate use of feed. One type of livestock used to supply animal protein is cattle. Cows are classified as livestock that is easy to cultivate, especially in rural areas, because they have grass and leaves that can be used as animal feed. Therefore, many people raise livestock as a business. Dairy cows are livestock that produce the animal protein in the form of milk or meat. Milk contains various nutrients such as protein, fat, carbohydrates (lactose), vitamins, and minerals. In addition, feces and urine can be used as fertilizer, biogas, and compost in daily life. Many of the advantages that can be obtained from this business are the potential for increasing income (Ervina et al., 2019).

MATERIALS AND METHODS

Research sample

The materials used in this study were 49 dairy farmers who joined the Empowered Livestock Farmers Group as many as 27 people and the Aura Livestock Farmers Group as many as 22 people who were in Tegal Seruni Hamlet and Pentongan Hamlet, Samiran Village, Selo District, Boyolali Regency.

Data analysis

The data analysis method used in this research is descriptive and quantitative methods that are used to describe the characteristics of respondents and data on aspects of access to information, aspects of access to capital, aspects of access to raw materials, aspects of access to equipment, aspects of access to activities, controls, and benefits and is used to analyze data of the sample and the results.

RESULTS AND DISCUSSION

Table 1. The income per month of Livestock Farmer Group

Component	KTT Berdaya = 27			KTT Aura = 22		
	Unit	Selling price	Total sales	Unit	Selling price	Total sales
Admission (A)						
a. Milk sales (liters)	2.850	6.500	18.525.000	1.860	6.500	12.090.000
b. Sales of dairy products						
Milk candy (pcs)	400	15.000	6.000.000	320	15.000	4.800.000
Milk assorted flavor (bottle)	300	13.000	3.900.000	240	16.000	3.840.000
Milk sticks (pcs)	400	10.000	4.000.000	240	10.000	2.400.000
Dodol milk (pcs)	120	10.000	1.200.000	80	10.000	800.000

Moringa milk soap (pcs)	120	10.000	1.200.000	0	0	0
Mozzarella cheese	0	0	0	120	35.000	4.200.000
c. Cattle manure	1.640	200	328.000	1.100	200	220.000
Cost (B)						
a. Maintenance:						
Pollard	108	4.500	486.000	60	4.500	270.000
Bran	108	4.000	432.000	60	4.000	240.000
b. Daity products:						
Raw materials	15	544.000	664.000	15	442.000	546.000
Packaging	3	5.900	460.000	3	5.900	364.000
Income (A-B)			Rp 35.153.000/month			Rp 28.350.000/month

Source: Processed primary data (2021)

The selling and processing activities of milk in the Berdaya Cattle Farmer Group and the Aura Livestock Farmer Group are mostly carried out by women and at the initiative of women (wives). The dominance of women (wives) in dairy cattle business activities in the Berdaya Cattle Farmers Group and Aura Livestock Farmers Group shows that women (wives) also make a large contribution in the form of energy in carrying out the activities of the production process and marketing of dairy farm products. The highest revenue is in the Berdaya Livestock Farmers Group from the sale of fresh milk to the Dairy Cooperative as much as 2,660 liters/month with a total nominal value of Rp. 18,525,000/month, while in the Aura Livestock Farmers Group from the sale of dairy products in the form of milk candy, various flavors of milk, sticks milk, and mozzarella cheese as much as 1,000pcs/month with a total nominal value of Rp. 16,260,000/month. The income received by farmers in the Empowered Livestock Farmers Group is Rp. 1,300,000/month. Meanwhile, the income earned by farmers in the Aura Livestock Farmers Group is Rp. 1,289,000/month. The income from the dairy cattle business in the Empowered Cattle Farmer Group and the Aura Livestock Farmer Group is relatively low. Regency/City Minimum Wage (UMK) as stated in Central Java Governor Decree No: 560/68 the Year 2019 dated 19 November 2019 concerning Minimum Wage in 35 (Thirty-Five) Regencies/Cities in Central Java Province in 2020, where MSE in Regency Boyolali, which is Rp. 1,942,500. The people's dairy cattle business is generally only used as a side job apart from farming as the main business. Farmers/breeders will sell the livestock if they need a large amount of money at any time (Priyono, 2008).

CONCLUSION

The income of dairy cattle in the Berdaya Cattle Farmer Group and the Aura Cattle Farmer Group is low due to the small scale of business, limited marketing of dairy products in the domestic market, Boyolali and Solo. Breeders have not dared to market their products to the modern market or retail, breeders still use the traditional maintenance system. This type of business was chosen because it aims to increase family income and have jobs other than raising dairy cattle such as farmers, drivers, laborers, and the private sector.

ACKNOWLEDGEMENT

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AB03

Motivation of Cattle Farmers in Collective Action through Farmer Group in Bantul District

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ABSTRACT

Smallholder farmers join the group because of the urge to maintain their livestock business. Within the group, smallholder farmers carry out joint activities in carrying out group functions. The purpose of this study was to identify the level of motivation of smallholder farmers to take collective action in fattening beef cattle through farmer groups. Smallholder farmers take collective action related to the motivation of the farmer group to function as a learning class, motivation as a vehicle for cooperation, and motivation as a business unit. The method used in this research is descriptive analytic. The results showed that descriptively the level of motivation in groups as a means of learning, motivation as a vehicle for cooperation and motivation as a business unit has a high level of motivation. The three group functions make contributions that are not much different in their implementation in collective activities within the group.

Keywords: Collective action, farmer motivation, farmer group, fattening beef cattle

INTRODUCTION

Motivation is encouragement or support to do something. According to Kartono (2008), motivation is (1) the force that causes behavior for a particular purpose, (2) the basic reasons, thoughts and supports that encourage someone to do something and (3) the main ideas that influence a person's behavior. Farmers by joining groups and doing joint activities are expected to get a high motivational boost because in the group they will get new knowledge, encouragement to get cooperation between breeders, and encouragement to improve their livestock business. This study aims to identify the level of motivation of smallholder farmers to take collective action in fattening beef cattle through group functions, namely motivation as a means of learning, motivation as a vehicle for cooperation, and motivation as a business unit.

MATERIALS AND METHODS

Research design and sample

This research is a case study in a farmer group. The selection of the breeder group was purposive sampling with group criteria, including the group of beef cattle breeders located in Kapanewon Bantul, Bantul Regency with the main business pattern being for fattening and has been established for a relatively long period of at least 20 years and is still actively carrying out the function groups in collective action activities. The research takes place from June to October 2021. The research material is farmers who are members of the group and until now are still actively participating in group activities.

Research instrument and analysis

The basic method used in this research is descriptive method, namely the method in researching a group of people, an object or condition. Descriptive research is used to present a comprehensive picture of a symptom or event and the condition of an object of research, in this case members of the farmer group. In this study, the application of the descriptive method was carried out with a quantitative approach carried out by survey techniques using a questionnaire tool containing questions about collective activities carried out as an interpretation of group motivation. The motivation scale is measured based on a Likert scale with five score answers to questions (Gardner and Lambert Model, 1972).

RESULTS AND DISCUSSION

Level of Motivation of Farmer's Groups in Collective Activities

Regulation of the Minister of Agriculture Number 273/Kpts/OT.160/4/2007 concerning Guidelines for Farmer Institutional Development states that there are three categories of joint activities in farmer groups, namely: a) as a learning class; The livestock farmer group is a place for teaching and learning for its members to improve their knowledge, skills and attitudes so that their productivity increases, their income increases, and their lives are more prosperous. b) as a vehicle for cooperation; Farmer groups are a place to strengthen cooperation between fellow farmers in farmer groups and between farmer groups and with other parties. Through this collaboration, it is hoped that the farming business will be more efficient and better able to deal with threats, challenges, obstacles, and disturbances. c) as a production unit; Farming business carried out by each member of the farmer group, as a whole must be seen as a single business unit that can be developed to achieve economies of scale, both in terms of quantity, quality, and continuity.

Motivation in carrying out group functions, namely groups as a means of learning, groups as a vehicle for cooperation, and groups as business units collectively have a high level of motivation. The percentage distribution of categories of motivation types and levels of group motivation can be seen in table 1.

Table 1. The level of motivation by type of motivation as a means of learning, motivation as a vehicle for cooperation, and motivation as a business unit (%)

Motivation	High	Low
Motivation as a learning tool	100	0
Motivation as a vehicle cooperation	98	2
Motivation as a business unit	99	1

Source: Processed primary data (2021)

Smallholder farmers by making joint agreements that are developed through the support of joint activities in livestock activities are also often called collective action. Animal husbandry activities carry out their group functions on a regional basis, so a collective agreement is needed in managing farmer groups. Collective action with high motivation due to awareness of learning facilities motives, cooperative vehicle motives, and business unit motives is expected to be able to improve the quality and quantity compared to individual smallholder farms.

Contribution of Group Motivation in Collective Action

Third of them, motivation as a means of learning, motivation as a vehicle for cooperation, and motivation as a business unit contribute to motivation that is not significantly different as shown in Figure 1. Motivation as a learning tool is the motive of farmers to exchange experiences and information between fellow farmers and people from outside the group. Breeders carry out an educational interaction process that encourages farmers to carry out collective activities in groups through collective intelligence to solve common problems.

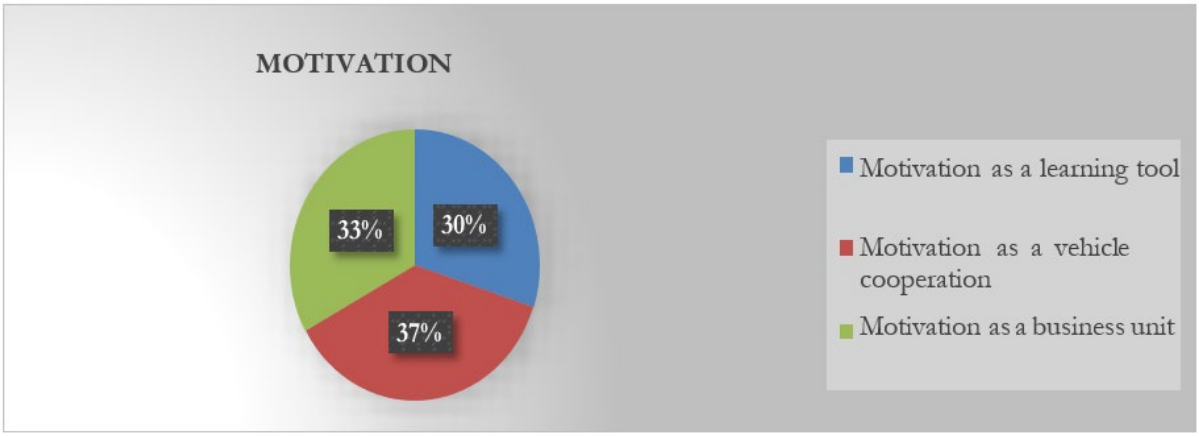

Figure 1. Contribution of motivation given from each group motivation

Motivation as a vehicle for cooperation is very important in agriculture. In groups, it is closely related to *gotong royong*, or working together with a number of people to solve a problem that is considered useful for the public interest. "*Gotong royong*" is a hereditary heritage and culture that must be preserved.

Motivation as a business unit in a group can increase production efficiency, for example in the procurement of production facilities, savings and loans, and livestock sales. Breeders can improve their ability to analyze market potential to develop livestock business and improve their ability to manage their livestock business by joining groups.

CONCLUSION

Farmer groups are a form of collective action to overcome the various risks faced by smallholder farmers in competing in business. With smallholder farmers who join groups with high motivation, they will get a means of learning livestock innovation, groups as a vehicle for cooperation in raising livestock, and farmers jointly increasing their livestock business in order to also improve the family economy.

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AB04

Pineapple Supply Chain during COVID-19 in Malaysia: A Preliminary Study

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ABSTRACT

Starting in March 2020, the world has been watching for the COVID-19 pandemic outbreak. This COVID-19 outbreak is caused by the SARS-CoV-2 virus which has a huge impact on humans today. It not only affects the health of the world with the death toll that has hit millions of people, but the outbreak has also affected the social and economic sectors. The impact of the outbreak is reflected in the closure of schools and educational institutions, business premises, domestic and international trade centres. This shows poverty rates, job losses and low of income. These constraints can also affect the smooth running of the supply chain, especially Malaysia. However, this situation does not completely impede the agricultural sector in Malaysia, such as the pineapple industry. Hence, the purpose of this study is to generate the framework for the pineapple supply chain during COVID-19 pandemic in Malaysia. This research utilizes library research. This paper has various implications to the pineapple industry. In addition, the study significantly contributes to the practitioners in industry towards the strategies and recommendation to strengthen and expand the pineapple sector in Malaysia during the pandemic, in line with the implementation of the National Agrofood Policy 2.0 by Ministry of Agriculture and Food Industries (MAFI) with eligible agency in pineapple industry matters, Malaysian Pineapple Industry Board (MPIB).

Keywords: COVID-19, Malaysian Pineapple Industry Board (MPIB), National Agrofood Policy 2.0, pineapple, supply chain, strategies.

INTRODUCTION

Pineapple (Ananas comosus) is one of the important crops that contributed to Malaysia's economy. Out of more than 2000 of tropical fruit plant species, pineapple ranked third after banana and citrus as the leading crops that consumed as edible fruit (Jaji, Man & Nawi, 2018). This fruit is recognized and widely known across global consumers for its mixed taste of sweet and sourly. As one of the crops that is cultivated in Malaysia, it is considered as the country's oldest agro-based export-oriented industry (Lun, Wai & Ling, 2014). Furthermore, pineapple plays an important role in improving socioeconomic development of the country, as a contribution to the national gross domestic product (GDP). This is strengthened by the fact that Malaysia has 12 main varieties of pineapple; "*Ananas Comosus*" or "AC" which are certified and regulated by the Department of Agriculture of Malaysia (DOA).

In the late of the year 2020, Malaysia is struck with the spreading of COVID-19 pandemic, a novel type of coronavirus that attacks the respiratory system and causes infected people to suffer with pneumonia. The

infection is so swift, easily transmitted from one person to another and deadly since it causes a dramatic spike in death tolls and hospitalization. The outbreak of COVID-19 pandemic has had a significant effect ton the global economy and supply chain including Malaysia. In general, COVID-19 did not paralysed Malaysia's economy and caused complete breakdown of the food supply chain in particular pineapple industry, but it is indeed that severing many sectors and resulted in disruption on the supply chain. There are several factors that contributes to the disruption of the food supply chain in Malaysia, and this is crucial because this can be an alarming indicator for the country's food security concern.

MATERIALS AND METHODS

To address the challenges on the pineapple supply chain during COVID-19, this paper is presented to achieve three objectives, which are to elaborate on the factors of the food supply chain in Malaysia. Secondly, in this study, the researchers aim to analyse the impact of COVID-19 towards the food supply chain pertaining to pineapple industry and thirdly is to propose the strategies that can be put forward as lessons learnt from the crisis-a post-crisis management perspective. To accomplish the stated objectives, this study utilizes full library research, where data is collected from literatures gathered from main databases such as Science Direct, Emerald, Scopus, Google Scholar and other secondary sources. The data is analysed using content analysis approach to extract relevant information to attain the research objectives. This study was also conducted as a review study on the topics of pineapple supply chain in relation to COVID-19 in Malaysia.

RESULTS AND DISCUSSION

Food supply chain is crucial for any society to ensure that the food security is not put in jeopardy. A shortage and disrupted food supply chain means that the food is not distributed to the population and people do not receive enough food to survive. Though COVID-19 does not halt the entire ecosystem, it does cause significant disruption of the food supply chain in Malaysia. According to Abu Dardak (2021), agriculture-related activities such as vegetable production and short-term crops such as pineapples are expected to be dormant for several months. One of the factors that caused disruption is the imposition of lockdown and Movement Control Order (MCO) (Chin, 2020). According to Surendan (2020), the implementation of MCO has impacted on the food supply chains particularly in urban areas due to the restriction of traffic, roadblocks as well as the limiting of opening hours of the business operation. The heavy reliance on land transportation of food in the populated area causing the agro-based products failed to reach the consumers on time, in which later many of the produce is dumped and given away because fresh produce easily spoils due to its perishable nature. According to Manikam & Md Saad (2020), 200 farmers are reported to have lost RM400,000 a day due to incapable of selling their produce. To overcome the challenges of being unable to sell produce, it is beneficial that farmers embark into an e-commerce approach, i.e., utilizing internet platforms to reach the consumer directly. The market potential for pineapple industry has it based within local food, such as pastries and cakes and it is evident that online platforms observed a spike of demand especially for small scale (M Kamal, 2020). Social media and mobile applications are powerful tools for advertising and marketing both pineapple produce and pineapple-based products.

CONCLUSION

Pineapple and pineapple-based products are one of the promising and lucrative sectors for boosting Malaysia's economy. It is imperative to secure and to enhance the resiliency of the pineapple supply chain in Malaysia. COVID-19 pandemic has shown that the pineapple supply chain has a considerable effect, therefore more strategy needs to be brought to light in order to strengthen to minimise the risk and disruption. All components of the pineapple supply chain should work closely, especially the government agency and the industry players. Farmers, as the first line of the pineapple supply chain within the country, shall consider embarking on new technology to boost the marketability of pineapple and pineapple-based products.

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Scope
Agrotechnology (AG)

AG01

Identification of AFLP Marker Related to Low Salinity Tolerance in Postlarval-15 Black Tiger Shrimp (*Penaeus monodon*)

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ABSTRACT

This research is to identify DNA marker related to low-salinity stress in the black tiger shrimp using AFLP analysis. In the experiment, postlarval 15 (PL-15) of ten full-sib families were prepared by acclimation in 30 ppt salinity water for 14 days then suddenly transferred to 3 ppt salinity water for 30 days. Survived and dead shrimps were collected individually and categorized into 3 groups by the time courses consisting of a group of shrimps died within 1 day, a group of shrimps died during 2-7 days and a group of shrimps survived after 30 days stressed. Individual shrimp samples were used to extract the genomic DNA and pooled within a group. The pooled DNA samples were then analysed by AFLP with 45 primer combinations to identify polymorphism of tolerant and dead shrimps. The results showed that 32 polymorphic bands were observed from a total of 2,511 bands (1.27%) generated. Twenty-seven of all polymorphic bands were successfully cloned and sequenced. Of those, 9 showed NCBI database sequencing similarity. In conclusion, the results of this research demonstrated that the DNA marker would be developed as a marker for low-salinity tolerance in *P. monodon.*

Keywords: Black tiger shrimp; AFLP marker; low salinity.

INTRODUCTION

The black tiger shrimp (*Penaeus monodon*) has been one of the most economically important aquaculture species in Thailand for more than 30 years. The products of this shrimp species had been exported from Thailand and made an income of more than 10,000 million baht annually. In their life cycle, they migrate between estuarine and ocean (Spanings-Pierrot, 2000). They can tolerate the fluctuation of salinity especially in the estuarine region that would be very low, down to 5 ppt. However, the low salinity condition actually is not suitable for shrimp life. The survival rate of shrimp may be the effects from the adaptation of their physiological mechanisms to salinity change. It is expected that the identification of genetic variation by DNA marker would be one of the tools for genetic selection breeding programs for black tiger shrimp. Several techniques can be utilized to identify the genetic variations in the differential tolerance shrimp under low salinity stress. Of these, AFLP technique has been widely applied to study genotyping, population differentiation and genetic diversity in a wide variety of organisms (Wang, Tsoi, and Chu, 2004) This method is capable for generating high-volume markers in a short time without any prior sequence knowledge (*Whan, Wilson, and Moore, 2000).* AFLP technique is based on the selective PCR amplification of restriction fragments generated by specific restriction enzymes and oligonucleotide adapters of a few nucleotide bases (Vos, Hogers and Bleeke 1995). The aim of the present work was to identify the genetic marker in the differential tolerance of black tiger shrimp under low salinity stress.

MATERIALS AND METHODS

Salinity stress test

Ten full-sib families postlarvae-15 of shrimps were acclimated for 14 days in sea water at 30 ppt salinity. Salinity stressed test was due by immediately transfer each family to diluted seawater of 3 ppt for 30 days. Data collection was performed by observing dead shrimp every 6 hours and preserving them individually under -80℃. The test shrimp samples were defined into three groups, shrimps died within 24 hours, shrimps died within 24 hours to 7 days and survived shrimps after transfer to low salinity for 30 days. Genomic DNA was extracted using proteinase K incubation and pooled into each group DNA samples.

AFLP analysis

The pooled genomic DNA samples were used directly in the AFLP marker method as described by Vos et al. (1995). Genomic DNA was analyzed using 45 AFLP primer combinations of E-ANN/M-CNN and E-ANN/M-GNN. The selective amplification products were then electrophoresed on 5% denaturing polyacrylamide gel and stained using silver nitrate.

DNA sequencing and sequence analysis

The polymorphic AFLP fragments were re-amplified and purified by PCR Purification kit. The fragment was ligated into pGEM®-T vector and transformed into *E. coli* (DH10B) using electroporation method. The clones were then sequenced, and sequences were analysed using Blast for similarity search against a public DNA database.

RESULTS AND DISCUSSION

Salinity stress test

After being stressed under low salinity for 30-day, shrimp samples in family 4 survived in maximum, followed by family 1, family 2, family 3, family 8, family 7, family 6 and family 5 respectively. Of these, all shrimps in family 9 and 10 died within 1 day (Figure 1).

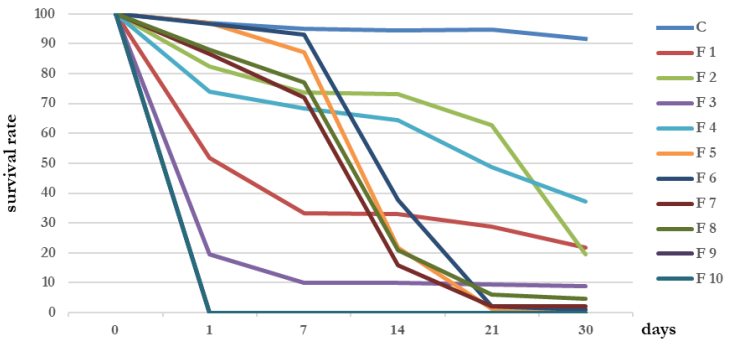

Figure 1. Survival rate of ten full-sib families the black tiger shrimp (*Penaeus monodon*) acclimated at 30 ppt salinity and then transferred to 3 ppt salinity.

AFLP analysis

From the stress test, shrimp samples were classified into 3 groups according to their tolerant time periods. A total of 32 AFLP bands generated from 18 primer combinations showed polymorphism among groups of samples (the data not shown).

DNA sequencing and sequence analysis

Observed all 13 sequences were significantly matched the known sequences in Genbank databases. Total of 9 sequences were significantly characterized as known sequences (Table 1, 2), some were functional in osmoregulation system. Therefore, improving the marker efficiency, should be converted into Sequence Characterized Amplified Regions (SCARs). The conversion of the dominant markers to SCAR by using PCR-based assay more robust because SCAR can detect a single locus and their amplification is less sensitive to reaction conditions.

Table 1. List of sequence homologues with the GenBank database using BLASTN program.

Fragment	Sequence homology	*Species*	Accession no.	E-value	%Identity
P2	RNA-binding protein lark-like	*P. monodon*	XM037917213	7e-136	98.92 (275/278)
P11-3	antilipopolysaccharide factor (ALF8)	*P. chinensis*	MH998633	4e-13	88.00 (66/75)
P13-1	phenoloxidase-activating factor 1-like	*P. monodon*	XM037933233	5e-18	96.77 (60/62)
P15-1	microsatellite sequence	*P. monodon*	GU137503	1e-85	94.79 (200/211)
P15-3	phenoloxidase-activating factor 1-like	*P. monodon*	XM037933233	4e-49	96.80 (135/142)
P23-4	microsattellite TUMXLV5.54 Sequence	*L. vanamei*	AF360026	4e - 16	86.75 (72/83)

Table 2. List of sequence homologues using BLASTX program.

Fragment	Sequence homology	*Species*	Accession no.	E-value	%Identity
P11-1	mariner transposase	*P. monodon*	QOJ42652	2e-25	70.67 (53/75)
P16-1	transposon Ty3-G gap-Pol polyprotein	*P. vannamei*	ROT69884	1e-12	58.14 (25/43)
P49	ovostatin-like	*P. monodon*	XP037800672	1e-05	72.97 (27/37)

CONCLUSION

AFLP analysis shrimp salinity stress-tolerance with 45 primer combinations provided 32 polymorphic bands. 27 fragments were re-amplified and cloned into *E. coli*. All 17 fragments were completed sequencing and the presented 9 sequences matched known sequences in databases.

ACKNOWLEDGEMENT

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AG02

Internet of Things (IoT) Approach to Detect and Modelling Fusarium Wilt Disease on Banana

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ABSTRACT

The continuing development of Internet of Things (IoT) is becoming progressively important in agriculture activities including plant disease identification. Consequently, the IoT technology will act as a game-changer in plant disease identification from manual to automated detecting plant disease. In ancient farming, most plant diseases identification were conducted manually based on the external symptoms which only can be done by experienced people and require more manpower to monitor the farms. Thus, this scenario brings difficulty for young or inexperienced farmers to identify the plant disease. This paper describes the development of IoT technology for detecting Fusarium wilt disease in bananas at the early stage of disease infestation under the greenhouse environment. Sensors will be equipped inside the greenhouse with microcontrollers, communication networks, and suitable protocols to capture soil parameters such as soil moisture content, pH, electrical conductivity (EC), and temperature. Then, all the measured data will be stored and managed properly using Thingspeak. To better understand the association of soil parameters with Fusarium wilt disease, a mathematical modelling will be done to simulate the disease progression using output data. As a result, this study will give insightful real-time data monitoring using IoT technology to determine the threshold of favourable soil conditions for Fusarium wilt disease occurrence.

Keywords: Environmental monitoring, fusarium, Internet of Things (IoT), sensors, smart agriculture

INTRODUCTION

As with other technologies, the Internet of Things (IoT) is rapidly being explored in the agriculture industry in Malaysia. From an agriculture perspective, IoT technology promises farmers to monitor their crops growth and field conditions through intelligent devices anywhere and anytime (Antony et al. 2020). Over the years, banana farming has faced significant global constraints in production. To date, Fusarium wilt disease or also known as Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) remains a critical threat to banana production loss for most banana cultivars globally (Olivares et al., 2021). Worries have been highlighted that banana production would be unable to serve the increasing global population, including Malaysia. By 2050, United Nations (UN) predicted the world population could reach up to 10 billion (Alexandratos & Bruinsma, 2012). This would put pressure in the food production industry to fulfil the demand of the population.

The central problem to be researched in this proposed study is the persistent and incurable Foc's infection for long generations. Thus, the impact is uncontrollably constrained to banana deficits and affected banana cropland (Viljoen, 2002). Additionally, a relatively little study was conducted to understand the underlying interaction of soil environments with Fusarium wilt disease progression. Thus, the knowledge on how soil properties are associated with the survival of Foc and the spread of Fusarium wilt disease to the whole banana population in the cultivated area remains unresolved. The main idea of the IoT concept in detecting Fusarium wilt disease is to alert the farmer regarding the presence of Foc in their banana cultivated area at the early disease infestation. The notification will be sent to the farmers through smart devices when the soil parameters exceed the threshold. To achieve the main objective, some specific objectives need to be done which include: 1) To characterise the soil properties of infested and non-infested soil of Fusarium wilt disease. 2) To measure the soil parameters of infested and non-infested under greenhouse study using an affordable IoT automated disease detection framework. 3) To develop a mathematical modelling for Fusarium wilt disease progression.

METHODS

The work undertaken in this research will be divided into three stages; laboratory analysis, experimental work under greenhouse, and data analysis with mathematical modelling. In this research, the focus is to implement an affordable IoT technology as a tool to alert farmers regarding the presence of Foc at the early disease of infestation. In the first stage of research work, the soil samplings for infested and non-infested Fusarium wilt disease from two different farms will be collected in 4 replicates from 0 - 15 cm depth using soil auger within quadrant 2m x 2m for each selected banana tree. Then, the parameters of collected soils will be evaluated using soil physicochemical analysis (pH, soil moisture and EC). In addition, the Foc isolation and identification also will be done to confirm the collected soil samples from the banana farm are infected by Foc. Only then, the result for physicochemical analysis will be used as predefined values for the IoT algorithm as it will alert the users when the measured parameters exceed the threshold.

This section provides a step-by-step guideline to implement an IoT system inside the greenhouse for data collection. First, the IoT framework will be designed according to the experimental requirements. The design must include all the hardware and software needed for this study (soil moisture sensors, soil pH sensors, soil EC sensors, soil temperature sensors, Raspberry Pi, Wi-Fi router, solar panel, SD card, AC adapter, Phyton programme and Thingspeak). Figure 1 presents the general idea of how the sensors are connected to the Raspberry Pi and other devices. Next, coding will be done using Phyton software which is the compatible language for Raspberry Pi. During the coding phase, all the pre-defined results of soil physicochemical analysis and API key for Thingspeak including the internet will be coded. Therefore, the proposed system could notify farmers of the presence of Foc and store data at Thingspeak. The connectivity enables the sensors to measure the soil data and transmit it to the Thingspeak using a Wi-Fi module. Then, the IoT framework will be tested to identify any errors in the IoT system. All the errors will be fixed before data collection and monitoring. This testing phase is essential for accurate data measurement and ensuring all the captured data can be stored at Thingspeak. During field tests at the greenhouse, maintenance activity is required throughout the study period to correct any errors or unexpected technical issues to ensure the IoT system continuously functions.

In the last phase, all the output data will be fitted to regression model to develop the best fitted model that can represent the growth curve disease epidemics This phase is important to make sure all the captured data is reliable and accurate to convince banana growers using this IoT system to alert the presence of Foc in banana cultivated areas. Then, mathematical modelling for Fusarium wilt disease epidemiological is vital to understand how soil properties are associated with the Fusarium wilt disease.

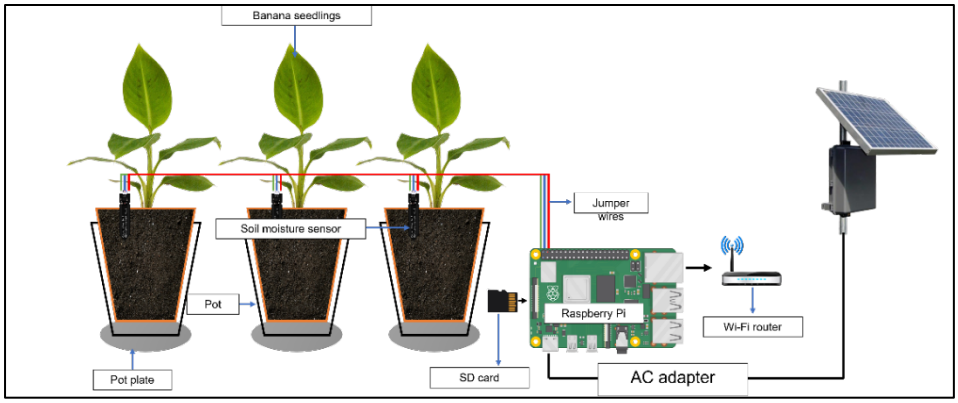

Figure 1. General idea of how the sensors be connected to the Raspberry Pi and other devices.

EXPECTED OUTCOME

By the end of this study, the soil properties of non-infested and infested soil samples from the field can be determined using lab analysis. Then, an affordable IoT system can be developed to monitor and detect Fusarium wilt disease progression in two different soils inside the greenhouse. After that, the captured data using the IoT system can be used for statistical analysis to evaluate the reliability and accuracy of the obtained data. Lastly, the IoT data also can be used to develop mathematical modelling to simulate the Fusarium wilt disease progression.

CONCLUSION

Fundamentally, this IoT automated Fusarium wilt disease detection system is becoming a great movement towards smart agriculture with a low-cost system, less manpower requirement, environmentally sustainable, and efficient power usage. It is a promising approach as the IoT is capable of connecting sensors and other useful devices with the help of communication networks and IoT platforms. In this study, the self-infestation method will be used to monitor the Foc development in the soils. By this monitoring, further knowledge regarding the soils and Fusarium wilt disease epidemiological could be demonstrated including the threshold for favourable soil conditions. As a result, this IoT framework has the potential to be applied in real banana farming and brought a positive impact to the banana growers to increase banana yield production. However, this study is only limited to the greenhouse condition of the same banana cultivar. As an expected result, the IoT system needs to be modified before embedding the IoT technology to the banana field.

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AG03

Optimisation of pH and Temperature for Protein Degradation by *Lactobacillus* sp.

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ABSTRACT

Fish meal is the common feed ingredient in aquaculture. However, its rising price has become an obstacle for many fish farmers. Soybean meal is a popular alternative to fish meal, but its usage is hampered by its low protein availability. Thus, this study aims to determine the optimum and temperature and pH of the probiotic, *Lactobacillus* sp, for protein degradation. *Lactobacillus* sp was isolated and identified from African Catfish gut and used to degrade soybean meal protein at different temperatures (room, 27°C; incubator, 40°C; and refrigerator, 4°C) and pH (pH 3, pH 4, pH 5, pH 6, and pH 7). The findings showed the highest protein enhancement at pH 7 and 40 °C. These findings have the potential to reduce the aquaculture feed cost, thus, benefiting fish farmers.

Keywords: *Lactobacillus* sp., soybean meal, protein degradation, pH, temperature

INTRODUCTION

Probiotics are good bacteria and yeasts present in the digestive system to maintain a healthy gut. These live non-pathogenic bacteria, such as *Lactobacillus*, can be found in yoghurt and other fermented foods. They are often introduced to the gastrointestinal tract to enhance microbial balance. Other advantages of probiotics include being a remedy for diarrhea and lactose intolerant individuals. Some strains like *Bifidobacterium* also help ease irritable bowel syndrome (IBS) symptoms.

Soybean meal is a popular fish meal alternative for fish farmers due to its high crude protein content (47 - 49% crude protein). However, most fish cannot directly use soy protein due to anti-nutritional factors. Thus, raw soy must be processed before it can be used, either through heating or fermentation. The crude protein will be degraded by bacteria enzymes into peptone via the fermentation process, improving its utilisation as fish feed. Therefore, this study aims to investigate protein degradation using *Lactobacillus* isolated from African catfish.

MATERIALS AND METHODS

Sample preparation and *Lactobacillus* sp. identification via Gram staining

The samples used in this study were the tested dietary catfish obtained from the tissue culture laboratory, Universiti Malaysia Kelantan, Jeli Campus, Kelantan, Malaysia. First, clave oil was used to euthanise the catfish and later cleaned with ethanol. Next, fish samples were aseptically dissected, and approximately 2.5 cm of the gut was excised. Then, the samples were weighed (1 g), cut, and the liquid gut sample was put into a test tube that contained 9 ml saline water. After that, the fluid sample was subjected to serial dilution (10^{-1},10^{-2},10^{-3},10^{-4} and 10^{-5}). Later, bacteria isolation was carried out using the spread plate technique at different dilutions in triplicates before conducting Gram staining according to Barile (2012) for identification.

Testing of reaction between *Lactobacillus* sp. and protein under different temperature and pH

A total of 100 µl of each sample was pipetted into three 96-well plates and analysed using iMark™ Microplate Absorbance Reader (BIO-RAD, USA) at a wavelength of 595 nm (initial reading). After that, each plate was wrapped with aluminium foil and placed at room temperature (27 °C), incubator (40 °C) or refrigerator (4 °C). The plates were read and restored in their respective temperatures at 5 min intervals until the 30th minute. Finally, a standard curve was created using the data obtained. Similarly, these steps were repeated for samples at different pH (pH 3, pH 4, pH 5, pH 6, and pH 7). All the samples were adjusted with a pH test kit using 1M HCL until they reached the desired pH. The data obtained were analysed using One-Way Analysis of Variance (ANOVA) and post hoc Tukey test at a significance level of $p < 0.05$ using the IBM Statistical Package for Social Sciences (SPSS) software version 25. The data were expressed as the mean ± standard deviation (SD) values.

RESULTS AND DISCUSSION

Bacteriocin produced by the lactic acid bacteria (LAB) is widely used as probiotics for human and animal consumption to avoid pathogen growth in the gastrointestinal tract (Audisio et al., 2001). Physicochemical factors, such as pH and temperature, impact bacteriocin production. However, the optimum pH and temperature conditions for high bacteriocin production rarely correlate with the best conditions for bacterial growth. In this study, the findings indicated that the protein degradation by *Lactobacillus* sp. was significantly affected by both pH and temperature ($p < 0.05$).

The highest enhancement of protein degradation by using *Lactobacillus* was at pH 7. Similarly, a previous study reported the optimum temperature for *Lactobacillus casei* ranged from pH 6.7 to pH 7. Furthermore, the cell number remains unchanged at pH 3 and pH 7. Meanwhile, it was found that 40°C is the best optimum temperature for *Lactobacillus* growth and the highest enhancement of protein degradation. In a hot climate or warm developing country, probiotics are remarkably efficient and effective even when the probiotics are stored at room temperature (28– 32 °C) (Rerksuppaphol, 2010). Meanwhile, at a temperature of 4°C, the live micro- organism remains viable for 24 months, while stability time was reduced to 4 weeks at room temperature (Rerksuppaphol, 2010). Lactic acid bacteria starter or probiotic cultures are usually preserved in a frozen or freeze-dried form, under the suitable condition to increase viability (Papadimitriou, 2016).

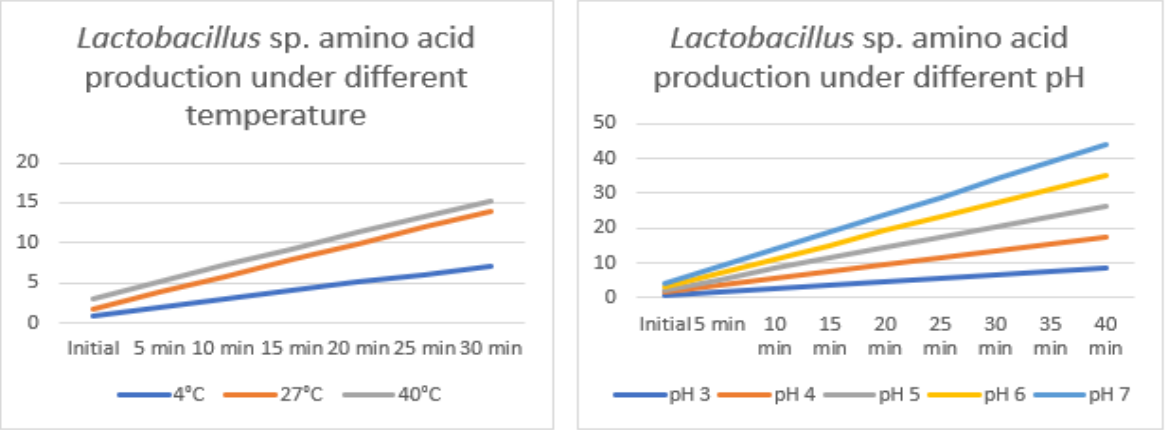

Figure 1. Total amino acid production by using *Lactobacillus* for different a) temperature and b) pH

CONCLUSION

The highest protein degradation enhancement by *Lactobacillus* was observed at pH 7 and 40°C. The utilization of probiotics in fish feed consumption, especially in the aquaculture industry, should be further investigated to identify the precise optimum condition for the lactic acid bacteria activity to produce more protein in fermented soybean meal. The findings will help in improving soybean meal as an alternative protein source for fish feed.

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AG06

Tissue Culture Technique for Rapid Clonal Propagation of Keladi Candik (*Alocasia longiloba* Miq.)

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ABSTRACT

Tissue culture protocol was established for the clonal propagation of *Alocasia longiloba* using seed as an explant. Seeds were taken from matured fruit of *A. longiloba* and cultured on modified MS medium containing different type and concentrations of plant growth hormones. The 6-benzylaminopurine hormone (3 mg/L) was the best hormone for shoot maximum induction. Maximum root percentage (95%), highest number of root/explant (11.41) and root length (9.61 cm) were achieved using 0.5 mg/L indole-3-acetic acid. Our protocol made it possible to produce large quantities of *A. longiloba* in short time and space.

Keywords: Plant tissue culture, mass propagation, *Alocasia longiloba*, medicinal plant, plant growth hormones

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INTRODUCTION

Plant tissue culture technique (PTC) has been widely used to obtain large numbers of plants from selected individual plants. PTC uses plant materials such as seed, leaf, stem, and rhizomes in a growing medium *in vitro*. Tissue culturing of medicinal plants is broadly used to produce natural products for herbal medicine and pharmaceutical industries (Hosseinzadeh et al., 2015). *A. longiloba* is a medicinal plant, belong to the family of Araceae, which consist of 114 genera and around 3750 known species. *A. longiloba*, commonly known as "Keladi Candik" in Malaysia. The plant has been used traditionally (mostly fruit and petiole) to treat wound and inflammation (Hamzah et al., 2019). It has also been used for cough and high-fever treatment in India (Das, 2018). Our previous studies have shown that *A. longiloba* has possessed *in vitro* xanthine oxidase inhibitory and high antioxidant activities (Abdulhafiz et al., 2020). Due to scarcity of *A. longiloba* plant, there is a dire need to establish micropropagation (tissue culture) protocol for mass propagation of this important medicinal plant.

MATERIALS AND METHODS

Plant material collection and seed treatment

The fruit of *A. longiloba* was collected from plants growing in a natural population in Kota Bharu, Kelantan, Malaysia (6.1211° N, 102.3178° E). The specimens of *A. longiloba* fruit were authenticated by Dr.

Zulhazman Hamzah from Faculty of Earth Science, Universiti Malaysia Kelantan. The seed was carefully separated from the fruit and air dried at room temperature with frequent turning. The dried seeds were then used to establish *in vitro* micropropagation. Sulfuric acid treatment at different concentrations (10, 20, 30 and 40%) was applied for 15 minutes to improve seed germination. Then the seeds were sterilized with sodium hypochlorite (5.25%) and culture on MS media. Germination rate was compared with non-treated seeds.

Shoot and root induction

The *in vitro* proliferated excised shoots within 4 weeks of culture were taken to induce multiple shoots using different concentrations (0, 1, 2, 3, 4 and 5 mg/L) of 6-benzylaminopurine (BAP). Single shoot (2-3 cm) from multiple-shoot culture was isolated and cultured onto a culture vessel containing twenty millilitres of MS medium fortified with various concentrations (0.5-2 mg/L) of indole-3-acetic acid (IAA) for rooting.

RESULTS AND DISCUSSION

Improving seed germination by sulfuric acid treatment

A. longiloba seeds having low germination rate triggered by their hard seed coats, which prevents absorption of water into the embryo. Hence, sulfuric acid (H_2SO_4) was applied to improve germination. H_2SO_4 treatment improved seed germination. While, the non-treated seeds produced less germination rate. Maximum germination percent (87.50 ± 5.59%) was achieved on seeds treated with 30% sulfuric acid after 19-day, followed by (79.16 ± 7.68%) germination on seeds treated with 20% H_2SO_4 after 20-day (Figure 1). H_2SO_4 decreased the number of days to seed germination in the range of between 17-37% and increased the germination percentage by 80 to 250%. H_2SO_4 treatment at 30% was the optimum concentration and was found to be sufficient to release seed dormancy and speed up the germination process.

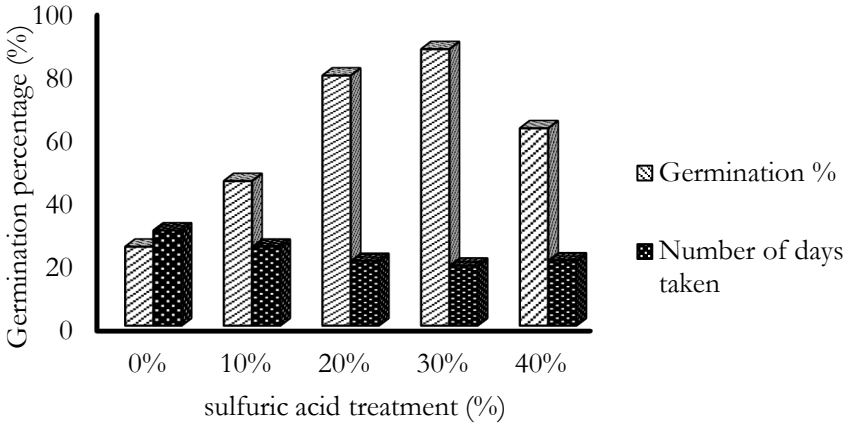

Figure 1. Improvements of seed germination capacity of *A. longiloba* by sulfuric acid treatment.

Multiple shoots induction

The highest shoot number (18.33) was observed at 3 mg/L BAP hormone treatment, followed by 13.7 shoots/explant recorded in 4 mg/L BAP and the lowest (3.66, 4.50 and 7.91) shoots per explant was achieved in 1 mg/L, 2 mg/mL and 5 mg/L BAP respectively (Table 1).in overall, BAP treatment 3 mg/L was found to induced maximum shoots.

Table 1. Effects of cytokinins on multiple shoot induction of *A. longiloba* using excised shoots.

BAP (mg/L)	Shooting response (%)	Number of shoots/explant	Shoot length (cm)
1.0	83.33	3.66 ± 0.49^{cd}	3.87 ± 0.36^{a}
2.0	100	4.50 ± 0.58^{cd}	3.21 ± 0.28^{ab}
3.0	100	18.33 ± 2.33^{a}	3.05 ± 0.44^{ab}
4.0	100	13.75 ± 2.14^{b}	2.82 ± 0.28^{b}
5.0	100	7.91 ± 1.32^{c}	2.44 ± 0.31^{b}

*Means followed by the same letter (s) within each column are not significantly different $p<0.05$ (DMRT, n=6).

In vitro root formation and acclimatization

Maximum rooting percentage (95%), highest number of root (11.41) and root length (9.61) achieved from the explants in culture vessels fortified with 0.5 mg/L IAA. The rooting percentage produced with 0, 1 and 2 mg/L of IAA were 80%, 90% and 85% respectively (Table 2). All the *in vitro* plantlets obtained from *in vitro* rooting were transferred to the nursery for the acclimatization process. Top soil and peat moss (1:2) was an optimum media.

Table 2. Effect of IAA hormone on *in vitro* root formation.

IAA (mg/L)	Root response (%)	Root number/explant	Root length (cm)
0.0	80.0	6.91 ± 0.63^{b}	9.30 ± 1.18^{a}
0.5	95.0	11.41 ± 2.16^{a}	9.61 ± 1.43^{a}
1.0	90.0	8.83 ± 1.11^{ab}	7.53 ± 1.02^{ab}
2.0	85.0	8.58 ± 1.51^{ab}	6.51 ± 1.08^{b}

*Means with the same letter within the same column are not significantly different at $P < 0.05$ (DMRT, n=12)

CONCLUSION

A successful micropropagation protocol was established for *A. longiloba*. BAP and IAA hormones at the concentration of 3 and 0.5 mg/L were found to give the best shoot multiplication rate and rooting response, respectively. A combination of top soil and peat moss at 1:2 ratio was an optimum for acclimatization.

ACKNOWLEDGEMENT

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AG07

Effects of Combined Fertilizer Sources on Pest Population and Plant Growth Performance of Chilli

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ABSTRACT

Several studies have reported the adverse effects of inorganic fertilizers while encouraging the use of organic fertilizer in agriculture. Biofertilizers are highly studied to explore their potential. Chilli (*Capsicum annuum* L.) is one of the most profitable crops in Malaysia. A field experiment was conducted on chilli to study the growth performance and pest population with different treatments of organic and inorganic fertilizer at Kg. Chong, Sik, Kedah in September 2020 to January 2021. 11 different fertilization treatments incorporating *Saccharomyces cerevisiae* as biofertilizer on Chilli Kulai 1033 was conducted. Results obtained showed that soil chemical properties have improved as the organic and biofertilizer increased. No significant differences in the pest population have proven that fertilization did not influence the pest infestation. Among the treatments, T10 and T11 have the highest agronomic efficacy than other treatments. NPK increased the fruit length yet failed to have any significant difference in the fruit weight. Therefore, the best treatment is T11 (2 tan/ha of NPK with poultry manure (10 t/ha) with 5g/L of *S. cerevisiae)* which influences the yield and growth performance of chilli compared to other treatments.

Keywords: Fertilizers, chilli, biofertilizer, pest

INTRODUCTION

Chilli (*Capsicum annuum* L.) is an economically profiting crop belonging to the family *Solanaceae*. In Malaysia, chilies are being cultivated commercially in Johor, Perak, and Kelantan. Fertilizers are used to improve soil fertility but intensive inorganic fertilizer usage in agriculture causes so many health problems and unrecoverable environmental pollution. Thus, to reduce and eliminate the adverse effects of chemical fertilizers on human health and the environment. Pandey (2014) has reported that nearly 35 species of insects and mites are the pest of chilli. Nevertheless, the continuous use of chemical fertilizers alone is not the best way to environmental sustainability. Organic manures have the capability of supplying a range of nutrients and improving the physiological and biological properties of soil. However, at a big scale of crop production, these nutrient sources are not adequate. Another important source of fertilizer is bio-fertilizer, widely known as eco-friendly, low-cost input and not only improves crop growth but also improves fertilizer efficiency. The combination of these three sources of fertilizers is one of the best methods to increase agriculture quality production and reduce the function of agrochemicals on soil and the environment.

MATERIALS AND METHODS

Experimental treatments and materials

There was a total of 11 treatment applications consisting of combinations of biofertilizers (*S. cerevisiae)*, poultry manure (PM), and inorganic fertilizers (NPK). The fertilization treatments were T1, (Control): (No manure + No fertilizer); T2, (NPK(12-12-17) + Poultry Manure (PM)): (2 tan/ha + 10 t/ha); T3, (NPK + PM + *S.cerevisiae*): (1 tan/ha + 10 t/ha + 1g/L); T4, (1 tan/ha + 10 t/ha + 3g/L); T5, (1 tan/ha + 10 t/ha + 5g/L); T6, (1.5 tan/ha + 10 t/ha + 1g/L); T7, (1.5 tan/ha + 10 t/ha + 3g/L); T8 (1.5 tan/ha + 10 t/ha + 5g/L); T9, (2 tan/ha + 10 t/ha + 1g/L); T10, (2 tan/ha + 10 t/ha + 3g/L); T11, (2 tan/ha + 10 t/ha + 5g/L). In this study, the chilli plants were grown from the chilli seeds Kulai 1033. The product was commercially available known as Complex Blue Special with content of 12:12:17:2+TE. Nearly 2 sacks of 50 kg fertilizer and 16 packs of poultry manure (PM) were purchased from a local agriculture shop in Jeli, Kelantan. The poultry manure (PM) was applied a week before the transplantation and NPK was applied 1st, 7th, and 13th week. For biofertilizer, foliar was prepared according to Nassef (2016). A total of 9 seedbeds were prepared with the width and length of 1.5 m and 63.25 m respectively. The 6- week seedlings were transplanted 60 cm apart in a single row. Observations on sucking pest complex nymphs and adults were recorded on 10 random plants on monthly basis. The efficacy of different treatments in pest control was judged by assessing the number of pests before and after the foliar and pesticide applications. Various taxonomic keys of identifications related to chilli insect pests and other arthropods by different authors were used for identification purposes in this study. Growth characters like plant height, girth length, and days to first flowering were taken and the data were recorded at the different growing stages (15, 45, 75, 105, and 165 DAT) (Singh, Choudhary, Sharma, Rawat, & Jat, 2016). The results were complemented by the MS. Excel package 2018 and statistically analysed by multivariate using SPSS software for all the treatments.

RESULTS AND DISCUSSION

Pest population

This study recorded 14 natural enemies throughout the growing stages. Out of fourteen, four species were from the order Hemipteran in four families. These include *Aphis possypii* (Glover) from, *A. viridigrisea* from the family of Cicadellidae which recorded the second-highest populated pest, *Bemisia tabaci* (Gennadius)from Aleyrodidae*, Helopeltis collaris Stal* from Miridae family. Besides, 28.75% of the species were from the Lepidoptera order. Among the three species which are *Spodoptera litura*, *S. exigua* (Hübner) and *H. armigera* (Hubner.) belong to the same family, Noctuidae. The other Lepidopteran was *Archips purpurana* from the family Tortricidae. Two Coleoptera species *Epitrix tuberis* and *Leptinotarsa decemlineata were* also recorded which belong to a similar family, Chrysomelidae. *Scirtothrips dorsalis* (Hood), the highest populated pest from Thripidae order, Thysanoptera family was also recorded. Species from Acari order were also recorded. This species belongs to Tarsonemidae which is widely known as Broad mite or yellow mite. Leaf-cutting ant was also recorded during this experiment from a family of Formicidae that included in the Hymenoptera order. Similarly, field cricket, an Orthoptera species, was also recorded that belongs to the Gryllidae family. Among the major and minor pests identified in the chilli plantation, three species were found to be dominant including *A. possypii, S. dorsalis, and B. tabaci*as they recorded high relative abundance. These pests have more frequently occurred as they recorded relative abundance in the range of 20.88 to 42.03 %. Following these species, nine pests were found to be subdominant as their relative abundance was recorded in between 0.5-10% which are *P. latus, E. tuberis, S. exigua, A. purpurana, L. decemlineata, Atta spp., H. collaris, H. armigera, and S. litura.* They were found to be the next frequently occurring as they appeared with the relative abundance in between 0.57 and 1.7 %. However, *A. viridigrisea and Gymnogryllus spp* were found to be the least abundant as they occurred less than 0.5%. These pests are categorized as rare since their appearances recorded 0.11 to 0.22% of relative abundance. In terms of relative abundance, the 14 pests were found to be in the following order of *A. possypii* > *S. dorsalis* > *B. tabaci*as >*E. tuberis*> *A. purpurana*> *P. latus*> *H. armigera*> *L. decemlineata*> *S. exigua*> *H. collaris*> *Atta spp*> *S. litura*> *A. viridigrisea*> *Gymnogryllus spp.*

Effects on chilli plant growth parameters

The plant height of chilli at 15, 45, 75, 105, 135, and 165 Days After Transplanting (DAT) which were influenced by various application rates of organic and inorganic fertilizer with the addition of biofertilizer at different concentrations were recorded. The data revealed that, chilli plant recorded mean plant height during 15 DAT (8.76 cm), 45 DAT (22.99 cm), 75 DAT (32.29 cm), 105 DAT (37.61 cm), 135 DAT (43.83 cm) and 165 DAT (50.56 cm). At the growing stage of 15 DAT, T11(RFD + NPK (2 tan/ha) + *S. cerevisiae* (5g/l), 11.30 cm) recorded significantly ($p \leq 0.05$) higher over the T1(control, 8.00 cm) and T2 (standard, 9.28 cm) by 29% (3.3 cm) and 22% (2.02 cm) respectively. No significant was found in the difference among other treatments at 15 DAT and 45 DAT, 105 DAT and 135 DAT. Meanwhile, during 75 DAT, the highest plant height was recorded by T10 (40.54 cm) and T11 (39.49 cm) which were at par level with each other. These treatments were superior to T2 (standard, 37.04 cm) by 3.5 and 2.5 cm respectively. At 165 DAT was T10 (59.24 cm) recorded the highest plant height yet the differences were statistically insignificant with the other treatments. Mostly, T11 recorded the highest plant height during all the growing stages. There was a significant difference between the plant girth with 11 treatments applied at various. At 15 DAT, T6 (0.24 cm) and T5 (0.24 cm) exhibited thicker stems than other treatments and outgrowing T2 (standard, 0.22 cm). Nevertheless, all the differences among the treatments were not statistically significant. During 45 DAT, T10 (0.61 cm) recorded the highest girth length, followed by T11 (0.56 cm). these treatments were at par level with T2 (0.52 cm) and T9 (0.51 cm). The highest plant girth was recorded during 75 DAT, 105 DAT and 165 DAT were by T10 (1.04 cm), T11(1.03 cm), and T11(1.54 cm) respectively where the difference between the plant girth was insignificant with other treatments. Among the treatments during 135 DAT, the significantly highest plant girth was recorded by T11(1.44 cm). These were followed up by T2 (1.19 cm) and T10 (1.15 cm) which were insignificant with each other. The results of this parameter show that the treatments with greater NPK content produce thicker plant stem.

The difference between the treatments and the days to attain the first flowering were significant. Nevertheless, among the treatments, the minimum days for the first flowering was by T11(25 days) and T10 (25 days) where the difference between these treatments was insignificant. Even though the blooming stage was accelerated with the amount of NPK application, T11 and T10 with the addition of biofertilizer induce the plant to bloom earlier compared to the T2 by three days. Similarly, all the treatments were significantly superior to the T1 (control, 46 days). The minimum days to attain first flowering was the treatment with the highest NPK application (2 tan/ha). Besides, the interaction effect of biofertilizer application and the treatments did not show significant results. Hence, it is evident that NPK plays an important role in inducing the first flowering rather than the biofertilizer application. The increase in growth attributes such as plant height, plant girth, and days to first flowering revealed the consequence of the cumulative effects of plant growth characteristics. In general, all the treatments were significantly superior to T1(control) while some outgrown the T2 (standard). The highly superior treatment that attained the greatest growth attributes was T11. These growth effects are due to the increased amount of nutrient absorption such as N, P, and K by plants leading to an increase in the formation of plant metabolites that build up the plant tissues. These results corroborate the findings of Singh et al (2016). The biofertilizer application influences a positive response on the chilli plant. Similarly, various doses of NPK fertilization and their combinations with biofertilizers (*Azospirillum* and *Phosphotica)* have a differential positive effect on the growth attributes. These current findings are also supported by the results obtained by Chowdhury (2015), he mentioned foliar fertilizer, Calsol has significantly induced the growth parameters of beetroot and emphasizes elaborated the mechanisms which involved the bioavailability of macro and micronutrients, production of growth hormones, and reduction of the phytopathogens' growth.

CONCLUSION

The combination of the poultry manure and inorganic fertilizer (NPK) with the addition of biofertilizer application enhances the growth of chilli plants in this study by producing the highest plant height (50.56 cm), maximum plant girth (1.54 cm), and minimum days to first flowering (25 days) were recorded by T11. This proved instead of a sole application, combined organic and inorganic application with biofertilizer able to affect and increase the growth of plants. As in terms of the pest population, *A. possypii was* found to be the highest infested pest. Nevertheless, the inorganic fertilizer applied for plant growth has also negatively

impacted the insect pest population. Hence, at certain times, pest control measures such as the use of chemical insecticides and other pest management options are also important.

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AG09

Rice Straw Biochar Application Enhances Soil Nitrogen Availability for Efficient Rice Plant Growth

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ABSTRACT

Urea fertilizer is used widely as a nitrogen contributor. However, N loss through ammonia volatilization from applied urea has become an intense problem to agriculture. A pot experiment was conducted to determine the effect of rice straw biochar on soil (1) total N, exchangeable NH_4^+, and available NO_3^- (2) physical growth performance of rice plants. The treatments evaluated were: (T0: soil only, T1: soil + 175 kg ha^{-1} urea, T3: soil + 175 kg ha^{-1} urea + 5 t ha^{-1} rice straw biochar, and T4: soil + 175 kg ha^{-1} urea + 10 t ha^{-1} rice straw biochar. The addition of rice straw biochar at 5 - 10 t ha^{-1} in the pot experiment significantly increased the soil total N availability by 33.33% - 46.67%. Treatments T3 and T4 also had significantly increased exchangeable NH_4^+ and available NO_3^- in the soil over T1. The soil availability nutrients increment in soil was attributed to the higher adsorption capacity of the rice straw biochar. Increment in soil nitrogen availability significantly increased the rice plant height, tiller number, and panicle number because of effective rice plant ammonium absorption. Rice straw biochar at 5 - 10 t ha^{-1} can improve the productivity of rice plants by increasing N availability in soil.

Keywords: Ammonia volatilization, biochar, urea, nitrogen loss, ammonium, nitrate

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INTRODUCTION

Nitrogen (N) is one of the primary essential plant nutrients for both crop growth and it is required in a large amount and remains as a critical nutrient supplement throughout a plant life cycle. Due to this, agricultural field needs N fertilizer application to increase the crop growth and yield production. However, rapid volatilization of ammonia (NH_3) from applied N fertilizers (urea) creates drawbacks to agricultural sectors. Around 60% of NH_3 volatilize from the surface applied urea fertilizer, which reduces ammonium (NH_4^+) in the soil (Rochette et al., 2009).

In order to minimize NH_3 loss and increase NH_4^+ availability, biochar can be used as one of the possible options. Biochar, namely biomass-derived charcoal is a highly aromatic substance that has been thermally decomposed under charring conditions (Lehmann et al., 2009). Agricultural waste such as rice straw can be utilized to produce biochar. Biochar is highly porous, alkaline and has a large surface area. Large surface area of biochar helps in binding cations such as NH_4^+ which directly increase cation exchange capacity (CEC) (Lehmann et al., 2009). Biochar can retain more nutrients in the soil for efficient plant uptake. However, there is less information on the use of biochar with a large surface area and high negative charges to adsorb NH_4^+ from soil applied with urea. Hence, this study was carried out to determine the effect of urea fertilizer amended with rice straw biochar application in improving soil N availability and rice plant growth performance in tropical acid soil.

MATERIALS AND METHODS

Pot experiment

A pot experiment was conducted in a netted house located at the Universiti Malaysia Kelantan Jeli Campus, Malaysia. Rice plant MR297 cultivar was used as a test crop in the pot experiment, and the seedlings were planted in pots which were filled with 5 kg sieved soil. The rice straw biochar was produced by using biochar kiln. The biochar rates of 5 t ha^{-1} and 10 t ha^{-1} were mixed thoroughly with the soil 24 hours before transplantation of 7th day rice seedlings into the pot. Three rice seedlings were planted in each pot and the water level in each pot was maintained at 3 cm from the soil surface. After seven days of transplantation, N, P, and K fertilizer in the form of urea, Christmas Island Rock Phosphate (CIRP), and Muriate of Potash (MOP) was applied at the rate of 175 kg ha^{-1}, 97.8 kg ha^{-1}, and 130 kg ha^{-1}, respectively. The lists of treatments evaluated in the pot experiment are listed in Table 1.

Table 1. Treatments evaluated in pot study.

Treatment	Description
T1	5 kg soil (Negative control)
T2	5 kg soil + 175 kg urea ha^{-1} + 97.8 kg CIRP ha^{-1} + 130 kg MOP ha^{-1} (Positive control)
T3	5 kg soil + 175 kg urea ha^{-1} + 97.8 kg CIRP ha^{-1} + 130 kg MOP ha^{-1} + 5 t rice straw biochar ha^{-1}
T4	5 kg soil + 175 kg urea ha^{-1} + 97.8 kg CIRP ha^{-1} + 130 kg MOP ha^{-1} + 10 t rice straw biochar ha^{-1}

The pot experiment was carried out in a completely randomized design with three replications in a net house. At the heading stage (70 DAT) before harvest, the plant height, number of tillers and the number of panicles were recorded. The soil samples from pots were collected immediately upon plant harvesting. The soil samples were analyzed for pH, total N, exchangeable NH_4^+, and available NO_3^-. Soil pH was measured in a ratio of 1:2.5 (soil: water) by using a digital pH meter. The total N was determined by using the Kjeldahl method (Bremner, 1965). The method described by Keeney and Nelson (1982) was used to extract exchangeable NH_4^+ and available NO_3^-, after which the ions were determined via steam distillation

RESULTS AND DISCUSSION

Effect of rice straw biochar application on soil nutrients

The addition of rice straw biochar had significantly improved soil nutrients by adsorption mechanism. At 70 DAT, the soil N, NH_4^+ and NO_3^- were significantly higher in treatments amended with rice straw biochar (T3 and T4) in comparison to T1 and T2. Rice straw biochar has a higher affinity to bind and retain more N, NH_4^+ and NO_3^- in soil. This is due to the nature of rice straw biochar which is highly porous and has a large surface area (Lehmann et al., 2009). Rice straw biochar also significantly increased soil pH compared to T1 and T2. The soil pH increment might be due to the proton exchange in between biochar and soil.

Table 2. Effects of rice straw biochar on soil total N, exchangeable NH_4^+, and available NO_3^- of pot experiment

Treatments	pH	Total N (%)	Exchangeable NH_4^+ (mg kg^{-1})	Available NO_3^- (mg kg^{-1})
T1	5.8 ± 0.13 [a]	0.07 ± 0.02 [a]	23.3 ± 2.34 [a]	25.6 ± 6.18 [a]
T2	6.2 ± 0.03 [a]	0.15 ± 0.01 [b]	31.3 ± 5.24 [a]	38.5 ± 2.02 [a]
T3	6.9 ± 0.09 [b]	0.20± 0.01 [c]	73.5 ± 2.01 [b]	66.5 ± 2.02 [b]
T4	6.9 ± 0.14 [b]	0.22 ± 0.02 [c]	94.5 ± 2.02 [c]	89.0 ± 2.01 [c]

Mean values within columns with different letter(s) indicate significant difference between treatments by Tukey's test at $p \leq 0.05$. Columns represent the mean values ± SE.

Effect of rice straw biochar application on rice plant growth performance

The increment in soil available inorganic NH_4^+ directly increases rice plant growth performance. The effect of rice straw biochar treatment on the growth performance of rice plants is shown in Table 3. The plant height, and panicle number were significantly increased upon application of rice straw biochar rate 10 t ha^{-1} (T4) compared to other treatments. Treatments T3 and T4 had significantly increased the production of tiller numbers compared to T1 and T2. This gives a clear idea that the application of biochar aids the growth of rice plants. Uzoma et al. (2011) reported that the addition of biochar increases the growth performance of plants because biochar adsorbs nutrients and releases it slowly for plants uptake.

Table 3. Effects of rice straw biochar on plant growth performance of rice plant MR297 cultivar.

Treatments	Height (cm)	Tiller Number	Panicle Number
T1	41.9 ± 0.19 ^{a}	2.00 ± 0.33 ^{a}	1.00 ± 0.02 ^{a}
T2	76.1 ± 2.92 ^{b}	3.00 ± 0.34 ^{a}	2.00 ± 0.33 ^{a}
T3	81.4 ± 0.72 ^{c}	7.00 ± 0.35 ^{b}	5.00 ± 0.57 ^{b}
T4	97.1 ± 1.16 ^{d}	7.00 ± 0.58 ^{b}	9.00 ± 0.58 ^{c}

Mean values within columns with different letter(s) indicate significant difference between treatments by Tukey's test at $p \leq 0.05$. Columns represent the mean values ± SE.

CONCLUSION

The addition of rice straw biochar effectively increased the presence of nitrogen in the soil. It increased NH_4^+ and NO_3^- ions in the soil for efficient rice plant uptake. The increment in soil nutrients improved rice plant growth performance. Hence, rice straw biochar at the rate of 5 - 10 t ha^{-1} (T3 and T4) can be used to adsorb more nutrients from the applied urea fertilizer and release it slowly for efficient rice plant uptake.

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AG12

Evaluating the Suitability of Heavy Metal Content in Sawdust, Paddy Straw and Oil Palm Frond for Mushroom Cultivation

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ABSTRACT

Abundant agricultural biomass can be used as alternative substrates for mushroom cultivation due to the sawdust prices have been increasing and can affect the cost of the mushroom production. Mushrooms have been observed to absorb and store a variety of chemical elements where they can lead to contamination towards the edible fruiting bodies. This heavy metal was analysed by AAS using dry ashing method. Results were demonstrated that heavy metal content for paddy straw is follow WHO/FAO safe limit quantity Cu, Fe, Zn and Pb were 0.1433mg/L, 1.5993mg/L, 0.8090mg/L and 0.0233mg/L, respectively. However, arsenic content for paddy straw recorded the lowest amount between the treatments which is 2.0423mg/L and nearly reaching the permissible limit.

Keywords: Agriculture biomass, substrates, mushroom, heavy metal

INTRODUCTION

As the agriculture sector grows, crop yields rise, resulting in an increase of the agricultural biomass such as paddy straw, coconut husk, corn silage, sugarcane bagasse and so on. Poor management of agricultural wastes can increase various problems especially towards the environment. To control the environmental pollution caused by the unpractical agro-waste management, the waste is reused as a substrate for mushroom cultivation. In this study, detection and comparison between different agricultural biomass of paddy straw and oil palm frond with the commercial substrate were made based on the composition of heavy metal in the agriculture waste for alternative as mushroom substrates.

MATERIALS AND METHODS

Study area and experimental materials

The study was conducted at the postgraduate laboratory at Universiti Malaysia Kelantan, Jeli Campus. The agricultural biomass used were sawdust of rubber tree, paddy straw and oil palm frond. The sawdust from rubber tree sources was purchased in sawmill in Kampung Jedok, Tanah Merah, Kelantan (5.8300^{o}N, 101.9390^{o}E), the paddy straw was collected from paddy grower in Bachok, Kelantan after harvesting time and the oil palm frond (OPF) was collected from oil palm estate in Felda Kemahang, Tanah Merah, Kelantan (5.8053^{o}N, 102.0062^{o}E).

Preparation of biomass samples

The sawdust obtained already in ground state while the OPF was cut into blocks using cutter machine and the size were reduced into 5 cm using machete. For paddy straw, it was cut for 5 cm long using chaff cutter. The OPF and paddy straw were cleaned using deionised water to remove any debris and drained. Then, all of the biomass samples were allowed to dry in oven at 70 ^{o}C for 24 hours and ground. For paddy straw and OPF, the raw samples were ground using miller until small size then were proceed to electric grinder for fine particles. The samples (i.e., sawdust, paddy straw and OPF) were ground using electric grinder and were sieved using siever (1.0 mm). The fine samples were stored in polypropylene plastic airtight containers at room temperature (25 ^{o}C) before analysed.

Analysis of heavy metal in agricultural biomass

The analysis was followed by the dry ashing method by Gebrelibanos et al., (2016). The prepared powder samples were weighed 0.5 g approximately into crucible and ashed at 500 ^{o}C for 4 hours until a white or grey ash residue was obtained. The residue was dissolved in 2 mL 69% nitric acid and filtered into a 50 mL volumetric flask using Whattman paper number four sized 90 mm. The residue on the filter paper was washed using deionised water to make sure all of the element entered the volumetric flask and made up until mark with deionised water. Same method was applied for the blank sample without including the samples. Then, four serial dilution processes were done towards the samples. The samples were preserved using 13.5 mL of 5% nitric acid. Then, the samples were analysed for zinc (Zn), copper (Cu), iron (Fe) and lead (Pb) analysis using Atomic Absorption Spectroscopy (AAS). While for arsenic (As) element analysis in the samples, about 1 mL from the stock samples were transferred into test tubes. Then, 1 mL of concentrated hydrochloric acid was added into the same tubes followed by 1mL of 5% KI + ascorbic acid solution. Next, the samples were placed at ambient temperature for 45 minutes and were diluted using 7 mL of deionised water. After that, the samples were subjected to four times serial dilution. The samples were analysed using Atomic Absorption Spectroscopy (AAS) for arsenic detection. The results were obtained and recorded.

Statistical analysis

All of the data were subjected by multiple comparisons of the ANOVA test and were ranked by Duncan's homogeneity subsets if the F test will be significant at the 95% probability level by using Statistical Package for Social Science Software (SPSS software) (IBM SPSS Statistics Version 21).

RESULTS AND DISCUSSION

Heavy metal analysis

Fertilisers and insecticides are frequently used in Malaysia, particularly in the agricultural sector for productivity booster (Casey, 2017). These agricultural activities resulted in significant amounts of wastes from phosphate fertilisers or pesticides that were released into the environment, contaminating soil and water. The plants have the ability to uptake the contaminated water and soil. The transportation of contaminants from one trophic level to another in the food chain can result in the accumulation of contaminants in the biomass and can remain in the organism (Tangahu et al., 2011). So, to find an alternative mushroom substrate by using agriculture biomass, heavy metal analysis was done in this study. As shown in Table 1, the mean concentration of copper (Cu) in oil palm frond was the highest (0.2180 mg/L) and the least was in paddy straw (0.1433 mg/L). The result from this study was found to be comparable based on the result reported by Oviasogie et al. (2011) where the mean concentration of Cu on oil palm frond and leaves of 60 years palm recorded the highest mean which is 3.91 mg/L. The bioaccumulation of Cu in oil palm is due to the sufficient bioavailable and reserved amount of Cu in the soils. However, low Cu content is due to the deficiency of Cu elements in the plants. The availability of Cu in the soil is also low and causes the uptake of Cu by the plant is low and leads to a deficiency of Cu. The concentration of Cu

for this study achieved the permissible copper value of plants by FAO/WHO 2015 which is 30 mg/L (WHO/FAO, 2015).

Table 1. Heavy metal content in agricultural biomass for mushroom cultivation substrates

Treatment	Concentration of Heavy Metal Content in Agriculture Biomass				
	Cu mg/L	Fe mg/L	Pb mg/L	Zn mg/L	As mg/L
Sawdust	0.1913	1.3736	0.0633	0.3046	3.3200
Paddy Straw	0.1433	1.5993	0.0233	0.8090	2.0423
Oil Palm Frond	0.2180	2.1123	0.0210	0.2156	6.5003

The result for iron content is stated in Table 1 where the mean concentration of iron (Fe) in oil palm frond is the highest (2.1123 mg/L) and the sawdust recorded the lowest concentration which is 1.3736 mg/L. The amount of iron content in the study is good in agreement with Olafisoye et al. (2020) where the amount of iron content in oil palm sampled in Agbarho is 1.29 mg/L. The utilisation of fertiliser is one of the factors that contributes towards the iron content in the plants and become essential in chlorophyll synthesis (Rout et al., 2015). However, the iron content recorded in this study is below the permissible level of 48 mg/L (WHO/FAO, 2015). Zinc has an important role in both primary and secondary metabolites. Mushrooms can uptake the zinc ions for their growth. Table 1 shows the highest concentration of zinc content recorded by paddy straw which is 0.809 mg/L while the lowest zinc content recorded by oil palm frond which is 0.2156 mg/L. The paddy straw has the highest zinc content due to the application of fertiliser of zinc sulphate (Amanullah et al., 2020). However, the zinc content recorded in this study is below the permissible level of 60 mg/L (WHO/FAO, 2015).

The mushroom fruiting bodies also have a tendency to absorb the heavy metal from the substrates because they can act as an effective biosorbent of toxic metals (Nilanjana et al., 2005). The heavy metal concentration in the mushroom fruiting bodies varies between species and substrate composition (Udochukwu et al., 2014). This study shows the result for lead (Pb) content (Table 1) in sawdust is the highest (0.0633 mg/L) compared to paddy straw and oil palm frond (0.0233 mg/L and 0.0210 mg/L) respectively. However, lead content recorded in this study is below the permissible level of 2.0 mg/L (WHO/FAO, 2015). From the result in Table 1, the oil palm frond is high in arsenic (As) content (6.5003 mg/L) compared to sawdust and paddy straw (3.32 mg/L and 2.0432 mg/L) respectively. This study has contradicted the result with Abedin et al. (2002) where the arsenic in paddy straw recorded the highest content which is 91.8 mg/L. The organic arsenic compound is usually used as pesticides (Tangahu et al., 2011). The oil palm frond recorded the high arsenic content might be due to the application of the pesticides on the oil palm plantation. The arsenic comes from the pesticides and leaches into the soil and has been uptaken by the tree. However, arsenic content recorded for paddy straw in this study is the nearly permissible level of 0.2 mg/L (WHO/FAO, 2015).

CONCLUSION

It is found that mushroom cultivation depends on the suitable cultivation substrates that are based on and heavy metal accumulation in the substrates. This study showed that paddy straw nearly at the safe limit for the substrate for mushroom cultivations.

ACKNOWLEDMENT

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AG13

Colchicine-Induced Variation in Xanadu (*Philodendron xanadu*) as Commercial Feature Aspects

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ABSTRACT

In the ornamental plant market, new characteristic of plants is a critical criterion for consumers making purchase decisions. *Philodendron Xanadu* is shiny green, leathery leaves, each with multiple lobes. Presently, the characteristics of the plant are not attractive, thus leading to less attention obtained from the consumers. Therefore, it is imperative to apply breeding methods to improve *Xanadu* features. One strategy to accelerate breeding is polyploid induction, which could improve variegation and plant size. The first two groups of sterile seedings of Xanadu were soaked in 2.4 and 4.8 mg/l of colchicine solution for 24 hours and then were cultured on free hormone MS media. The last two groups were cultured on MS media with 2.4 and 4.8 mg/l of colchicine. Cultured seedlings of all treatments were transplanted into soil and their growth after four weeks of transplanting were recorded. It was found that 2.4 mg/l colchicine for soaking or adding into MS media gave the highest average shoot length whereas average leaf number is the lowest when treated with 4.8 mg/l colchicine in MS media. Besides, average leaf width was reduced in all treatments compared with control.

Keywords: Colchicine, variation, Xanadu, *In vitro* culture

INTRODUCTION

Currently, many species of ornamental plants are widely popular, particularly plants with different characteristics such as spotting or variegated leaves, plant sizes, larger or smaller leaves are gaining popularity. Xanadu is a popular ornamental which is suitable for potted plants or planted in the ground to decorate the garden or use the leaves for flower arrangements in various parties such as a wedding ceremony. Importantly, it also helps to purify the air and absorb toxins in the home as well. Hence, the breeding of Xanadu gives new characteristics. It is considered to create another opportunity to expand the market of Xanadu. Mutations can be caused by both intrinsic and extrinsic factors. Intrinsic factors are mutations caused by abnormalities within the plant's genome. Most of them are caused by errors in recombination DNA replication, DNA repair, the lack or increase of DNA fragments causes plants to produce abnormal gametes or is caused by the interaction of transposable elements or mutator genes. Exogenous factors are often due to deficiency of certain nutrients, temperature, sudden temperature change or radiation that exists in nature (Brown, 2002). There are also several chemicals that can induce mutations, such as chemicals with alkene groups, base analogues, and colchicine (Davidson, Pertens, & Zhao, 1983). In plant genomics, tissue culture methods are used to assist in the testing of chemically or radiation-stimulated seedlings. Therefore, in this experiment, the use of the chemical as colchicine to induce genetic variation in sterile conditions was investigated. This will result in the appearance of the Xanadu that is different from the original plant and can continue to select the good cultivars.

MATERIALS AND METHODS

Preparing colchicine treatment

Colchicine was obtained from Colchicine brand oral gout medication (1 tablet contains 0.6 mg colchicine, lactose, magnesium, stearate, and starch) purchased over the counter from a local pharmacy. The first two group of treatment was prepared by adding 1 tablet of colchicine into 250 and 125 ml. of sterile water to obtain 2.4 and 4.8 mg/l of colchicine solution which were used for soaking cleaned-culture Xanadu Seeding for 24 hours

The second two group of treatment was prepared by directly adding 4 and 8 tablet of colchicine into 1 liter of MS media to obtain MS media added 2.4 and 4.8 mg/l of colchicine which were used for directly culturing cleaned-culture Xanadu seeding.

Induction of variation of Xanadu seedling by colchicine

The first two group of sterile seedings of Xanadu were soaked in 2.4 and 4.8 mg/l of colchicine solution for 24 hours and then were cultured on free hormone MS media. The last two groups were culture on MS media added 2.4 and 4.8 mg/l of colchicine. All treated cultures were incubated at 25 ± 2° C under a 16-hour photoperiod with illumination provided by cool fluorescent lamps at an intensity of 60 $\mu molm^{-2}sec^{-1}$ (TLD 36 w/853350 lm Phillips, Thailand). Some quantitative traits were recorded at four weeks after transplanting and all were subjected to statistical analysis. Means, Standard error were computed.

RESULTS AND DISCUSSION

Cultured seedlings of all treatments were transplanted into soil and recoded their growth after four weeks of transplanting. It was found that 2.4 mg/l colchicine for soaking or adding into MS media gave highest average shoot length whereas average leaf number is lowest when treated with 4.8 mg/l colchicine in MS media. Besides, average leaf width reduced in all treatment compared with control. (Table 1, Figure 1). It seems that at the same concentration, to treat colchicine by soaking affected on seedling growth more than adding directly into media. This existence is because when soaking, the seedling as explant could absorb colchicine thoroughly into cell and diffused to various parts of the cell ((Taychasinpitak, Pinthong, & Pensuriya, 2016). Moreover, high concentration of colchicine tended to reduce all features.

Table 1. Some quantitative traits presented when treated with or without colchicine

Treatments	Average shoot length	Average root number	Average leaf number	Average leaf width
T1: No colchicine	2.70±0.70	3.93±0.43	8.13±0.44	1.46±0.22
T2: Soaking in 2.4 mg/l colchicine for 24 hours	2.94±0.53	4.13±0.96	8.86±0.69	1.37±0.32
T3: Soaking in 4.8 mg/l colchicine for 24 hours	2.78±0.30	3.73±0.86	8.40±0.64	1.31±0.19
T4: Adding 2.4 mg/l colchicine in MS media	2.94±0.37	4.10±0.27	8.33±0.62	1.35±0.20
T5: Adding 4.8 mg/l colchicine in MS media	2.80±0.27	3.86±0.18	7.8±0.44	1.34±0.18

CONCLUSION

Our research intended to find an appropriate method and concentration for exposing in vitro explants to colchicine solution from gout medication tablets to induce variation in ornamental Xanadu. It was found that the growth of colchicine-treated plantlets was slightly different from the control. Particularly, shoot

length increases when treated with colchicine at 2.4 and 4.8 mg/l whereas leaf width decreases when treated with both concentration

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AG14

The Macronutrient Evaluation of the Recycling and the Modifying of Sajor-caju Mushroom Waste

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ABSTRACT

This study aimed to determine the macronutrient of Sajor-caju mushroom waste for cultivating the lettuce. This experimental design was a completely randomized design with five treatments and ten replications with the macronutrients (N, P, K) after fermentation with the portion of the Sajor-caju mushroom waste and other substrates. The level of Sajor-caju mushroom waste (100% and 50%) were mixed with the different agricultural waste (coco-coir and rice husk) and chicken dung. The results showed that the growing media with the highest macronutrients after fermentation and the highest portion of Sajor-caju mushroom waste contained 2.92%N, 2.10%P, and 1.35%K classified in the organic fertilizer criteria due to the Department of Agriculture, Thailand.

Keywords: Sajor-caju mushroom waste, macronutrient, growing media, organic fertilizer

INTRODUCTION

Mushroom cultivation in Asian countries started a thousand years ago. Thailand is a particularly optimal environmental condition for growing tropical mushrooms. The sajor-caju mushroom (*Pleurotus sajor-caju*) is most likely cultivated in southern Thailand but since only edible parts of the fungal are harvested, the remaining substrate contains edible fungal residues and approximately 75-85% of unused nutrients. For every one kilogram harvested, approximately 25% is left behind and is treated as waste that is a source of nutritious organic waste which may be worth reusing (Li *et al*., 2020). Mushroom waste substrate can be converted into valuable material by composting (Garg *et al*., 2006) and the sajor-caju mushroom waste has the potential to be composted. Sustainable agriculture supports compost and vermicompost as alternatives incorporated into organic crop production (Castelo-Gutiérrez *et al*., 2016).

The various utilization of the sajor-caju mushroom waste not has been used as a substrate for culturing only the same or other edible fungi such as *Pleurotus abalonus* and *Auricularia polytricha* (Sripheuk, 2007) but has been also used for producing bacteria (Wu *et al*., 2014). Large amount of mushroom waste has been used as fertilizer because it has all essential characteristics of organic manure after recomposting by natural weathering or any other process. This study had been done to evaluate the macronutrient from recycling and modifying *P. sajor-caju* waste as a growing media for vegetable production.

MATERIALS AND METHODS

Measurement of physiological and chemical characteristics of sajor-caju mushroom waste

The sajor-caju mushroom waste were collected for analyses physiological and chemical characteristics; organic carbon (OC), total nitrogen (total N), C/N ratio, total phosphorus content (as P_2O_5), total potassium content (as K_2O), organic matter, pH, and electrical conductivity (EC).

Experimental treatments and design

Experimental treatments comprised five different growing media and control. The sajor-caju mushroom waste (SMW), chicken dung, coco-coir, and rice husk were mixed in different ratio (v/v) and added with/without NPK fertilizers to prepare different growing media. The treatments included 100% SMW, fermented 100% SMW+NPK, SMW+coco-coir (1:1), SMW+rice husk (1:1), Mixed and fermented of SMW+coco-coir (1:1), and mixed and fermented of SMW+rice husk (1:1). The experiment was arranged in a completely randomized design (CRD) with ten replications. Organic carbon (OC), total nitrogen (total N), C/N ratio, total phosphorus content (as P_2O_5), total potassium content (as K_2O), organic matter, pH, and EC were measured.

RESULTS AND DISCUSSION

Physiological and chemical characteristics of sajor-caju mushroom waste

The total nitrogen of sajor-caju mushroom waste as 0.31%, total phosphorus content as P_2O_5 0.23% and total potassium as K_2O 0.26% and other physiological and chemical composition was showed in Table 1. The *Pleurotus* species reveal high efficiency in the degradation of a wide range of lignocellulosic residues and others into mushroom protein. Kwak *et al.* (2007), spent mushroom substrate is a nutrient rich organic by product of mushroom cultivation where the primary source in mushroom substrates is wood sawdust, one of the most common sources which is routinely used for mushroom cultivation.

Suitability of sajor-caju mushroom waste as growing media

The physiological and chemical composition of all growing media used in this study is shown in Table 1. All the media were slightly acidic in nature with pH values ranging from 5.7-6.8. The pH values of different growing media varied significantly (Table 1). Growing media containing 100% SMW showed that the highest pH level (7.4), while 100% SMW+NPK showed the lowest acidity level (5.7). When SMW was mixed with coco-coir and rice husk with fermented and non-fermented process, pH values were higher as compared with fermented 100% SMW+NPK but lower as compared with 100% SMW. Electrical conductivity (EC) also varied significantly among treatments (Table 1) that values ranged from 2.59 to 5.53 dS m^{-1}. The 100% SMW contained the lowest percentage of nitrogen (0.31%), phosphorus (0.23%) and potassium (0.26) compared to other media. On the other hand, nitrogen content in fermented 100% SMW + NPK was the highest (2.92%) among all growing media. The total phosphorus as P_2O_5 of media, the mixed and fermented of SMW+coco-coir (1:1) contained the maximum amount of P (2.32%), which was statistically similar to the total phosphorus of SMW+coco-coir (1:1) (2.27%). The total potassium of mixed and fermented SMW+coco-coir (1:1) is highest (1.79%) compared with other growing media. The nutrient elements in fermented SMW with both coco-coir and rice husk showed a better quality of growing media than 100% SMW. According to Jigme (2015), the chicken manure contains high nitrogen, phosphorus and potassium content that can increase the microorganisms in the growing media.

Table 1. The physiological and chemical composition of 100% SMW and growing media.

Characteristics	100%SMW	*Growing media 1	Growing media 2	Growing media 3	Growing media 4	Growing media 5
1. Organic matter (%)	85.85a**	78.79b	75.95bc	71.80c	69.98d	72.75c
2. Electrical conductivity (dS m^{-1})	2.59d	5.53a	4.60b	2.86c	4.81b	5.10b
3. pH	7.4a	5.7d	6.1c	6.8b	6.3c	6.0c
4. Total nitrogen (%)	0.31d	2.92a	2.24bc	2.30bc	2.17c	2.30bc
5. Total phosphorus as P_2O_5 (%)	0.23d	2.10b	2.27a	1.65c	2.32a	1.86c
6. Total potassium as K_2O (%)	0.26d	1.35b	1.46b	1.08c	1.79a	1.27b
7. C/N ratio	160.6a	15.65c	19.67b	17.65b	18.70b	15.87c
8. Organic carbon (%)	49.79b	45.70b	44.05b	40.70c	69.98a	72.25a

*Growing 1 = fermented 100% SMW+NPK, growing 2 = SMW+coco-coir (1:1), growing 3 = SMW+rice husk (1:1), growing media 4 = Mixed and fermented of SMW+coco-coir (1:1), and growing media 5 = mixed and fermented of SMW+rice husk (1:1).

**Mean with the same letter within the same row are not significantly different ($P < 0.05$) according to LSD test.

CONCLUSION

In conclusion, *Pleurotus sajor caju* waste has the potential to become a growing media for vegetable production without fertilizer feed. The fermented 100% SMW + NPK is most suitable for the production of lettuce in this study. The lettuce can grow through the harvest without fertilizer but they use the macronutrient in the growing media. More study should be applied to improve the fermentation formula with essential nutrient elements.

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AG15

Dihaploidization of Anther and Ovary Cultures of Rock Melon (*Cucumis Melo* L.) as the Basis for Production of Hybrid Cultivars

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ABSTRACT

Melons are usually consumed during summer or hot weather. In Malaysia, many hybrid rock melon seeds are purchased and imported from foreign lands for their excellent quality and traits. A lot of money is spent for that purpose as more seeds must be purchased for the next plantation since the seeds obtained from the fruits of hybrid seeds cannot retained the same quality as the mother plants. In fact, the imported seeds especially F1 heterozygous seeds can only be grown for one generation as they will undergo segregation if grown for the next season. Plus, the price of hybrid seed is not encouraged and may increase the expenditure of input. The production of own hybrid cultivars of rock melon is essential in long term agriculture industries in Malaysia, but, dihaplodization method can be conducted as a short-term alternative to obtain the seeds with excellent traits. This study is to attempt in producing genetic materials as basis in producing hybrid cultivars of rock melon in Malaysia by haploid generation (haploidization) by tissue culture followed by double haploid (dihaploidization) method by using colchicine. (The comparison between hybrid plants and dihaploid plants towards disease susceptibility might be considered as responding variables to verify the difference in traits quality).

Keywords: Rock melon, haploid, anther culture, ovary culture, dihaploid

INTRODUCTION

Melon is one of the most consumed tropical fruits worldwide because of its scrumptious and appetizing texture, color, and taste (Zainal et al., 2019). In Malaysia, the Glamour cultivar from *reticulatus* var. is suggested as one of the most popular rock melons where it is widely consumed by people from all over the country (Zainal et al., 2019). In Malaysia, many seeds for plantations, especially hybrids, are purchased and imported from foreign lands for their excellent quality and traits. The seed prices are expensive; thus, the problem arises when we need to purchase more seeds for the next plantation as the seeds obtained from the fruits of imported seeds cannot retained the same quality as the mother plants. In fact, the imported seeds especially F1 heterozygous seeds can only be grown for one generation, as they will undergo segregation if grown for the next season. In terms of rock melon, the Glamour cultivar of rock melon that are heterozygous hybrids from Japan can be included as the example. Since they can only be grown once, the superior fruit quality resides within those cultivars cannot be retained *via* normal propagation method. This cultivar exhibits superior quality over the inbred ones because it is F1 heterozygous that have the heterosis effect (hybrid vigor). To produce melons with the same quality, our country would have no choice but to purchase the seeds for the next plantation. Due to that particular reason, a lot of money is spent on the high seeds price and seed importation. Malaysia has great opportunities to develop and produce its own hybrid cultivars.

But then, depending on the conventional repeated selfing will not guarantee a fast-developed cultivar. The development of the inbred lines requires six to eight generations of self-fertilization or selfing and selection until the preferred level of homozygosity is obtained. This long process affects the release of the new cultivar on the commercial term as it might take more than ten years to do so from the time of the first cross (Chaikam et al., 2019). Therefore, the conventional repeated selfing application is less likely to be eligible to obtain new melon cultivars at a faster rate. Compared to conventional repeated selfing that needs eight generations, in vitro induction of haploid plants followed by chromosome doubling to produce dihaploid or double haploid (DH) plants is the fastest way in producing homozygous lines. Double haploid (DH) is produced from haploid but undergone artificial doubling to form homozygous diploid. In other words, a dihaploid contains two similar homologues, thus, the amount of the recombination genetic information is comparable to a backcross (Khan et al., 2015). In a backcross, researchers can transfer the desired gene from donor parent (DP) onto the recurrent parent (RP) that possesses a preferred genetic background (Vogel, 2009). Conveniently, the process might be skipped in double haploid (DH) method as it is expected that most of the excellent traits from the hybrid parents can be retained without further repeated selfing.

MATERIALS AND METHODS

Production of haploid rock melon lines from anther and ovary cultures

Anther culture: Male flower buds (10-15 mm in length) containing microspores at the middle to late uninucleate stages were collected from healthy mother plants of 35-45 days old. The male flower buds were first immersed in 70% (v/v) alcohol for 1 min, 2.5% (v/v) sodium hypochlorite solution for 5 min followed by rinsing with distilled water for several times. The anthers then were separated and placed in 100 x 15 mm petri dishes containing 20 ml of culture medium. Anther cultures were incubated at 25 ± 2 ℃ under 16 h photoperiod with a light source providing 60 μmol $m^{-2}s^{-1}$ light intensity. Calli, 30-35 days culture initiation, will be transferred onto fresh media for embryo induction. Callus and embryo induction media used in our experiments will be MS (Murashige and Skoog, 1962) supplemented with 3% sucrose, 6 g/l agar, growth regulators of 2,4-D (2.26 μM), BAP (4.44 μM) a kinetin (4.64 μM) for callus induction and with 0.54 μM α-naphthalene acetic acid (NAA) and 13.32 μM BAP for embryo induction on calli. Media will be adjusted to pH 5.7 prior to autoclaving at 121℃ for 20 min.

Ovary Culture: Unpollinated ovaries were harvested one day before anthesis. The ovaries were first immersed in 70% (v/v) alcohol for 1 min, 2.5% (v/v) sodium hypochlorite solution for 5 min followed by rinsing with distilled water for several times. Individuals' ovules were isolated from the ovaries and placed in 20 ml of culture medium. Cultures were incubated at 25 ± 2 ℃ under 16 h photoperiod with a light source providing 60 μmol $m^{-2}s^{-1}$ light intensity. Calli, 8 weeks after culture initiation, will be transferred onto fresh media for embryo induction. Callus and embryo induction media used in our experiments will be MS (Murashige and Skoog, 1962) supplemented with 3% sucrose and 6 g/l agar. The callus derived from ovules on medium supplemented with 1 μM BAP + 2 μM NAA were cultured on MS medium supplemented with 1.0, 2.0, 5.0 and 10 μM BAP/TDZ/KN/2-iP for shoot regeneration. Shoots regenerated from callus were cultured on MS medium supplemented with 0.5, 0.1 and 2.0 μM NAA/IAA/IBA for induction of roots. Media were adjusted to pH 5.7 prior to autoclaving at 121 ℃ for 20 min.

Production of rock melon dihaploid lines by colchine treatment

In vitro: Single nodes, shoot tips or axillary buds will be dissected aseptically and placed onto culture medium containing PGR and 500 mg/L colchicine (pre-dissolved in 2% DMSO and diluted in distilled water before being added to the medium). The culture containing explant will be shook at 100-150 RPM in an incubator at 27℃ for 18-36 hours. The explant then will be removed from medium, washed several times with distilled water and cultured on new medium containing PGRs (IAA (5μM), BAP (5μM), ABA (1μM) and AgNO3 (30μM)). Cultures will be kept in growth chamber at 27℃ under 16h photoperiod. Regenerated plantlets from the cultures will be transferred to new media (~3 weeks after the first culture). Plantlets with adequate root will be transferred to small pot of soilless medium and those with inadequate root will be cultured in rooting media containing IAA before being potted. Complete regenerated dihaploid plantlets will be transferred to greenhouse for acclimatization.

In vivo: 5–10 cm tip of apical stems or shoot tips of 30-40 cm length plants will be prepared by removing the leaves and tendrils to prevent the haploid shoot from growing. The prepared parts then will be wrapped with cotton pieces submerged in 1% colchicine solution, for an hour in the dark. The cotton pieces will be covered with aluminum foil; thus, moisture evaporation can be avoided. The treated parts will be rinsed with distilled water as soon as exposition time elapsed and the stems were then left to grow for a certain time; approximately until new leaves grown from the treated plant parts.

Screening of rock melon haploid and dihaploid lines

Flow cytometry and manual inspection will be used to identify the ploidy level of plants.

RESULTS AND DISCUSSION

It is expected that haploid plants can be generated via anther and ovary culture and dihaploid plants can be produced by applying colchicine as the anti-mitotic agent. Flow cytometry and manual inspection were expected to provide accurate result to determine ploidy level (haploid and dihaploid).

CONCLUSION

This study will provide beneficial information on the effectivity of the anther and ovary cultures in the production of haploid plants of *C. melo* and at the same time verifying the efficiency of in vitro and in vivo dihaploid plants production by chromosome doubling using colchicine treatments. This study also will provide a more comprehensive insight regarding the competency of dihaploid plants to be used as basic materials for producing hybrid cultivars of rock melon (Comparison towards disease susceptibility to be considered.

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AG16

Calcium Release Pattern of Calcium Amendments Applied to Strongly Acid Soils

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ABSTRACT

Ca-amendments are a well-known agricultural technique for maintaining soil nutrient levels and improving soil characteristics in order to support crop production. In a laboratory incubation experiment, the rate of Ca and the release of Ca, Mg, and K from hydrated lime, quicklime, calcium carbonate, and dolomite were studied. The Jeram and Bungor series of acid soils were tested. Ca-amendments were combined with the two soils at a rate of 20 g kg^{-1}, packed in leaching columns, and incubated wet at room temperature. For a total of 24 weeks, leaching with deionized water was performed every two weeks. Ca and Mg levels in leachates were determined. Calcium content in hydrated lime and quicklime is released more quickly than calcium content in dolomite. The quicker Ca release rate in hydrated lime might be attributed to its greater Ca content as compared to dolomite. The slower rate of Ca release in dolomite may be explained by its low Ca concentration, which is known to delay Ca release and therefore nutrient release. Throughout the incubation phase, all Ca-amendments released positive Ca. The release of cations from the Ca-amendments occurred in the following order: Ca>Mg. Throughout the incubation time, the cations were progressively released from the Ca-amendments materials.

Keywords: Calcium amendments, Magnesium, Acid Soil

INTRODUCTION

Calcium amendments can be used an ideal source of Ca nutrient source for plant growth. The importance of Ca as plant nutrients is more realized in acid soils than neutral soils. In acid soils, acid sensitive crops do not give high yields unless these are suitably fertilised with Ca (Verma, 1995). Soil Ca is a highly leachable nutrient compared to Mg, and greater amounts are often found in the subsoil than in the upper parts of the soil profile, especially in older and highly weathered soils. As the Ca-amendments on acid soils is reflected directly on pH, it is worthwhile to study its release pattern (Senthurpandian et al., 2009). Although many studies have been carried out on Ca releasing capacity of different soils (Cofie and Pleysier, 2004; Senthurpandian et al., 2009), the relevant information available on the oil palm growing soils are generally very much limited and particularly not available in the Ultisols of Malaysia. It is known that external application of fertilizers serves as the major source for Ca to meet the requirements of plants. Therefore, characterization of Ca-amendments and their pattern of release are important where nutrition is concerned. A knowledge of the Ca patterns as well as the release rate of essential plant nutrients in these amendments are necessary for efficient soil and crop management. In view of the foregoing, the study was conducted with the following objective to evaluate the Ca and Mg release patterns from the application of Ca-amendments when applied to strongly acid soils.

MATERIALS AND METHODS

Calcium release experiment

Calcium amendments and dolomite treatments were replicated three times and arranged in randomized completely block design in the laboratory. For control treatment, there was using soil-silica sand mixture. A second 5-mm glass wool pad was put on top of the soil mixture to prevent dispersion and to reduce interference during leaching. To hold the final mixture during the incubation phase, glass leaching tubes (3.5 cm diameter and 9.5 cm length) coated with 5 mm of glass wool pad and covered with a thin coating of silica sand were employed. To increase soil aeration during incubation, the Ca-treated soil was combined with 20 g of acid-washed silica sand. The various Ca-additions were thoroughly mixed with 20 grammes of air-dried soil to yield an equal rate of 20 g kg^{-1} dry soil. Leaching was accomplished by carefully flowing 100 ml of deionized water through the test tube in 5 increments. The leaching procedure was then repeated every two weeks for a total of 24 weeks. Leaching was accomplished by carefully flowing 100 ml of deionized water through the test tube in 5 increments. Extractions were performed on soil samples. An atomic absorption spectrophotometer was used to determine the calcium concentration in the filtrate (GBC model 908AA GBC INC, Australia). 5 g of soil was placed in wide mouth plastic bottles, to which 25 mL of 1 N ammonium acetate was added (Hanway and Heidal, 1952), maintained over horizontal shaker for 5 minutes, and filtered using Whatman No. 1 filter paper. Following the collection of the filtrate, 25 mL of 1 N ammonium acetate was added to the same soil solution for the second extraction. The Ca release experiment leachates were also tested for Ca concentrations to determine the rate of release of these nutrients from the impacts of Ca materials. The percentage of nutrients released was calculated using the same formula as:

$$\frac{\text{Ca released in treated soil} - \text{Ca released in control}}{\text{Ca added in amendments}} \times 100$$

RESULTS AND DISCUSSION

Calcium release

Figures 1A and 1B show the amounts of Ca released biweekly in the Bungor and Jeram soils treated with Ca-amendments. In the Jeram soil, hydrated lime and quicklime exhibited similar patterns of Ca release, characterized by a gradually declining amount of Ca released from the start of incubation up to the 16th week followed by a steady release towards the end of the incubation period. In the Bungor soil, the amount of Ca released from the hydrated lime declined sharply from the start of incubation until the 8th week and then followed by a steady release up to the 24th week. Quicklime Ca, on the other hand, was released continuously from the start of incubation up to the 10th week. After a sharp decline on the 12th week, it then followed a release pattern almost identical to that of hydrated lime Ca towards the end of the incubation period. Calcium from the Ca-amendments exhibited a gradual but steady release during the entire incubation period in both soils.

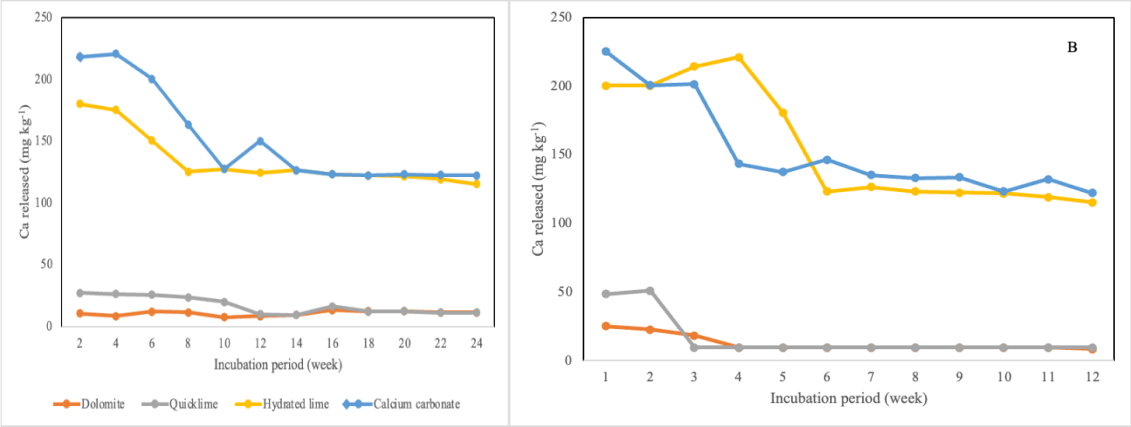

Figure 1. Net amounts of Ca released bi-weekly in A) Jeram and B) Bungor soil treated with Ca-amendments in relation to incubation time.

Figures in parentheses 1A and 1B are the percent of Ca released from Ca-amendments treated soil, expressed as net amount of Ca per total Ca in Ca-amendments. Relative to their original Ca content, however, quicklime had the highest percentage (50.3%) of Ca released in the Jeram soil and hydrated lime (51.4%) in the Bungor soil. Dolomite had the lowest percentage of Ca released in both soils. The fast release rate of Ca in Ca-amendments may be explained when Ca content reflected directly on pH (Senthurpandian et al., 2009). This could be because the both of Bungor and Jeram soil are strongly acidic and hence the Ca released would be greater (Verma and Palani, 1997; Wang et al., 2004).

CONCLUSION

Results of the study indicated that all the Ca-amendments showed net Ca release. Calcium in the hydrated lime was released faster than in the dolomite. Possible reasons for the slower rate of Ca released in the dolomite could be the lower Ca concentration. The gradual and steady release of nutrients in the decomposing Ca-amendments suggests that they could be successfully used as supplements to inorganic fertilizers in supplying nutrients to growing crops.

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AG18

The Effect of *Blaptica dubia* on *Oreochromis* sp. Growth

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ABSTRACT

High demand on both animal-based and plant-based proteins in fish feed have inexorably led to the overuse of natural resources and the subsequent price fluctuation in fish and feed production. The Food and Agriculture Organization of the United Nations (FAO) recommended insects as a substitute to protein source in livestock feed (Van Huis et al., 2013). In this experiment, red tilapia or *Oreochromis* sp. has been used as an animal model. *Oreochromis* sp. fingerlings were purchased from a private hatchery farm. All fish were acclimatized to the laboratory condition for seven days before the experiment began. there are five different treatments in this study with five different combinations of Dubia cockroaches every diet, *Balaptica dubia* and commercial pellet, namely T1 (60% *B. dubia* + 40% commercial pellet), T2 (70% of *B. dubia* + 30% commercial pellet), T3 (80% of *B. dubia* + 20% commercial pellet), T4 (90% of *B. dubia* + 10% commercial pellet) and T5 (100% of *B. dubia*). The control group of fish was fed with commercial pellets. Survival and growth rate of experimental fish were monitored weekly for continuous fifteen weeks. The present study showed the result that there was significant difference ($p < 0.05$) among the treatment diets towards the growth of red hybrid tilapia, where Treatment T5 showed the best result in terms of growth rate which is 12±1.18%.

Keywords: protein source, fish feed, *Oreochromis* sp.*, Balaptica dubia*, growth.

INTRODUCTION

Aquaculture production has been rapidly growing food producing sector over the last three decades and its growth is predicted to continue. With this growth comes high demand on protein for fish feed and competition for feed inputs, especially protein related, with other forms of livestock. Fish meal and soybean meal are the requisite ingredients found in most forms of fish feed as the source of protein. (Lee et al., 2017). However, high demand for both plants based and animal-based proteins in fish feed can lead to the overuse of natural resources and the subsequent price fluctuation in feed and fish production. The Food and Agriculture Organization of the United Nations (FAO) recommended insects as an alternative protein source in livestock feed (Van Huis et al., 2013). In this circumstance, insects have good potential meeting the rising demand in meat products and replacing fishmeal because they are a reliable source of protein, vitamins, high digestibility, and they are part of the natural diet of fish, human poultry and pigs (Mj et al., 2017).

MATERIALS AND METHODS

Fish tank set up

Red hybrid tilapia fingerlings were purchased from a private hatchery farm. All fish were acclimatized to the laboratory condition for seven days before the experiment started. The fingerling fish were fed with commercial diets two times per day at the rate of 2% of the total body weight and the palatability of the feed was recorded. The fish tanks were equipped with aeration and water exchange was carried out twice a week. Growth rates were monitored and recorded weekly. The initial weights of *Oreochromis* sp. fingerlings ranged from 1.2g to 1.5 g. Water quality parameters of each fish tank were monitored using multiparameter sonde (YSI, USA). The temperature of experimental tanks were maintained at 25-28 °C, pH of water at 6.0 - 8.5 and dissolved oxygen ranged from 5 to 7 mg/L (Lee, et al., 2017).

Preparation of experimental diet

This experiment consisted of five different combinations of Dubia cockroach, *B. dubia* and commercial pellet, namely T1 (60% *B. dubia* + 40% commercial pellet), T2 (70% of *B. dubia* + 30% commercial pellet), T3 (80% of *B. dubia* + 20% commercial pellet), T4 (90% of *B. dubia* + 10% commercial pellet) and T5 (100% of *B. dubia*), each treatment has three replicates, while the control group of fish was fed with commercial pellet. Fifty fingerlings of tilapia fish or *Oreochromis* sp. with average initial weight of 1.427±0.037 g was put on feeding trials in respective tanks with approximately 25 L of water volume, for a period of 100 days. Growth rates of experimental fish were monitored weekly for continuous fifteen weeks.

Statistical analysis

The results were statistically analyzed and presented as mean ± standard error by using One-Way Analysis of Variance (ANOVA) test and followed by Tukey Post Hoc to determine the significant differences in mean ($p < 0.05$) using Statistical Package for the Social Sciences (SPSS) 16.0.

RESULTS AND DISCUSSION

The growing value and restricted sources of raw material to produce fish feed has emerged as a major constraint to the development of the aquaculture industry as one of the food security tools to generate nutrition for humans. As a result, scientists are advised to discover opportunity protein supply for aquaculture in preference to dependence on fish meal to formulate fish feed. Hence, this study was carried out to compare the feasibility of *B. dubia* meal as alternative feed for fish meal in tilapia farming. From the present study, the highest growth rate was recorded from T5 with mean and standard deviation of final weight were 44.39±8.769 where the lowest growth rate was T2 with mean and standard deviation of final weight were 25.427±3.750.

Table 1: Growth and survival rate of tilapia fingerlings that received five different treatments

Treatment	Initial weight (g)	Final weight (g)	Survival rate (%)	Growth rate (%)
C	1.37±0.157	24.327±3.624	63.33±1	8.2±0.3
T1	1.263±0.143	28.76±4.994	67.78±0.833	8.3±0.31
T2	1.283±0.162	25.427±3.750	62.22±0.972	8.6±0.43
T3	1.457±0.148	31.163±1.224	60±1.12	9.1±0.4
T4	1.45±0.113	34.75±1.051	47.78±0.972	10.1±0.44
T5	1.423±0.190	44.39±8.769	43.33±1	12±1.18

T1 (60% *B. dubia* + 40% commercial pellet), T2 (70% of *B. dubia* + 30% commercial pellet), T3 (80% of *B. dubia* + 20% commercial pellet), T4 (90% of *B. dubia* + 10% commercial pellet) and T5 (100% of *B. dubia*)

This research was the first attempt on the application of *B. dubia* meal and commercial pellet combination to feed red hybrid tilapia, *Oreochromis sp.* fingerlings. From this study, the growth result showed that the highest increasing weight was T5 among all the treatments including control. Partial replacement of protein source in fish feed is possible as shown by the results in present study, where T1 which consisted of 60% *B. dubia* meal and 40% commercial pellet in feed formulation was found to have a higher survival rate

compared to other treatment groups. However, in the present study, the *Oreochromis sp* fingerlings were notably showing stunted growth when fed with a formulation diet consisting of less than 80% house *B. dubia* meal.

CONCLUSION

Insects are a sustainable source of protein for animals and for human consumption. The present study revealed that, formulated feed using *B. dubia* in high amounts (>60%) may give harmful effect to the red hybrid tilapia fingerling on the liver and growth performance. Hence, by using *B. dubia* as a full replacement of other protein sources not recommended. The use of *B. dubia* as fish feed will need further study to evade this problem.

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AG20

Response on Vegetative Growth and Yield of Butternut Squash (*Cucurbita moschata*) Cultivar Waltham under Different Bokashi Dosage

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ABSTRACT

Malaysia's population is growing year after year. Even though Malaysia has a rich biodiversity, Malaysia still imports food to support our communities. Vegetables, meat, and fruits are popular imported foods. The vegetables still imported from other countries to support demand in Malaysia such as onion, garlic, broccoli, mushrooms, okra, snake beans, potatoes, tomatoes, and chilies. Butternut squash is one of the new imported vegetables. Malaysians are beginning to accept butternut squash as added food in the menu. Butternut squash were cooked in varieties of recipes in Malaysia such as boiled, baked, steamed, 'masak lemak,' and 'sira.' Butternut squash cultivation was cultivated in Indonesia since 2013. Therefore, we can expect that the butternut squash could be grow in Malaysia because the season in Malaysia is the same as the equator. However, there are lack of knowledge about the cultivation of this crop in Malaysia. So, the aim of this study was to learn about the butternut squash response in vegetative growth and yield by using Waltham with different dosages of bokashi. The study was carried out in Universiti Malaysia Kelantan Jeli Campus, Malaysia. The factorial Randomized Completely Block Design (RCBD) was used in three replications for the study. The different dosages were used such as fertilizer of 0 kg/ha, 4000 kg/ha of bokashi, 8000 kg/ha of bokashi, and 12 000 kg/ha of compost. Then, the parameter was observed such as plant height, number of leaves, width of leaves, length of leaves and yield. The application of different bokashi dosages and Waltham had a significant impact on vegetative growth and yield. Waltham with an application rate of 8000 kg/ha produced the high plant growth and yield. This experiment could provide knowledge to assist farmer in cultivating butternut squash by using effective dosage of fertilizer to get high production.

Keywords: Bokashi, butternut squash, Cucurbita moschata, growth, yield

INTRODUCTION

The Ministry of Agriculture (MOA, 2015) mentioned that the vegetable production in Malaysia has steadily increased each year such as 623 tons (2009), 872 tons (2010), 938 tons (2011), 974 tons (2012), 1,434 tons (2013), and 1,439 tons (2014) however Malaysia still imports these vegetables to meet population needs. Vegetables imported from other countries include onion, garlic, broccoli, mushrooms, okra, snake beans, potatoes, tomatoes, and chillies. Butternut squash is also one of the vegetables imported from other countries, particularly Australia. Since 2013, butternut squash has been cultivated in Indonesia. Moreover, the climate in Indonesia and Malaysia is close to the Equator. So, we expected that butternut squash would be able to be cultivated in Malaysia. In Kelantan, there was a large amount of idle land with 5, 494 hectares. Pahang has the idle land (34,293.27 hectares), followed by Johor with 17,855 hectares, Perak with 14,507 hectares, Terengganu with 12,309 hectares, and Negeri Sembilan with 10,309 hectares. These lands had low soil fertility and needed to be improved. Soil improvers with bokashi may be a viable alternative for low-

income farmers who own degraded farmlands. Wijayonto *et al.*, (2016) mentioned that the bokashi fertilizers made from various types of agricultural material could improve soil chemical properties and increase crop yield.

MATERIAL AND METHOD

This study was carried out at the Agro Techno Park, Universiti Malaysia Kelantan Jeli Campus, Kelantan, Malaysia. The experiment used Randomized Completely Block Design. The cultivar Waltham of butternut squash was used in this experiment. There were four-level fertilizer dosages (B1 = 0 kg/ha, B2 = 4000 kg/ha, B3 = 8000 kg/ha, and B4 = 12 000 kg/ha of compost). The planting beds were prepared, with a size of 100 cm x 400 cm and a total of 8 plants. The chemical fertilizers by using 400 kg/ha NPK and 150 kg/ha urea, were applied to all treatments. Then, the application of 0 kg/ha, 4 000 kg/ha, 8 000 kg/ha and 12 000 kg/ha of compost were applied. The planting bed was covered with silver shine after all fertilizers were applied. Seed sowing was done in the tray. After two weeks, the seedling was transferred to the planting bed. The trellis was established. The planting distance was 50 cm x 100 cm. When the fruit colour changed from green to creamy yellow, the butternut squash was harvested. The weight of butternut squash was measured and recorded. Besides that, the parameters of vegetative growth and yield were also measured. The vegetative growth measured was such as plant height, number of leaves, length of leaves and width of leaves. The plant height of a butternut squash was measured from the stem's starting point above soil to the shoot tip. Plant height was measured from the first to the eighth weeks. Number of leaves were counted by the total number of leaves per plant. The number of leaves was determined by counting the leaves that sprouted from the stem above the soil level until they reached the shoot tip. From week one to week six, the number of leaves was counted and recorded. The other vegetative such as width of leaves and length of leaves were measured by randomly choosing three biggest leaves from each plant. Finally, the yield was determined by total weight of butternut squash per plant. Data obtained were analyzed by using Statistical Package for the Social Science (IBM SPSS) version 23 by using Tukey's test ($P<0.05$) to separate the means of plant height, leaf number, length of leaves, width of leaves and yield.

RESULT AND DISCUSSION

Table 1. Mean Vegetative Growth and Yield of Waltham

Dosage of Bokashi	Plant Height (cm)	Number of Leaves	Length of Leaves (cm)	Width of Leaves(cm)	Yield per Plant (g)
0 kg/ha	234.73 ± 12.99^{b}	62.0 ± 8.60^{b}	26.53 ± 1.49^{a}	33.8 ± 2.17^{a}	849.25 ± 157.37^{b}
4000 kg/ha	325.88 ± 22.14^{a}	85.75 ± 13.07^{a}	25.79 ± 1.45^{a}	33.0 ± 2.29^{a}	1106 ± 266.20^{a}
8000 kg/ha	353.10 ± 31.79^{a}	86.25 ± 3.86^{a}	27.0 ± 1.47^{a}	34.22 ± 2.71^{a}	1316.50 ± 449.12^{a}
12 000 kg/ha of compost	251.18 ± 37.52^{b}	82.75 ± 10.81^{a}	24.22 ± 2.62^{a}	29.63 ± 5.28^{b}	934.25 ± 405.63^{b}

From the result in Table 1, there were significant differences between plant height, number of leaves, width of leaves and yield. Meanwhile, the length of leaves was not significant. Dosage of bokashi with 8 000 kg/ha were higher in plant height, number of leaves, width of leaves and yield. Meanwhile, dosage of bokashi with 0 kg/ha was lowest in plant height, number of leaves and yield per plant. Besides that, dosage of compost with 12 000 kg/ha were lowest in length of leaves and width of leaves. Increased plant height is related to increased nutrient use by plants (Baldotto & Baldotto, 2016). There was little variation in plant growth in the absence of bokashi. Furthermore, the addition of bokashi resulted in an increase of favourable microorganisms in the substrate, which may have accelerated the cycling and availability of nutrients. Because bokashi contains nitrogen fixers, the availability of N and other nutrients increases (Álvarez-Solís et al., 2016). The use of bokashi is very effective in improving plant vegetative growth. Bokashi significantly increased plant growth and development (Wijayanto et al., 2016). Waltham achieved the highest yield with an application of 8000 kg/ha of bokashi. Pei-Sheng and Hui-Lian, 2002 reported that using bokashi fertiliser

resulted in increased photosynthetic rate, transpiration rate, and mesophyll conductance. As a result, bokashi fertiliser increased yield per plant significantly (Pei-Sheng & Hui-Lian, 2002).

CONCLUSION

The butternut squash of Waltham was able to grow in the climate condition of Malaysia particularly in Jeli area. Then, the bokashi 8000 kg/ha gives higher plant growth such as plant height, number of leaves, length of leaves and width of leaves. Besides that, the result also showed that by using 8 000 kg/ha resulted in higher yield of Waltham.

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AG21

Isolation and Characterization of *Pediococcus pentosaceus* HLV1 from Idly Batter, a Fermented Food of South India

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ABSTRACT

The present study was aimed to isolate and characterize thermolabile *Pediococcus pentosaceus* from idly batter. From 10 idly batter samples totally of 50 lactic acid bacteria were isolated. Ten strains were selected for further evaluation through the screening process finally 1 isolate performing well at above 35º C was selected for further characterization. The selected culture was studied for its phenotypic and physiological characters. The resulted sequence was confirmed as *Pediococcus pentosaceus* by NCBI-BLAST analysis. Sequence analysis of the present bacteria showed a maximum identity of 99% and above with *Pediococcus pentosaceus* Ni1142, at both nucleotide and amino acid levels. The culture was named *Pediococcus pentosaceus* HLV1 and submitted to NCBI Genbank (Accession number MH921241). The isolated strain can utilize a wide range of carbohydrate substrates including glucose, fructose, sucrose, lactose, maltose and xylose. The major fermentation products from glucose, lactic acid. The optimal fermentation parameters were identified through one factor one-time experiments. The isolated culture optimally grows and produces lactic acid with glucose as carbon source, yeast extract as nitrogen source, at pH 7 and 40º C temperature. Using the above optimal conditions lactic acid from with agro-industrial waste, mango peel was studied and at the end of the fermentation 8% (v/v) of lactic acid was produced.

Keywords: Isolation of LAB; idly batter; optimization; mango peel fermentation

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INTRODUCTION

Lactic acid (LA) is one of the 12 most promising value-added building blocks that can be derived from the microbial fermentation process and can be used for the production of various commodity and speciality chemicals (DOE, 2004). Lactic acid (2-hydroxypropanoic acid, CH3-CH(OH)-COOH) is a natural organic acid widely distributed in nature. Production of optically pure D- or L-lactic acid can be possible only by the selection of appropriate microorganisms. The global lactic acid market size was valued at USD 2.22 billion in 2017 and is estimated to reach USD 8.77 billion by 2025 with 18.7% growth annually.

Lactic acid bacteria have been isolated from a variety of sources. In most fermented foods, especially foods of/in India, the fermentation is connected with cereals and legumes by Lactic Acid Bacteria (LAB). Idly batter is a combination of dehulled black gram and milled/powdered rice, the preparation is different in various regions of South India mainly, the ratio/measure/measurement of rice and black gram, also the time of soaking and fermentation of the batter. In the leavening process of batter and acid production, heterofermentative Leuconostoc meseteroids and homofermentative *Streptococcus faecalis* and probiotic *Lactobacillus Plantarum*, *Lactobacillus lactis* and *Pediococcus pentosaceus* are major lactobacillus candidate organisms that present in batter. Various studies carried on lactic production from different agro-industrial residues like sugarcane bagasse, date juice and coffee mucilage. Mango peel proved as one of the cheap feedstocks

for ethanol and butanol production with a good amount of fermentable sugars (Reddy et al., 2019). Reports on utilization of mango peel for LA are very scarce. Hence in the present study, it was used for the LA production by the isolated LAB.

MATERIALS AND METHODS

Isolation and molecular identification of lactic acid bacteria

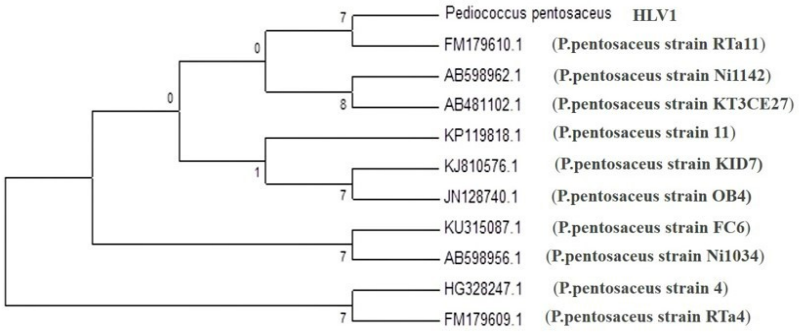
For the isolation of LAB, 1gm/1ml of idly batter was taken and serially diluted using sterile distilled water. These diluted samples were plated using the spread plate technique on deMan Rogosa Sharpe (MRS) agar plates and incubated at 37° C for 24hrs. The colonies were identified based on Gram staining, size, shape, and motility of the organism by using a microscope (Leica, Mumbai, India). The physiological characteristics were determined to depend on different biochemical tests such as catalase, oxidase, hydrogen sulfide(H_2S), indole, urease, methyl red, and Voges Proskauer, the results were confirmed with Bergey's manual (Cato et al., 1986). For the molecular identification and characterization fresh cultures were prepared and from fully grown culture DNA was isolated. The 16S rRNA gene was amplified using primers of forward: 27F (5-AGAGTTTGATCCTGGCTCAG-3) and reverse: 1492R (5-GGTTACCTTGTTACGACTT-3) for the amplification. BLAST and multiple alignment program Clustal W software were used for the data sequence analysis. The phylogenetic tree was constructed by using the MEGA 7 software (Reddy et al.,2019).

Lactic acid production from mango peel

Mango peel was procured from the local mango pulp industry (Varsha Fruit Pulp Industries Ltd, Koduru, Kadapa, India). It was macerated using a mixer (Panasonic mixer, Model:340) the liquid containing sugars were extracted with the help of cheesecloth by squeezing after enzymatic digestion with 1% (v/v) pectinase, Trizyme 50 (Triton Chemicals, Mysore, India). The extraction medium pH was adjusted to 7.0 and was supplemented with peptone (0.3%) to know the effect of nutrients on fermentation and unsupplemented medium acted as the control (Reddy et al., 2011). The cell growth was measured using a UV spectrophotometer at 600 nm. Lactic acid was determined using high-performance liquid chromatography (HPLC).

RESULTS AND DISCUSSION

For the isolation of lactic acid bacteria samples were prepared from overnight fermented idly batter. Total 38 isolates of lactic acid-producing bacteria were selected and performed Gram's staining. The results of biochemical tests methyl red were positive and remain oxidase, hydrogen sulfide, indole, urease, and Voges Proskauer were negative. From those 6 isolates which have more clear zones with bromocresol purple, $CaCO_3$ were selected for further screening and characterization. All 6 isolated cultures were gram-positive, cocci, arranged in tetrads and pairs and catalase-negative. Most of the investigators have used fermented batters, fruits, vegetables and milk products for the isolation of lactic acid producing microorganisms. Mostly lactic acid bacteria are non-motile, cocci or rod in shape homo or heterofermentative and catalase negative. The selected culture was subjected to 16S rRNA gene sequencing analysis. The DNA of the isolated strain was subjected to PCR amplification of 16S rRNA and a 649 bp nucleotide sequence was obtained. The obtained sequences were analyzed by comparison with the Gene Bank Database. The isolated culture was showed 99% similarity with strain *P. pentosaceus* Ni1142 (Accession no. of AB598962.1) in BLAST analysis. The isolated culture was named as *Pediococcus pentosaceus* HLV1 and the sequence was submitted to NCBI Databank and the Accession number is MH921241.1.



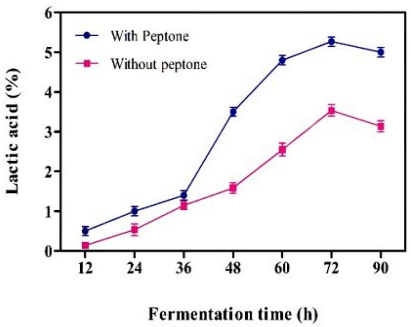


Figure 1. Phylogenetic tree derived from neighbour-joining analysis **Figure 2.** Lactic acid production from mango peel

Lactic acid production from mango industrial waste

Lactic acid production from fresh mango peel was carried with a newly isolated LAB strain. The fresh mango peel contained 25% total solids and 7.2% reducing sugars. At the end of fermentation 5.2%(v/v) LA was produced in peptone supplemented mango peel extract medium and 3.2% in an un-supplemented medium. This shows the isolated strain requires an additional nitrogen source for the effective utilization and production of LA from mango peel. The nutrient supplement would provide required nutrients that overcome nutrient deficiencies and produce more fermentation metabolites by keeping the microorganism in the log phase for longer periods. The clear idea on influence of nutrient supplementation required more experiments with different nutrients in different amounts and in different feeding methods. In a nutrient unsupplemented medium the fermentation and utilization of carbohydrates are slow because of insufficient growth nutrients in the peel medium. Jawad et al (2012) investigated the production of LA from Mango peel and reported a very low yield, 17.4 g/l through the spontaneous anaerobic fermentation with indigenous microbial consortium and with direct fermentation of blended mango peel.

CONCLUSION

Lactic acid producing bacteria was successfully isolated and based on 16S rRNA gene sequencing, the isolates were indicated 99% homology to *P. pentosaceus* and named as *P. pentosaceus* strain HLV1. The isolated strain showed better growth and lA production with glucose as carbon source, peptone as nitrogen source, initial medium pH 7.0 and at 40^{o}C. The isolated strain was effectively inhibited by both Gram-positive and Gram-negative bacteria in a preliminary evaluation. Mango peel was effectively utilized and produced 5.2% of lactic acid by the supplementation of peptone. In conclusion, the results indicate that the isolated LAB, *P. pentosaceus* strain HLV1 is able to grow and produce LA at high temperature (45 ^{o}C) and have survivability at 50 ^{o}C with utilization of mango peel like agro-industrial biomass for lactic acid production.

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Scope
Animal Science (AS)

AS04

High-Tech Herbal Medicine: Sustainable Ruminant Formula for the Future

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ABSTRACT

Recent breeding advancements in estrus synchronization offer the farmers the valuable potential to capture the economic benefit through manipulation of the length of the estrus cycle. Estrus synchronization with Control Internal Drug Release (CIDR) has been employed commercially in the dairy and beef industry. Nevertheless, herbal treatment has gained prominence among the producers, considering its low cost and therapeutic properties. Therefore, the objectives of the present study were (1) to measure the expression of estrus signs in female goats (2) to identify the follicle size in each treatment. Twenty-four non-pregnant female goats (n=24) were randomly assigned into three estrus synchronization protocols and primed with Treatment A (Herbs; n=8), feeding herbs with TMR for seven consecutive days. Treatment B (Herbs+Vitamin E), the female goats were injected once with Vitamin E prior to the onset of feeding herbs with TMR for seven days. Treatment C (CIDR; n=8), an intravaginal device containing 0.3 g progesterone were inserted for ten days. All the female goats were exposed to buck for estrus detection immediately after treatment via visual observation four times daily. Simultaneously, the follicle pattern was confirmed with ultrasound scanning after 24 h onset of treatment and repeated every 48 h until ovulation. The study revealed that the herbs + vitamin E group (Treatment B) had the highest ($P>0.05$) occurrence of estrus signs at 72.92% than in herbs (Treatment A) and CIDR (Treatment C) at 41.67% and 64.58%, respectively. There was no significant effect ($P>0.05$) between ovarian follicular diameter and days to ovulation. The mean diameters of the ovulatory follicles observed in herbs+vitamin E were the highest at 9.0 mm compared to herbs 8.50 mm and CIDR 8.76 mm. Lastly, the study demonstrates that the novel approach of herbs + vitamin E is the most efficient tool for eliciting estrus, while herbal supplement offers a novel approach for effective estrus synchronization.

Keywords: Estrus synchronization, follicle diameter, corpora lutea, ultrasonography

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INTRODUCTION

The livestock industry has been an integral part of Malaysia agricultural production system and plays a critical role in socio-economic development. However, the challenge in breeding has garnered worldwide attention due to poor fertility rates in female goats having devastating effects on livestock production. To address the urgent and pertinent issues on meat sustainability, the Malaysian Government was introduced the National Agrofood Policy 2021-2030 (DAN 2.0). Previously, controlled internal drug release (CIDR) has been prevailing in fostering reproductive schemes by synchronizing luteinizing hormone (LH) pulse

frequency and ovulation. To date, there has been a rapid turnover into herbal formulations as sources of fertility-enhancing properties for estrus detection and conception rate in animals. Therefore, the objectives of the present study were to measure the expression of estrus signs in female goats and identify the follicle size in each treatment.

MATERIALS AND METHODS

Location, animals and their management

This study has been conducted at the animal farm, Kaprima Hulu Seladang Valley Farm, located at Setiu, Terengganu. Twenty-four female Boer cross goats with good body condition scores were assigned randomly into three treatments: Treatment A (Herbs; n=8), feeding herbal supplementation with TMR would take seven days continuously. Treatment B (Herbs+Vitamin E), the female goats were synchronized with injection Vitamin E prior to the onset of feeding herbs with total mixed ration (TMR) for seven days continuously. Treatment C (CIDR; n=8), the goats were performed using CIDR, were implemented on Day 0 and removed on Day 10. All the goats were observed for the behavioural manifestation of estrus signs for 45 minutes four times daily. Consequently, the size of the follicle was identified starting 24 hours after treatment onset and repeated every 48 hours until ovulation using a real-time B-mode ultrasound DP-10 scanner.

Scoring of estrus signs

Different behavioural signs observed during estrus were assigned with a score depending upon their frequency or intensity of expression, as opposed by Dash (1980). The total score for each animal observed was classified into three classes weak (1–11), moderate (12–22) and intense (23–33).

Outcome measure and analysis

Statistical analyses were performed using the statistical software package IBM SPSS Statistics for Windows, Version 25.0. The size of follicles and estrus response was measured using the Chi-square test at $P<0.05$.

RESULTS AND DISCUSSION

Manifestation of estrus signs

The first novel finding of this study observed no significant differences in the manifestation of estrus signs between each treatment ($p>0.05$). Initially, the estrus response exhibited by the female goats in treatment herbs+vitamin E was the highest at 72.92% compared to treatment herbs and CIDR at 41.67% and 64.58%, respectively (Table 1). These findings further support the notion of Parivzi and Ellendorff (1982) notion that the cinnamaldehyde derived from cinnamon improves norepinephrine that can increase the release of nitric oxide to stimulate the hypothalamic axis and activate the gonadotropin hormone (GnRH).

Table 1. The total percentage of Boer goats exhibit a degree of estrus signs

Estrus signs	Degree of expression (%)												Chi-square (X^2)	Sig.
	Herbs				Herbs+Vitamin E				CIDR					
	W	M	I	Total	W	M	I	Total	W	M	I	Total		
Stand heat	25.00	8.33	66.67	12.5	29.17	18.75	52.08	37.50	33.33	25.00	41.67	50.00	0.03	0.09
Mount	72.22	11.11	16.67	37.50	23.33	11.67	65.00	50.00	37.50	14.58	47.92	62.50	0.98	0.76
Tail wagging	5.00	11.67	83.33	62.50	2.38	10.71	86.90	87.50	10.71	11.90	77.38	100.00	0.53	0.94

Sniffing genitalia	61.1 1	13.8 9	25.0 0	37.5 0	33.3 3	19.4 4	47.2 2	50.0 0	43.7 5	8.33	47.9 2	37.5 0	0.22	0.80
Vulva swelling	10.4 2	14.5 8	75.0 0	50.0 0	8.33	14.2 9	77.3 8	62.5 0	5.95	9.52	84.5 2	87.5 0	0.95	0.86
Mucus discharge	16.6 7	18.7 5	64.5 8	50.0 0	3.13	6.25	90.6 3	100. 00	2.08	18.7 5	79.1 7	100. 00	0.32	0.83

w-weak, m-moderate, i-intense, %-percentage, sig-significant

As illustrated in Figure 1, the relation between ovarian follicular diameter and days to ovulation was insignificant, X^2 (1, N=24) = 0.24, p>0.05. The mean diameters of the ovulatory follicles observed in herbs+vitamin E, herbs and CIDR were 9.0 mm, 8.50 mm and 8.76 mm, respectively. The preovulatory follicle diameter in CIDR synchronized was larger than Bukar et al. (2010) in Boer goat at 7.61 mm.

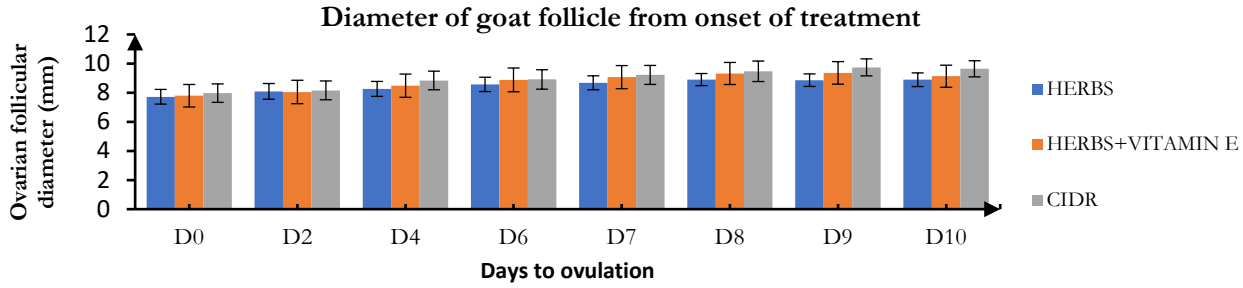

Figure 1. The diameter of Mean ± SEM of ovarian follicular diameter from the onset of treatment to days of ovulation

CONCLUSION

Among the three estrus synchronization protocols used in this study, the herbs+vitamin E had comparatively higher effectiveness in achieving satisfactory estrus signs and follicle size than the modified herbs and CIDR. Therefore, meticulous attention to detail in the modified herbs is crucial to create a novel opportunity in the estrus synchronization program.

ACKNOWLEDGEMENT

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AS05

Strategy Analysis on Small Ruminant Livestock Business in Pandemic Covid-19 (Case Study of HPDKI Farmers, Banyumas, Indonesia)

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ABSTRACT

Since the beginning of 2020, the world has been shocked by a deadly virus outbreak, namely the Corrona Virus-19 (covid-19), which originated from Wuhan, China. This virus continues to spread to various areas, including in Banyumas, Indonesia. This virus not only attacks public health but also has a negative impact on the economic sector. The policy issued by the government to mitigate the spread of the covid-19 virus, disrupt the course of business activities, and changes in market conditions is no exception in the livestock business sector. This research aims to determine the business strategy of small ruminants in HPDKI Banyumas farmers during the Covid-19 pandemic. There are three types of small ruminant businesses studied; sheep and goat breeding, sheep and goat fattening, and dairy goats. Each type of small ruminant business has a different impact. The primary data and present study were collected using various techniques such as survey methods, FGDs, questionnaires, and observations. This study consisted of farmers HPDKI Banyumas selected by the census as many as 38 farmers, policymakers, and experts in the livestock business. The following analysis tool uses IFE, EFE, IE, and SWOT matrix analysis. The results showed, during the pandemic, the dairy goat and fattening of goat and sheep business is in maintained and survived condition. The resulting generic strategies were market penetration and product development. This strategy requires farmers to maintain product quantity and penetrate existing markets. In comparison, the goat breeding business is in a harvest or divestment condition with a generic strategy of shrinking and divesting. This strategy requires farmers to minimize production costs and reduce some of their less productive assets**.**

Keywords: Goat, Sheep, Business, Strategy, Covid-19

INTRODUCTION

According to a report from the Organization for Economic Co-operation and Development (OECD) (2020), in general, the impact of the COVID-19 pandemic has disrupted industrial businesses in three ways; production supply chains, distribution, and market conditions. The livestock sector is affected by feed supply, slaughtering, meat processing, and distribution processes (FAO, 2020). Jannah (2021) The COVID-19 pandemic has increased the demand for fertilizer and goat's milk, but the demand for meat and livestock for fattening has decreased. Besides that, the concentrate price has increased, and farmers are short of tofu waste, usually used as feed ingredients. Therefore, this study aims to analyze alternative strategies for the sheep and goat business in HPDKI Banyumas farmers. HPDKI is Himpunan Peternak Domba Kambing Indonesia (The Indonesian Sheep and Goat Farmers Association). This association spread across various regions of Indonesia. In this study, the area studied is in Banyumas regency in Central Java, Indonesia.

MATERIALS AND METHODS

The primary data and present study were collected using various techniques such as survey methods, FGDs, questionnaires, and observations. The secondary data was obtained from the local Department of Animal Husbandry (Dinas Peternakan) and the Central Statistics Agency (Badan Pusat Statistik, BPS). This study involved three respondents consisting of HPDKI Banyumas Farmers who were determined by a census of 38 members, policymakers, and experts in the small ruminant livestock business. The data is processed and analyzed using Internal Factor Evaluation (IFE), External Factor Evaluation (EFE), Internal-External (IE) matrix to obtain the right alternative strategy, and SWOT matrix analysis for strategy development.

RESULTS AND DISCUSSION

The following is the result of matrix IE analysis which shows the strategic position of the three types of small ruminant business:

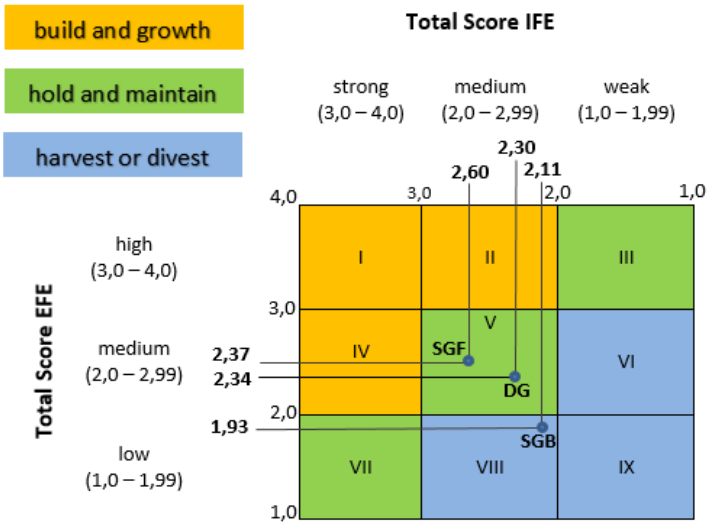

Figure 1. The result of Matrix IE. SGF: Sheep and goat fattening business, DG: Dairy goat business, SGB: Sheep and goat breeding business

The IFE score of the dairy goat business is 2.30, which describes being in a condition of stability in facing the weaknesses and strengths of the business they have. The total EFE score is 2.34, which illustrates that the dairy goat business faces opportunities and threats during this covid-19 pandemic in average strength. Based on the cell position in Figure 1, the IE matrix is in cell V, which means that the dairy goat business is in a state of holding and maintaining. So, the appropriate generic strategy for the penetration of the dairy goat business is market and product development. Meanwhile, the total score for the fattening business for sheep and goats is 2.60, while the result for the fattening business is 2.37. The matching results based on the IE matrix in Figure 1 show that the sheep and goat fattening business is in the same cell v as the dairy goat business with the same strategy category. Strategy development is done by using swot matrix analysis. Each type of business has different strengths, weaknesses, opportunities and threats, so the specific strategies produced vary.

The sheep and goat breeding business show an IFE score of 2.11 which means it is in a stable internal condition. Still, the EFE score shows a 1.93 result which illustrates that this business is in a weak response strength condition in facing the opportunities and threats in the COVID-19 pandemic. Based on the position of the cells in the matrix IE, the breeding of sheep and goats is in cell VIII, which means harvesting or divestment with the appropriate generic strategy is shrinking or divesting. The development of specific strategies for these three types of small ruminant livestock business is based on swot analysis. The swot analysis of each type depends on their business's opportunities, threats, strengths, and weaknesses.

CONCLUSION

These studies showed that the dairy goat business and fattening of sheep and goat business maintained and survived during the pandemic. The resulting generic strategies were market penetration and product development. This strategy requires farmers to maintain product quantity and penetrate existing markets. In contrast, the goat breeding business is in a harvest or divestment condition with a generic strategy of shrinking and divesting. This strategy requires farmers to minimize production costs and reduce some of their less productive assets.

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AS06

Effect of Guanidinoacetic Acid with Different Protein Levels on Growth Performance of Broiler Chicken

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ABSTRACT

Guanidinoacetic acid (GAA) is involved in the de novo synthesis of creatine. These reactions improve cellular bioenergetic for broiler muscle growth and performance. This study was conducted to evaluate the effect of GAA on broiler growth performance fed a low-protein diet. A total 1.176-day old chick unsexed broiler (Lohman Indian River strain) were randomly allocated to 2×3 factorial arrangement with seven replicate pens of 28 birds each. The dietary treatment consisted of two levels of protein (high and low protein in each growth phase) and three levels of GAA (0, 600, and 1200 g/ton). Results showed that GAA with different protein in pre-starter phase increased feed intake (FI) and feed conversion ratio (FCR), in starter phase increased FI and body weight (BW), and increased BW only in finisher phase ($P<0.05$). GAA level did not affect to FI, BW, FCR, (index performance) IP, depletion in pre-starter phase and starter phase, but in finisher phase reduced BW and IP ($P<0.05$). Low protein diet reduced FI and FCR also increased BW and IP in pre-starter phase, starter phase ($P<0.05$). But in finisher phase low protein diet reduced FI, BW, and IP but increased FCR ($P<0.05$). Supplementation GAA with different protein levels increased FI and decreased FCR in the pre-starter phase, increased BW in the starter phase and finisher phase.

Keywords: Broiler Chickens, Creatine, Guanidinoacetic Acid, Performance, Protein Level

INTRODUCTION

Broiler chicken makes a very large contribution as meat producing livestock. Every year chicken meat always increases, consumption of broiler meat has increased per capita per year in 2016 (5.1 kg), in 2017 (5.7 kg), in 2018 (5.6 kg), in 2019 (5.7 kg) and in 2020 reaching 6.0 kg (Kementan RI, 2020). Feed is the main contributor to economic and environmental costs of poultry production schemes. Currently, broiler feeding startegies wold be better and more appropriate if they provide nutrients to feed that not only to support their growth and development but also reduce the cost and environmental impact of overfeeding. It can be overcome by replacing high protein feed with amino acids (Lysine, Methionine, Threonine) which are currently considered more economical and feasible in several respects, so the search for most influential amino acids is still being sought (Dilger et al., 2013). GAA is formed when glycine receiver a guanidine group from the amino acid L-arginine in a biochemical reaction catalysed by L-Arg:Gly-aminidinotransferase. This initial reaction mainly takes place in the kidney. In the second reaction that takes place in the liver, GAA is methylated by S-adenosyl methionine and is converted into creatin. The role of GAA and its relationship with arginine was reviewed in order to define a replacement ratio between GAA and arginine for broiler diet formulation, the ratio being of how much arginine could be spared or replaced by GAA. Dilger et al. (2013) reported that GAA can be used as an effective substitute for arginine in poultry

feed. In this study, aims to determine the effect of GAA with different protein levels on growth performance of broiler chicken in each phase.

MATERIALS AND METHODS

The materials used in this study were basal rations, experimental rations with pre starter phase using 23% and 21% CP, starter phase using 21% and 19% CP, finisher phase using 19% and 17% CP. Each added guanidino acetic acid (GAA) 0 g/ton, 600 g/ton and 1200 g/ton. This study was conducted to evaluate the effect of GAA on broiler growth performance fed a low-protein diet. A total 1.176-day old chick unsexed broiler (Lohman Indian River strain). The birds were given different treatments for each phase. Data were analyzed using analysis of variance (ANNOVA) followed Duncan's test to determine the difference between mean values.

RESULTS AND DISCUSSION

Effect of GAA with different protein levels on performance in pre starter phase

The present study was designed to test the efficacy of GAA with different level protein. For supporting growth performance in broiler each phase. The effect of GAA with different protein level, significantly ($P<0.05$) decreased FI ($P<0.05$) and FCR ($P<0.05$) in pre-starter phase. The result showed the highest FI in P0 (23% CP + 0 g/ton GAA) with an average 27.17 and the lowest FI at P3 (21% CP + 0 g/ton GAA) with an average 24.74. The greatest FCR at P0 with an average 0.81 and the lowest at P3 with an average 0.66. The first seven days of life are determinant for the optimum development of broilers. Therefore, good results should be pursued during this period, as losses are not recovered with compensatory growth until the end of the cycle (Borges *et al.*, 2021). On the other hand, when GAA was added to diets containing adequate arginine concentration, improvement was observed in both weight gain and FCR. Results confirmed that dietary inclusion of GAA can reduce arginine use, thus improving animal performance (Borges et al., 2021). In this study, a significant difference in FCR was observed due to GAA supplementation with different protein levels, which had received GAA converted feed to BW more efficiently. Borges et al. (2021) reported that inclusion of 0.20% GAA in broiler preinitial diets proved efficient for improving FCR until 14 days of age, performance improvement can be linked to the fact that GAA inclusion saves amino acids for endogenous synthesis of creatin so they can be used for protein synthesis and muscle growth instead. There was no significant effect of GAA on growth performance ($P>0.05$) in pre-starter phase. Difference protein levels showed significant ($P<0.05$) result for FI, FCR, BW, and IP. Crude protein 21% better than 23% in pre-starter phase. It according with study by Maksoud et al. (2011) reported significantly higher growth in broiler fed 21% CP supplemented with crystalline EAA compared to those fed 23% protein diet. The decreased FI with low CP may be due to depressing of CP or amino acid in excess of dietary requirement.

Effect of GAA with different protein levels on performance in starter phase

Table 3 shows the growth performance for grower phase (11 to 22 days of age). A significant two-way interaction GAA with different protein levels was observed for BW ($P<0.05$). The highest BW was observed in P0 (21% CP + 0 g/ton GAA) with average of 630.6 and the lowest BW was observed in P3 (19% CP + 0 g/ton GAA) with an average 555.7. It is suspected that the most optimal creatine synthesis in starter phase is in 21% CP and 0 g/ton GAA. The addition of GAA in sufficient CP will interfere the availability of creatine and muscle energy metabolism (phosphocreatine and creatine) to recycle ATP followed reduced body weight. The creatine and phosphocreatine system functions as a rapidly mobilizable reserve of high-energy phosphate in cells that have high and variable energy demands (particularly skeletal muscle, the brain, macrophages and sperm cells) to recycle ATP. It concluded that birds fed arginine deficient diet and excess arginine had lower BW than optimum CP and arginine. Similarly, recent study demonstrated Ale et al. (2019) concluded that bird fed arginine deficient died had lower BW and poor FCR than birds fed optimum arginine diets. The addition of GAA showed non-significant ($P>0.05$) result on performance of broiler chicken in starter phase. It is according with Mousavi et al, (2013) reported the

effect of GAA supplementation on BW gain was not significant throughout the experiment. On the other hand, in study Ale et al. (2019) reported supplementation with 0.6 and 1.2 g/kg of GAA decreased the adverse effect of arginine shortage on daily weight gain across pre-starter and starter periods. While difference protein level showed significant ($P<0.05$) result in FI, FCR, BW and IP. CP 21% showed better result than CP 19%. Srilatha et al. (2016) suggested levels of 21% and 19% CP appear to be adequate for commercial broilers during the pre-starter and starter phase, respectively. The mechanism by which GAA affects broiler performance is not fully understood (Dilger et al. 2018). GAA is a natural precursor of creatine, which is involved in cell energy metabolism, particularly in tissues with high and varying energy demand such as skeletal muscle.

Effect of GAA with different protein levels on performance in finisher phase

Table 4 shows the growth performance resultfor the finisher phase (23 to 35 days). For this period, GAA with different protein level showed non-significant result on performance ($P>0.05$). Ceylan et al. (2021) reported in their study of interactions between GAA and dietary energy level, there were no significant interactions between GAA and dietary energy level on various performance parameters. In study by Dilger et al. (2013) reported that the dietary GAA is an efficacious replacement for dietary arginin when fed to young chicks.

Supplementation GAA shows significant results for BW and IP ($P<0.05$), GAA 0 g/ton showed better result compared to 600 g/ton and 1200 g/ton in the finisher phase. In this study the addition of GAA decreased BW. In study by Lemme et al. (2007) reported that 41 days BW differences among graded levels of GAA supplementation and the non-supplemented control group were not significant, also reported that dietary supplementation of 0.06% GAA increased BW gain of female broilers raised to 42 days of age however the effects were not significant in male broilers. Difference in protein level shows significant results for FCR, BW and IP ($P<0.05$). The CP 19% showed better results in terms of FCR, BW, and IP compared to CP 17% in the finisher phase. Srilatha et al. (2016) reported that the birds fed on higher level protein in the finisher phase showed higher body weight gain and best FCR compared to the lower levels. It may be feeding lower protein levels in the finisher phase reduced the feed efficiency.

CONCLUSION

Supplementation GAA with different protein levels increased FI and decreased FCR in the pre-starter phase, increased BW in the starter phase and finisher phase. The mechanism by which GAA affected in broiler performance is not fully understood but, in this study, GAA did not improved growth performance, and high protein in diet showed better results than low protein in diet.

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AS07

In Vitro Ruminal Fermentation of Ration Supplemented with Calcium Soap

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ABSTRACT

The objective of this study was to investigate the effects of two types of supplemental calcium soap, flaxseed soap (FS) and the mixture of flaxseed and canola soap (FCS), on *in vitro* rumen fermentation of rolled barley as substrate. Each treatment had three levels of sub-treatments, 5, 10 and 20 mg FS and FCS, and 0.5 g of roller barley on dry matter basis (DM) was used as substrate. Gas production (GP) at 12, 24 and 48 h and *in vitro* digestibility of DM and crude protein (CP) at 48 h were measured during incubation. *In vitro* GP increased consistently with FCS content at 12 and 24 hours of incubation ($P<0.05$). There was no significant difference between GP and the concentrations of FS and LRS during 48-h incubation. The digestibility of DM and CP was not affected by the treatments and the level of supplementation. The results suggest that supplemental FCS activates *in vitro* rumen fermentation at an earlier stage of incubation under concentrated substrate conditions.

Keywords: Ca-soap, *in vitro* digestibility, flaxseed soap, mixture of flaxseed and canola soap and Concentrate substrate

INTRODUCTION

Fats in feed may be suitable for ruminants with high energy requirements, improve grain quality and reduce dust formation. Fats prevent rumen acidosis, facilitate the absorption of fat-soluble nutrients and allow the composition of meat or milk fat to be altered (Suharti et al., 2017). Inclusion, fat in the diet improves energy efficiency due to reduced methane production in the rumen and direct utilization of long-chain fatty acids in the metabolic pathways of fat synthesis. This is a difficult challenge because the microorganisms in the rumen alter the fatty acid profile of the diet through isomerization and biohydrogenation of unsaturated fatty acids (Harvatine & Allen, 2006). Calcium soap derived from long chain fatty acids from animal or vegetable fats is widely used as an energy source in ruminant diets. It has been reported that calcium soap remains relatively inert in the rumen under normal pH conditions, but completely dissociates under the acidic conditions of the abomasum. There is no known comparison between the source of calcium soap and different levels of feed as a dietary ingredient. In this study, the sources and amounts of calcium soaps were investigated on rumen fermentation using gas production technique.

MATERIALS AND METHODS

Experimental design

The experiment was laid out 3x2 factorial in a completely randomized design. There are two factors, namely two types of calcium soap (FS) and the mixture of flaxseed and canola soap (LRS) and three levels of calcium soap addition at 5, 10 and 20 mg in the diet. All samples were dried at 65 °C to constant weight and ground through a 1-mm sieve. Samples were individually weighed to 500 mgDM and added to each tube. The substrate was analyzed for dry matter (DM) and crude protein (CP) according to AOAC (1990). Rumen fluid was collected through the suction tube from three goats before morning feeding, normally fed concentrates and roughage at 2% of body weight. Then a special salivary preparation according to Menke and Steingass (1988) was performed. Gas production was measured after 1FCS2, 24 and 48 hours.

In vitro dry matter and crude protein digestibility

After 48 hours of incubation, the samples were centrifuged to recover the residues. These were oven dried at 105°C for 24 hours for determination of in vitro digestibility (IVDMD). Digestibility was calculated according to equation (1). The samples were analyzed for crude protein and digestibility was calculated according to equation (2).

$$\text{IVDMD}(\%) = \frac{\text{Initial DM input} - \text{DM residue} - \text{blank}}{\text{Initial DM input}} \text{ x } 100 \quad (1)$$

$$\text{IVCPD}(\%) = \frac{\text{CP substrate (mg)} - (\text{mgN x } 6.25)}{\text{CP substrate (mg)}} \text{ x } 100 \quad (2)$$

Statistical analysis

The data obtained were subjected to an analysis of variance using the SAS package (SAS, 1996). When significant differences appeared, means were separated using the Turkey test at a level of $P < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of feed

The DM contents were similar for all the diets, the EE contents FS (83.80%) and FCR (80.90%) were higher than rolled barley (1.86% DM basis). The CP content of substrate was 11.28% which was higher than FS and LRS (0.06 and 0.23% on DM basis). The NDF and ADF content of rolled barley were 31.12 and 7.14% on DM basis, respectively.

The gas production

Table 1 shows that gas production after 12 and 24 h differed significantly ($P < 0.05$) between treatments, which could mean that these types of calcium soap could be digested very rapidly during this period. Total gas volume after 12 and 24 h of incubation was statistically different ($P < 0.05$) between FCS administrations. FCS supplementation with 20 mg in the diet showed the highest value. This could be due to the fact that the grain of rolled barley is rapidly degradable in the rumen, thus promoting an environment rich in VFA and NH3-N, which is used by bacteria and protozoa for their development and multiplication. Gas production 48 h after incubation in both types of calcium soap did not differ significantly between treatments. These results could be due to the fact that the residual fractions were less readily available to the microbes in the rumen (Suharti et al., 2017).

Table 1. Effects of supplementary linseed soap and mixture soap on gas production

Supplementation	Level (mg)	Gas production (mL/500 mg DM)		
		12 hr	24 hr	48 hr
FS	5	41.33[ab]	90.67[ab]	114.00
	10	40.67[ab]	91.75[ab]	115.92
	20	41.67[ab]	92.17[ab]	117.17
FCS	5	38.67[b]	89.00[b]	115.00
	10	42.17[ab]	93.83[ab]	118.33
	20	43.92[a]	95.08[a]	117.08
SEM		0.422	0.474	0.468
Significance of supplementation				
Treatment (T)		0.642	0.267	0.255
Levels (L)		0.034	0.017	0.062
T x L		0.044	0.155	0.561

[a, b] Least squares mean with different lowercase superscripts in a column differ significantly ($P < 0.05$)
SEM = Standard error of means, FS = flaxseed soap and FCS = mixture of flaxseed and canola soap
[*] Least squares mean of non-supplementation was significantly differ from supplementation ($P < 0.05$)
[ns] Least squares mean of non-supplementation was not significantly differ from supplementation

In vitro digestibility

There was no difference in in vitro digestibility of dry matter (IVDMD) and crude protein (IVCPD) between treatments (Table 2). IVDMD and IVCPD were not affected by the FS and FCS supplementation, although feeding lipids may inhibit digestion in the rumen. In an in vitro study, the addition of fatty acids showed that each fatty acid reduced the digestibility of DM by 1.9-9.3% (Davison and Woods, 1960). In this study, it was found that the digestibility of DM and CP was not affected when the diet was supplemented with FS and FCS. One of the concerns with feeding barley to ruminants is that its lower fibre content may be associated with faster digestion in the rumen. In general, starch digestibility is high for all grain sources.

Table 2. Effects of supplementary linseed soap and mixture soap on *in vitro* digestibility

Supplementation	Level (mg)	*In vitro* digestibility (%)	
		Dry matter	Crude protein
FS	5	85.51	55.72
	10	85.90	54.45
	20	86.81	55.59
FCS	5	86.58	56.14
	10	86.33	54.11
	20	86.17	53.37
SEM		0.225	0.389
Significance of supplementation			
Treatment (T)		0.208	0.287
Levels (L)		0.419	0.113
T x L		0.060	0.264

S.E.M = Standard error of means, FS = flaxseed soap and FCS = mixture of flaxseed and canola soap
[ns] Least squares mean of non-supplementation was not significantly differ from supplementation

CONCLUSION

The study shows that *in vitro* GP increased consistently with FCS content at 12- and 24-h incubation ($P < 0.05$). The DM and CP digestibility were not affected by the treatments and the level of supplementation. Supplemental FCS activated in vitro rumen fermentation at an earlier stage of incubation under the condition of concentrated substrate.

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AS10

Single Nucleotide Polymorphism (SNP) in 5' franking region of Dopamine Receptor D2 (DRD2) Gene in Thai Native Chicken

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ABSTRACT

DRD2 is a protein receptor that play a role in many physiological functions of organisms. In poultry, DRD2 transmits the signals from dopamine neurotransmitters to regulate the reproductive system and maternal behaviours such as broodiness and maternal care. The objective of this study is to identify the single nucleotide polymorphism (SNP) in 5' franking region of DRD2 gene of Thai native chicken female in Narathiwat province, Thailand. Total genomic DNA of red blood cells from ten samples were amplified by PCR using specific primer DRD2 gene. The amplicons were detected by 3% agarose gel electrophoresis and nucleotide sequencing used to identify different genotype of SNP. The results show two positions of SNP at T-12,518C and G-12,543T respectively. This is the first report of SNPs of these positions in 5' franking region of DRD2 gene in Thai native chicken.

Keywords: DRD2, SNP, Thai native chicken

INTRODUCTION

Dopamine Receptor D2 (DRD2) is a G-protein coupled receptor localized to lactotrophs in the adenohypophysis and when coupled to dopamine maintain inhibition of prolactin secretion (Hansen et al., 2005). In avian, previous studies showed that dopamine (DA) played a role in PRL secretion and stimulating PRL secretion via DRD1 receptor on the hypothalamic level and inhibiting PRL secretion via DRD2 receptor on the pituitary level by remoting through vasoactive intestinal peptide (VIP) (Al Kahtane et al., 2003). The aim of this study was to identify single nucleotide polymorphism (SNP) in 5' franking region of DRD2 gene of Thai native chicken.

MATERIALS AND METHODS

DNA extraction

Genomic DNA was extracted from red blood cells of nine Thai native chicken females according to the method of Goodwin et al. (2007). Briefly, blood samples 50 µL were rinsed with 0.9 % NaCl. After centrifuge at 2,500 rpm for 5 min a 300 µL of solution l (0.03 M Tris-base, 0.20 M sucrose, 0. 10 M sodium chloride and 0.01M EDTA pH 8.0) was added. After that adding 600 µL of solution II (0.05 M tris-base, 0.05 M EDTA pH 8.0 and 2.5% SDS) and 300 µL of chloroform respectively. After that, 200 µL of saturated phenol was added to the mixture and centrifuged at 14,000 rpm for 15 min. The supernatant was

further added 500 µL of isopropanol and incubated -20°C for 10 min. The mixture was centrifuged at 14,000 rpm for 15 min and washed with 70% ethanol for 2 times. Finally, DNA pellet was dried at room temperature for 15 min and diluted with 50 µL of nuclease free water. DNA was analysed by 1% agarose gel electrophoresis and visualized in UV Transilluminator (Biorad).

PCR Amplification and DNA sequencing

The genomic DNA was used as a template to amplify the *DRD2* gene promoter with primers DRD2_F TGCACTTCAATCCTTCCCAGCTT and DRD2_R TTGCGCTGCCCATTGACCA under the following conditions: preheating at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s and extension at 72°C for 20 sec: with a final extension at 72°C for 10 min. The PCR products were identified by 3% agarose gel electrophoresis and showed three genotypes of this locus where the homozygous insertion (II) consisted of 187 bp, homozygous deletion (DD) composed of 165 bp, while heterozygous (ID) showed 187 and 165 bp. The PCR products of homozygous insertion and deletion were direct sequences. While, heterozygous was cloned in pGEMT easy vector (Promega, Madison, WI, USA) and sent to Macrogen, Seoul, Korea, for sequencing.

DNA sequence analysis

All obtained PCR sequences were compared against public NCBI database using the BLAST program (NCBI) to check that the sequence and CAP3 Sequence Assembly Program (http://pbil.univ-lyon1.fr/cap3.php) for assembly sequences.

RESULTS AND DISCUSSION

Insertion and deletion of 22 bp in 5' franking region of *DRD2* gene

In this study, a 22 bp Insertion/Deletion (INDEL) in 5' franking region of DRD2 gene were founded of Thai native chicken. In TC1 sample was founded two DNA bands composed of insertion (187 bp) and deletion (165 bp). While, other samples (TC2-TC9) were founded only one band (165 bp) as a deletion pattern of 22 bp (Figure 1a). The insertion of 22 bp (GTTGCTACCCTTAGCAAAGGCT) in promoter of DRD2 gene from Thai native chicken (Figure 1b), that nucleotide sequence was same previous reported. Datumada et al. (2020) showed the results of INDEL mutation of 22 bp in promoter region of *DRD2* gene. Moreover, INDEL mutation has been report at position I-38475D, I-38473D, I-38468D and I-38463D in 5' regulatory region of *DRD2* gene (Xu et al., 2010).

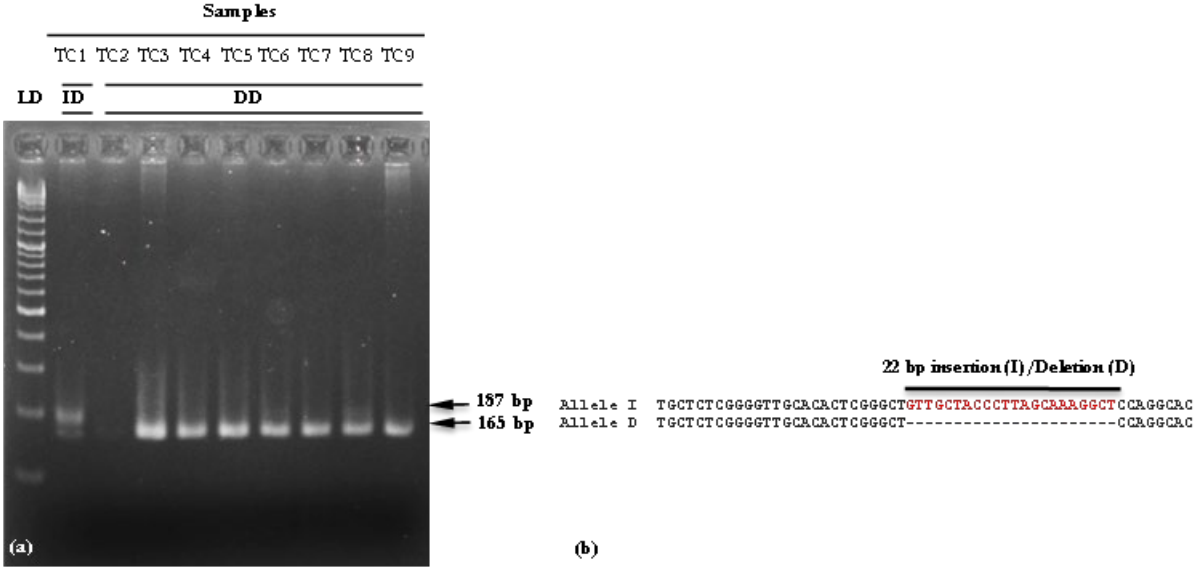

Figure 1. Genotyping of 22 bp insertion/deletion in promoter region of *DRD2* gene (a) PCR product detection by 3% agarose gel electrophoresis analysis. LD=ladder, ID=insertion/deletion, DD=deletion, TC=Thai native chicken (b) 22 bp insertion/deletion sequences

Single nucleotide polymorphism in 5' franking region of *DRD2* gene

Sequencing results showed two positions of nucleotide mutations in 5' franking region of DRD2 gene. The nucleotide comparison between obtained sequences and *Gallus gallus* breed Red Jungle Fowl isolate RJF #256chromosome 24, GRCg6a (22,894 bp) (Accession number: NC_006111) from NCBI data base were founded two positions of SNPs at T-12,518C and G-12,543T respectively (Figure 2). The variation of SNP was study reported in poultry, where the expression of DRD2 gene correlated with broodiness behaviours in turkey (Chaiseha et al., 2003). In chicken Xu et al. (2010) presented 27 variations in 5' regulatory region, exons and intron of DRD2 gene from 6 chicken population and suggested 2 SNPs of A-16105G and T+619C might be markers for breeding against broodiness.

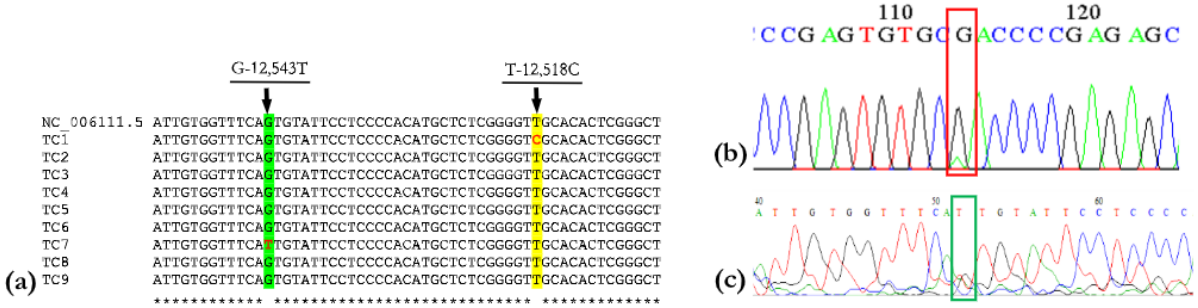

Figure 2. Nucleotide sequences of SNPs in 5' franking region of *DRD2* gene (a) Multiple sequence alignment of TC samples compared with *DRD2* gene of *Gallus gallus*. The SNPs at position -12,518 labeled in yellow and -12,543 shaded in green (b) Sequence diagram of the SNP reverse sequence at position -12,518 in red box (c) Sequence diagram of the SNP at position -12,543 in green box

CONCLUSION

The results of this research showed 2 SNPs at T-12,518C and G-12,543T on 5' franking region of DRD2 gene in Thai native chicken females.

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AS13

Antibacterial Activity of Ivory Snail (*Babylonia areolata*) Extractions against *Aeromonas hydrophila* and *Streptococcus agalactiae*

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ABSTRACT

This study is aimed to screen the presence of antibacterial activity of ivory snail (*Babylonia areolata*) against specific pathogens which are gram-negative bacteria, *Aeromonas hydrophila* and gram-positive bacteria, *Streptococcus agalactiae*. The crude extract of *B. areolata* was tested for inhibition of *A. hydrophilla* and *S. agalactiae* growth which tends to be a serious treat towards Malaysia aquaculture industry. Four different extraction solvents were used; ethanol, distilled water, hexane and methanol to extract the homogeneous body of *B. areolata* and the antibacterial activity were carried out using the agar well diffusion method. The antibacterial activity was measured accordingly based on the inhibition zone around the well. The present study revealed that *B. areolata* showed antibacterial activities against the pathogenic microbial forms. The finding showed the values of triplicate readings for the diameter of inhibition zone (mm) for pure extraction of homogeneous body of *B. areolata* using distilled water, ethanol, methanol and hexane against *A. hydrophila* and *S. agalactiae* on both TSA are 0.53±0.06, 0.57±0.06, 0.70±1.36E-16, 0.57±0.06 and 0.63±0.06, 0.60±0.00, 0.70±1.36E-16, 0.60±0.00, respectively. There was statistically significant effect of type of extraction solvent on diameter (inhibition zone at the $p<0.05$) in which at least one pair of means differ significantly. The methanol extracts of *B. arealata* possessed the highest activity against *A. hydrophila* and *S. agalactiae* compare to distilled water, ethanol and hexane. This finding showed that *B. areolata* contained antibacterial properties and medical value which reacted against isolated bacteria. The results obtained can be used as a guideline for further study related to the full structural component of antibacterial compound that derived from marine molluscs and other further research in this field in the future.

Keywords: *Babylonia areolata*; *Aeromonas hydrophila*; *Streptococcus agalactiae*; antibacterial activity; extraction

INTRODUCTION

Marine molluscs such as *Babylonia areolata* (*B. areolata)* contains bioactive compounds including sterols, nitrogenous compound, peptide, depsipeptides, polypropionate, macrolides, prostaglandins and fatty acid derivatives, sesquirterpene, alkaloids and miscellaneous compounds (Blunt et al., 2006). Molluscs is the second largest animal phyla in a marine environment, however, only a few studies have been carried out in term of antimicrobial peptides (AMPs) according to Sathyan et al., (2014). According to Bazes et al. (2009), they are potentially acts as an antimicrobial aid that can protect them from other harmful pathogens. Previous study by Kumaran et al., (2011), stated that invertebrates like mollusc also been proved to contain a diverse antibacterial compound in their homegenates body. *Aeromonas hydrophila* and *Streptococcus agalactiae* are among the most common bacterial pathogens of fish. Hence, this research was conducted to determine the antibacterial activities in *B. areolata* against *A. hydrophila* and *S. agalactiae*, and to compare the antibacterial

effect of using four different types of solvent in the sample extraction process which were distilled water, ethanol, methanol and hexane.

MATERIALS AND METHODS

Sample collection

The *B. areolata* was collected from Kemasin, Bachok, Kelantan, Malaysia**.**

Preparation of solvent extract

Dried and powdered *B. areolata* (75 g) were Soxhlet extracted with 300 ml of distilled water, ethanol, methanol and hexane for about 3 to 4 hours till completed extracted. The homogeneous body of *B. areolata* extracted in solvent was removed from the Soxhlet and then was concentrated to sticky dried in rotary vacuum evaporator below 50 °C and stored at -80 °C until needed for the bioassays.

Microbial strains and inoculum preparation

The microorganisms used in this study were pathogens namely *A. hydrophila* and *S. agalactiae*. To get desirable cell counts for bioassays, overnight grown bacterial cells were subcultured in fresh NA at 37 °C. Active cultures were prepared by inoculating fresh Tryptic Soy Broth, (TSB) medium with a loopful of cells from the stock cultures at 37 °C for overnight.

Well diffusion method

Antibacterial activity of *B. areolata* was evaluated based on the diameter of the clear inhibition zone surrounding the well that was filled with pure sample extract after incubated at 37 °C for 24 hours. The reading of the diameter of inhibition zone was measured in millimetre (mm) by using a ruler.

Statistical analysis

All analyses were carried out in triplicate. The data were statistically analyzed using one-way analysis of variance (ANOVA) to find out any significant differences among the experimental groups followed by Post Hoc test using Bonferroni test.

RESULTS AND DISCUSSION

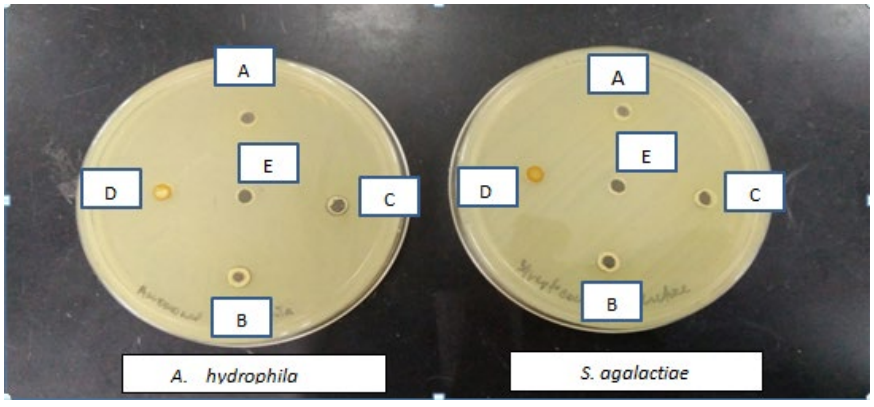

Figure 1. Inhibition zone of extraction of homogeneous body of *B. areolata* using (A) distilled water, (B) ethanol, (C) methanol and (D) hexane towards bacteria pathogens (*A. hydrophila* and *S. agalactiae*). A (E) control was used as comparison.

The finding showed the values of triplicate readings for the diameter of inhibition zone (mm) for pure extraction of homogeneous body of *B. areolata* using distilled water, ethanol, methanol and hexane against the selected bacteria on both TSA are 0.53±0.06, 0.57±0.06, 0.70±1.36E-16, 0.57±0.06 and 0.63±0.06, 0.60±0.00, 0.70±1.36E-16, 0.60±0.00, respectively. There was statistically significant effect of type of extraction solvent on diameter (inhibition zone at the $p<0.05$) in which at least one pair of means differ significantly. In this research, methanol extracts is proved to exhibit a high zone of inhibition compare to distilled water, ethanol and hexane. This finding showed that the effectiveness of inhibiting bacteria in inhibition zone was due to tendency of body compound of *B. areolata* that was more preferred to be extracted in less polar solvent which was methanol. Polar solvent can dissolve polar compound best while non-polar solvent dissolve non-polar compound best. However, the antibacterial activity depends upon of compound extract and the solvent itself (Sugesh et al., 2013). Hence, it can be concluded that the homogeneous body of *B. areolata* was extracted in higher amount and higher rate in less polar solvent.
Hence, the finding showed that *B. areolata* contained antibacterial properties which react against isolated bacteria as the extractions of homogeneous body of *B. areolata* using distilled water, ethanol, methanol and hexane showed visible clearing along the well. Therefore, *B. areolata* has been proved to contain antibacterial activity against both pathogens (*A. hydrophila* and *S. agalactiae*). It is believed that the protein extracted from tested *B. areolata* may potentially act as the antibiotic for aquatic organisms towards bacterial infections.

CONCLUSION

From the findings, it is proved that the extracts of *B. areolata* were able to inhibit the growth of both *A. hydrophila* and *S. agalactiae* in all solvents (distilled water, ethanol, methanol and hexane). However, methanol extracts were proved to exhibit a high zone of inhibition compared to distilled water, ethanol and hexane due to differ in terms of effectiveness of inhibiting bacteria in inhibition zone. Tendency of body compound of *B. areolata* is more preferred to be extracted in less polar solvent which is methanol. Further investigations to purify these active compounds should be considered to clarify their chemical composition.

ACKNOWLEDGEMENT

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AS14

Evaluation of Coconut Leaf Silage as an Alternative Feedstuff Forage for Ruminants

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ABSTRACT

Coconut leaves are commonly used by farmers in Madura land, especially in Pamekasan during the summer, but coconut leaves just a little treatment are only separated from the sticks and given directly to the cattle. Although coconut leaves have a high lignin content. Therefore the objectives of the study was to determine the effect of fermentation treatment on the nutritional value of coconut leaves. The materials used were coconut leaf, indigenous grass, elephant grass (*Pennisetum purpureum)*, rice bran, molasses, and moebilin (probiotic). The treatment used was a combination of feed materials based on coconut leaves with a variety of P1 100% coconut leaves, P2 50% coconut leaves + 50% indigenous grass, P3 50% coconut leaves + 50% elephant grass, P4 33.33% coconut leaves + 33 ,33% indigenous grass + 33.33% elephant grass. Each treatment was repeated three times, so that 12 trials were obtained. This study used a Completely Randomized Design (CRD), the data were analyzed using Analysis of Variance and it was continued with the Least Significant Difference (LSD) test. The difference between fresh forage before fermentation and after fermentation was analyzed using the T test. The results showed that the fermentation treatment based on coconut leaf materials had a significant effect ($P \leq 0.05$) on the dry matter content increased by 19%, crude protein increased 2% to 3%, crude fat increased by 2%, and crude fiber decreased 5%. The conclusion of this study is that fermentation treatment can improve the nutritional quality of based on coconut leaf materials, so that fermented coconut leaves or a combination of coconut leaves and forage (indigenous grass and elephant grass) are declared suitable as alternative feed materials for ruminants.

Keywords: Non-conventional feed, coconut leaf, nutritional quality

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INTRODUCTION

Coconut leaves are commonly used by farmers in Madura land, especially in Pamekasan during the summer, but coconut leaves just a little treatment are only separated from the sticks and given directly to the cattle. Although coconut leaves have a high lignin content. Therefore the objectives of the study was to determine the effect of fermentation treatment on the nutritional value of coconut leaves.

MATERIALS AND METHODS

Materials

The materials used coconut leaf, indigenous grass, elephant grass (*Pennisetum purpureum)*, rice bran, molasses, and *lignochloritic* bacteria (moebilin starter).

Methods

The treatment used was a combination of feed materials based on coconut leaves with a variety of P1 100% coconut leaves, P2 50% coconut leaves + 50% indigenous grass, P3 50% coconut leaves + 50% elephant grass, P4 33.33% coconut leaves + 33 ,33% indigenous grass + 33.33% elephant grass. Each treatment was repeated three times, so that 12 trials were obtained. This study used a Completely Randomized Design (CRD), the data were analyzed using Analysis of Variance and it was continued with the Least Significant Difference (LSD) test. The difference between fresh forage before fermentation and after fermentation was analyzed using the T test.

Research Implementation

Fresh materials of coconut leaves, indigenous grass, and elephant grass were prepared and nutrient quality was analyzed using Proximate Analysis. The fresh materials also were prepared to used in the fermentation process. The fermentation treatment was started with the coconut leaves, indigenous grass, and elephant grass were cut into small pieces (5-10 cm) and were placed on a clean and leveled place. 5 ml of *lignochloriitic* bacteria (as fermenter) was mixed with 1 teaspoon of water (5 ml) and used to spray on the treatment material evenly.5 ml of molasses was mixed with 2 teaspoons of water (10 ml) and used to spray on the treatment material evenly. Bran was sprinkled on the treatment material evenly as much as 2 grams. The treatment materials, bacteria, and rice bran were mixed together to taste. The treatment material was put into the plastic and tie tightly, double-layered plastic to prevent leakage and left for one week. The nutrient quality using Proximate analysis of the fermented materials.

RESULTS AND DISCUSSION

The results of the tests carried out, the composition of nutrients contained in fresh materials and fermented materials are presented in the following table:

Table 1. Nutrition Contents Fresh Materials

Material	Nutrition Contents (%)		
	Crude Protein	**Ether Extract**	**Crude Fiber**
Coconut Leaf	9,54	3,3	24,84
Indigenous Grass	8,95	1,85	25,79
Elephant Grass	11,88	1,36	32,72

Data source: Result test of Nutrition Laboratory University of Muhammadiyah Malang (2017)

Table 2. Nutrition Contents of Fermented Materials

Treatment	Nutrition Contents (%)					
	Crude Protein		**Ether Extract**		**Crude Fiber**	
	Before	After	Before	After	Before	After
P0	10,37	14,27	3,59	4,25	26,99	25,21
P1	10,14	14,27	2,82	4,52	27,78	24,24
P2	11,96	12,79	2,57	4,36	32,17	24,64
P3	11,28	13,54	2,40	4,40	30,97	25,13

Data source : Result test of Nutrition Laboratory University of Muhammadiyah Malang (2017)
Description : P1 = 100% coconut leaves, P2 = 50% coconut leaves + 50% indigenous grass, P3 = 50% coconut leaves + 50% elephant grass, P4 = 33.33% coconut leaves + 33 ,33% indigenous grass + 33.33% elephant grass.

The results showed that the fermentation treatment based on coconut leaf materials had a significant effect (P≤0.05) on the crude protein increased 2% to 3%, This is caused by the interaction between nutrients so that with the help of lignoclhoritic bacteria, which simplification lignin to be simpler. can lead to increased protein. The extract ether increased by 2%, this is due to other additions such as rice bran and molasses that make the increase. and crude fiber decreased 5%, this is due to the simplification of complex carbohydrates (polysaccharides) into simpler carbohydrates (Monosaccharide) by lignochloritic bacteria.

CONCLUSION

Based on the results of this research, it can be concluded that the fermentation treatment can improve the nutritional quality of coconut leaf-based feed materials, As well as the nutrition of coconut leaves to meet the needs of madura cattle's crude fiber so that it is potentially worthy of being an alternative feed material for crude fiber for ruminants.

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Scope
Food Security (FS)

FS01

Types and Source of Velvet Tamarind on Physical and Chemical Properties

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ABSTRACT

Velvet tamarind (VT) has a distinctive appearance and specific food in three southern border provinces, lacking post-harvest management information. The objectives of this study were to manage types and sources of VT on physical and chemical properties. The results found that types of VT influenced the dimensions and shape was significantly different ($p \leq 0.05$), while the thickness of shell VT was not different ($p > 0.05$). The Sawa velvet tamarind)SVT(was 3.32 cm which had the highest length value than purple velvet tamarind (PVT) at 2.49 cm and Keladeh velvet tamarind at 2.12 cm (KVT). The types and source of shell VT and pulp VT had different effects on moisture a_w content ($p \leq 0.05$). The shell of the VT was black, and the PVT species was purple different from the other VT. The VT pulp found that with the higher DPPH radical scavenging activity, there was a significant difference ($p \leq 0.05$) in the IC_{50} range 25.82-27.76 µg/ml, respectively. Total phenolic content (TPC) in pulp VT was significantly different ($p \leq 0.05$))341.46-367.29 mg GAE/ml(. The vitamin C value obtained from the pulp in the VT is an important health product. The sensory of pulp VT's overall score level was 6.90-7.44 (moderate preference). In conclusion, these data indicated that the VT could be used in the novel healthy food product in the future.

Keywords: Velvet tamarind, sensory test, physical, chemical properties

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INTRODUCTION

Velvet tamarind (*Dialium guineense*) is an important fruit in three borders southern province of Thailand and can be found in Southern Thailand. People in Yala, Pattani, and Narathiwat province know them as "Lukyee and Kerayee." It is a tree of about 30 m with densely leafy crown and smooth greyish bark. Leaves are hairy, and the flowers are usually whitish, while the fruits are less circular and flattened. The fruit's pulp is edible and sweet, with fairly high levels of ascorbic acid and fiber (Chedoloh, 2018). In addition, Velvet tamarind (VT) pulps are a fairly good source of minerals and antioxidants (Afolabi et al., 2018). However, it also lacks information on post-harvest management. Therefore, we are interested in studying the management of types and sources of VT on physical and chemical properties.

MATERIALS AND METHODS

Preparation of VT

The samples of VT fruits were collected from Yala, Pattani, and Narathiwat provincial areas from July to November of 2021. VT is separated manually to remove all foreign materials such as dirt, broken and fungal

infections. The fresh fruits were first sorted, cleaned, and weighed. Fresh fruit production and classification of VT were classified as SVT, PVT, and KVT.

Determination of physical and chemical characterization

The quality of fresh VT analytic is classified by shape and structure size (diameter, thickness, circumference, length) using micrometers and vernier caliper. Types of VT; SVT, PVT, and KVT are analyses of physicochemical, composition fruit, moisture, a_w, pH and vitamin C. The total soluble solids content (TSS) of pulp sample beverage was determined using AOAC method (AOAC, 2000). TSS was determined by refractometer to check sugar content. The color of VT samples were evaluated by measuring L*, a*, b parameters using a reflectance colorimeter. The antioxidant of velvet tamarind pulp was carried out by DPPH. The Folin-Ciocalteu assay was determined the TPC of the VT fruit pulp extract.

Texture analysis of dried VT fruits and flesh

Texture analysis of VT fruit was performed using a texture analyzer Brookfield's CT3 Texture Analyzer. Data was collected in Newton (N) for the braking force. Three samples of VT, with five replicates, have been analyzed.

Sensory Evaluation

Sensory testing will be performed in Yala Rajabhat University Sensory Lab. 50 non-trained panelists are selected to equally represent genders within age categories (18-45). Questions are done using a 9 points hedonic scale, with 1 corresponding with dislike extremely and 9 with like extremely.

Statistical Analysis

Statistical analysis followed a completely randomized design (CRD) for physical and chemical properties and The Randomized Complete Block Design (RCBD) for the Sensory test. A linear mixed model with treatments (type and source of velvet tamarind). A mixed model in SPSS software was employed. Duncan's new multiple ranges (DMRT) tests were used to compare means of treatments when statistical significance is at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results showed that the type and source had influenced the dimensions and shaped significantly ($p \leq 0.05$). The colors of pulp and shell were different as shown in Table 1. The SVT sample had the highest length value (3.32 cm) than other samples of VT. The type and source of shell and pulp VT had different effects on TSS, pH, L* a* b*, moisture, and a_w value ($p \leq 0.05$). Fruit VT were IC_{50} values within the tested concentration range (25.82-27.76 µg/ml). This scavenging activity of tamarind pulp may be related to their presence of flavonoids, condensed tannin, and polyphenols, thus contributing to their electron transfer/hydrogen donating ability (Obulesu & Bhattacharya, 2011). In addition, the high vitamin C value in VT is important for health. In the sensory test of pulp VT, the overall score level was 6.90-7.44 (moderate preference). Therefore, VT is good food. It can be made into many products.

Table 1. The sizes of velvet tamarind for SVT, PVT and KVT

Type of velvet tamarind		SVT	PVT	KVT
Shell	Shell thickness ^{*ns}	0.040±0.02	0.039±0.01	0.038±0.01
	a_w ^{*ns}	0.58±0.01	0.57±0.01	0.57±0.01
	Moistures (%)^{*ns}	19.89±0.30	19.28±0.45	19.02±0.37
	TSS (°Brix)	4.57±0.06^{b}	5.13±0.15^{a}	4.70±0.10^{b}
	pH	2.93±0.06^{b}	2.97±0.06^{ab}	3.10±0.10^{a}
	L^*	17.70±0.42^{b}	19.15±0.76^{a}	16.97±0.10^{b}
	a^*	0.97±0.10^{b}	2.55±0.72^{a}	1.24±0.12^{b}
	b^*	1.46±0.15^{b}	3.36±0.88^{a}	1.61±0.02^{b}
Seed	Width^{*ns}	0.83±0.11	0.78±0.09	0.84±0.07
	Length^{*ns}	1.08±0.08	0.87±0.07	0.91±0.05
	Thickness^{*ns}	0.37±0.04	0.37±0.05	0.38±0.05
	Circumference^{*ns}	1.82±0.07	1.85±0.16	1.79±0.06
	a_w ^{*ns}	0.63±0.02	0.63±0.01	0.63±0.02
	Moistures (%)^{*ns}	21.05±0.52	20.63±0.09	20.65±0.43
	TSS (°Brix)	3.23±0.06^{b}	3.07±0.06^{c}	3.57±0.06^{a}
	pH^{*ns}	6.23±0.23	6.47±0.06	6.33±0.06
	L^*	26.16±0.37^{b}	27.56±0.33^{a}	27.43±0.05^{a}
	a^*	13.08±0.10^{a}	12.39±0.49^{b}	12.88±0.01^{ab}
	b^*	10.91±0.16^{a}	11.33±0.29^{b}	11.89±0.02^{a}
Pulp	Width	1.73±0.14^{a}	1.61±0.04^{a}	1.32±0.12^{b}
	Length	3.32±0.20^{a}	2.49±0.08^{b}	2.12±0.08^{c}
	Thickness	1.96±0.14^{a}	1.72±0.04^{b}	1.26±0.07^{c}
	Circumference	5.14±0.07^{a}	4.52±0.28^{b}	3.83±0.29^{c}
	a_w	0.54±0.01^{ab}	0.52±0.01^{b}	0.55±0.01^{a}
	Moistures (%)	17.80±0.08^{b}	17.25±0.20^{c}	18.72±0.17^{a}
	TSS (°Brix)	7.37±0.06^{c}	9.63±0.12^{a}	8.37±0.06^{b}
	pH	2.87±0.05^{b}	3.06±0.05^{a}	2.97±0.05^{ab}
	L^*	21.22±0.10^{c}	21.67±0.02^{b}	22.68±0.37^{a}
	a^*	5.33±0.17^{a}	5.28±0.01^{a}	5.79±0.40^{a}
	b^*	5.32±0.13^{b}	5.44±0.02^{b}	6.18±0.56^{a}
	DPPH IC_{50} (µg/ml)	25.88±0.73^{b}	27.76±0.88^{a}	25.82±0.60^{b}
	TPC (mgGAE/ml)	341.46±4.02^{b}	367.29±7.79^{a}	351.05±10.92^{b}
	Vitamin C (mg/100ml)	22.65±0.60^{b}	28.33±1.00^{a}	28.10±0.81^{a}
	Hardness	1.82±0.06^{b}	3.71±0.55^{a}	1.67±0.15^{b}
	Crispness	2.05±0.30^{b}	4.80±0.67^{a}	1.80±0.18^{b}
	Color^{ns}	6.62±1.12	6.82±1.08	6.72±1.01
	Smell^{ns}	6.48±1.11	6.40±1.07	6.26±1.17
	Flavor^{ns}	6.68±0.91	6.72±1.14	6.82±0.87
	Sourness	6.96±0.64^{b}	7.48±0.61^{a}	6.38±1.10^{c}
	Taste	7.08±0.94^{b}	7.48±0.58^{a}	6.78±1.18^{b}
	Texture^{ns}	6.90±1.71	6.78±1.07	6.60±1.18
	Overall	6.90±0.79^{b}	7.44±0.50^{a}	6.96±0.90^{b}

Different characters in the landscape have significant differences ($p \leq 0.05$).
^{*ns} is Non- significant differences ($p > 0.05$).

CONCLUSION

The type of VT was different in shape and structure, also that physical and chemical composition in pulp VT important for quality control in SME. It results affect the development of a product in the future.

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FS02

The Challenges of Food Security: A Comprehensive Study on Safety, Sustainability, Transforming Food Systems and Machine Learning Based Approaches

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ABSTRACT

Due to the increasing population, the demand for food products has become a major concern that can be solved through the proper utilization of resources and the production of food products. The major factors that affect the food system includes increasing food shortage, decreasing quality, wastage, and loss of food products, limited natural resources, etc. According to the World Food Program (WFP), around 30% - 50% of food produced globally goes waste. Climate change is considered as one of the main factors that affect food security. Other factors such as urbanization, climate change, and natural disasters also affect food security. Family farming can help minimize food security. Numerous ways exist to achieve sustainability and food security, some of these include limiting food losses and waste, increasing the use of plant-based food, and recycling. The establishment and expansion of effective and inclusive governance institutions are key components of the strategy to transform food systems. One of the recent citations of Sood and Harjeet (2021) address the various aspects of image processing, which are used to minimize the issues faced by researchers when it comes to analysing food production and agriculture-related applications. Some of the active research areas related to food security are food tracing system, monitoring the growth of plants using modern machine vision techniques, use of internet for global food security, artificial intelligence will automate the human work for food security and image processing and deep learning collectively helped in achieving food security.

Keywords: Food security, machine learning, image processing, WFP.

INTRODUCTION

The concept of food security emanates from the world food conference (WFC), Food and Agriculture Organization (FAO), which came out in 1974. The FAO in 1996 defined and redefined in 2001 food security as the "when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food, which meets their dietary needs and food preferences for an active and healthy life" by Sood and Harjeet 2021. Food security is a concept conceptualized by the FAO/UNICEF (United Nations International Children's Emergency Fund) and the Food Authority of the US alienated food security into four pillars which resembles as; (1) It involves the availability of food for people to meet their dietary needs, (2) Food access is a basic human need that should be maintained in such a way that people should have enough resources to buy nutritious food, (3) When food is available, it is time to utilize it with proper hygiene and storage techniques will help in improving the efficiency of food processing and (4) Food stability, means households get constant food supply throughout the year.

Food safety

Food safety refers to the process of ensuring that sufficient food is available for all members of the family. This concept is defined as a situation where all members of the family have the opportunity to eat nutritious and safe food. A food safety policy should provide for an early warning system, rapid reaction to a crisis, and clear and open communication with consumers. In this regard, the European Union's policy on food safety can be considered exemplary. It is linked to the management of food quality and health. With the publication of the White Book on Food Safety by Brussels (2000). This White Book emphasizes the importance of coordinating government policies. One example of this is the formation of the European Food Authority, which would be a scientific research body that would monitor emergencies and coordinate responses in the case of a crisis. As this document sets out the European Union's priority in terms of food safety; also coordinates the management of responses in the event of an emergency. The White Book marked a significant step in the coordination of efforts related to food safety. It allowed for the establishment of a European Food Safety Agency (EFSA), which was created in January 2002, and which has its headquarters in Italy. The primary political challenge associated with food safety lies in finding the point of-equilibrium in the trade-off between economic development, environmental conservation, food safety and between local, national and international interests.

Food sustainability

The aims of food safety and security are complementary elements of our sustainable future. Sustainable development goals (SDG) including eradication of hunger and poverty, clean water, sustainable land use, responsible production and consumption, mitigating climate change, and sustainable life on land and water. The tools and strategies used to improve food security must align with the various health and safety considerations. One health approach is needed to assess trade-offs and achieving sustainability (Vagsholm et al. 2020). In addition, the control of food frauds is an emerging issue requiring attention. Getting the trade-offs right, between the security, safety, and sustainability of food production, will require careful balancing between multiple concerns and challenges.

Environmental sustainability is a key component of the 17 SDG. One key message is that it is a part of many social and economic goals. Unfortunately, focusing on one goal could result in undermining the other goals. Big data analysis can help improve the efficiency of food production and reduce waste. It can also ensure the quality and safety of the food we consume. The intensification of food production should align with the requirements for a long-term sustainable agriculture (Rockström et al. 2017). The long-term sustainable agriculture has to operate within its environmental boundaries to remain sustainable. The significant considerations of this approach include the ecological dimensions, resource footprints and resilience, the social dimension of food security, and improving livelihoods of the global food production systems. Agriculture and aquaculture should become more sustainable in order to meet the demands of a growing global population. Today the sustainability of our food security is challenged since between 30 and 50% of the food is lost or wasted in different stages of the food system, with consequent higher consumption of animal foodstuffs. However, this system could also contribute to increasing the environmental footprint.

Transforming food systems

We are aware that transforming the food systems provide nutritious and affordable food for all and become more efficient, resilient, inclusive and sustainable. Recent citations report and presents an unprecedented opportunity to transform the way we feed ourselves with the upcoming UN Food Systems Summit, the COP26 on climate change, and the Nutrition for Growth Summit (FAO 2021). The outcomes of these events will be able to gather vital commitments to improve the nutrition of all people also highlighted the need for better food systems to address the root causes of food insecurity and malnutrition.
The six transformation pathways are a set of recommendations that help guide the development of policies and investment portfolios that will enable the efficient transformation of food systems. They are based on a context-specific analysis that identifies which drivers will most affect food security and nutrition outcomes, and which combination will provide the most impact.

Machine learning process

Machine learning is capable of autonomously acquiring and integrating knowledge. It works by taking into account various features that are known as parameters of a given set of variables. There are various techniques used to classify and recognize images. Some of these include Support Vector Machine (SVM), Decision tree are widely employed in food recognition, weed detection, and also in food image classification and Artificial Neural Network takes input, pass this input to hidden layers by adjusting weights where the learning takes place and finally gives decision/prediction and also utilized for measuring the height of plant.

Due to the complexity of the data collection and the wide variety of image processing techniques used for analysing the food products, it is very challenging to implement the right techniques. This paper presents a comprehensive study on how Machine Learning techniques can improve the image processing efficiency and resolve the agriculture issues. After going through the various papers presented in the literature, the author Sood and Harjeet 2021 concluded that the combination of CNNs and shallow algorithm produced promising results in terms of both training and evaluation. Further, the authors also stated that the implementation of CNNs in Deep Neural Network has led to better results than the other techniques. Figure 1 depicts other techniques utilized routinely in detecting various food security areas in agriculture

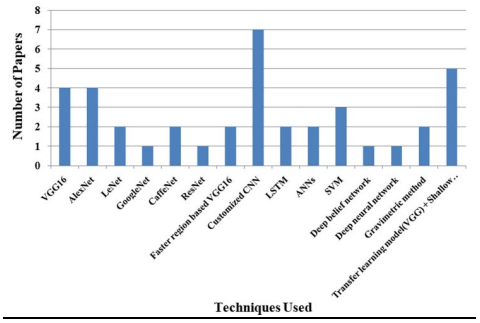

Figure 1. Tools/techniques used for food security in agriculture domain
(Source: Sood & Hargeet, 2021)

CONCLUSION

In this article, the authors have summarized a comprehensive survey on food security, safety, sustainability, transforming food systems, and machine learning based techniques for food products and agriculture domain. The concept of trade-offs and the decisions related to food safety and security should be supported by evidence-based studies. This paper aims to motivate researchers to improve, solve the efficiency and profitability of agriculture and food production by experimental approach in future studies.

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FS04

Effect of Salts, and Protease in Protein Hydrolysis of Black Soldier Fly Larvae (*Hermetia illucens*)

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ABSTRACT

The effectiveness of salt and protease in protein hydrolysis and protein denaturation of *Hermetia illucens*, Black Soldier Fly Larvae (BSFL) meal was studied. Two different treatments on the BSFL meal were applied simultaneously: 10% NaCl (Treatment 1); and 10% TAPzyme (Treatment 2). Each treatment received 5 grammes of BSFL meal. The samples were incubated under optimal conditions for hydrolyzing and denaturing the BSFL protein, resulting in smaller peptides. The Bradford assay technique was used to determine the percentage of protein concentration decreased. The percentage of protein concentration decreased in Treatment 2 was substantially higher ($p < 0.05$) than in Treatment 1. These findings suggested that the BSFL protein can be degraded by protease when compared to the control (sample C). This data revealed that the protease efficiently improves protein hydrolysis based on the percentage of protein concentration decreased.

Keywords: Black soldier fly larvae (BSFL), salt, protease, protein hydrolysis, protein denaturation

INTRODUCTION

Protein hydrolysis, also known as peptide bond breakdown, can be caused by either an enzymatic or chemical process. Enzymatic hydrolysis may be performed under mild conditions and has no negative impact on the nutritional value of the protein source. Furthermore, because various enzymes are active at different temperatures, they provide for temperature flexibility in the processes to be catalyzed. Protein denaturation, on the other hand, is defined as the loss of biological activity caused by structural changes in proteins caused by physical or chemical factors such as pH, temperature, salt, detergents, organic solvents, or chaotropic agents (Sinha & Khare, 2014). Secondary, tertiary, and quaternary structures are greatly influenced by denaturation, resulting in smaller peptides.

Recently, Thermostable Alkaline Protease Enzyme (TAPzyme) was identified in water samples from *Lojing* hot spring (Kelantan, Malaysia) to have the potential to be used as a laundry detergent component as well as an eco-friendly enzymatic dehairing of animal hides (Ibrahim et al., 2019). To date, TAPzyme has also been utilized as a catalyst to hydrolyze the protein of the black soldier fly (BSF), *Hermetia illucens* (Mohd Zuki et al., 2020). This study provides new insights into the effectiveness of salt and protease in conducting protein hydrolysis and protein denaturation of BSFL's protein.

MATERIALS AND METHODS

Protein hydrolysis of BSFL

The protein hydrolysis of BSFL was performed by using the Thermostable Alkaline Protease enzyme (TAPzyme) and sodium chloride (NaCl). TAPzyme with a specific activity of 2205 U/mg was introduced to treat the BSFL meal. The following treatments employed a total of 5 grammes of BSFL meal: control (C), 10% NaCl (T1), and 10% TAPzyme (T2). For 24 hours, the samples were put in an incubator shaker at 50 °C (C, T1 and T2) with pH 9 and a rotation speed of 150 rpm. To separate supernatant and pellet, the samples were centrifuged at 4000 rpm for 30 minutes at 25°C.

Determination of protein concentration

The protein concentration of BSFL's protein was measured using the Bradford assay. The absorbance of the sample (C, T1, and T2) was determined using a Shimadzu UV-Vis 1900 spectrophotometer, set at a wavelength of 595 nm. Based on the bovine serum albumin (BSA) standard curve (R2=0.9978), the protein concentration of BSFL's protein was calculated.

Determination of percentage of protein concentration decreased

The protein concentrations of untreated BSFL, (C_o) and protein concentrations of untreated BSFL, (C_1) were determined to calculate the percentage of protein concentration decreased, as shown in equation Eq. (1).

$$\text{Protein concentration decreased (\%)} = \frac{Co - C1}{Co} \times 100\% \quad (1)$$

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the percentage of protein concentrations decreased, followed by Tukey's HSD (real significant differences) for post-hoc testing to compare the significance (p) between the means of different BSFL meal treatments. The significant difference between the mean and standard error of the mean was declared at ($p < 0.05$) (SEM).

RESULTS AND DISCUSSION

There were significant differences in the percentage of protein concentration decreased across treatments, as shown in Table 1. Treatment 1 (10% NaCl) had the lowest percentage of protein concentration decrease (10.36 ± 0.84%) and was significantly different from all other treatments ($p < 0.05$), respectively. Treatment 2 (10% TAPzyme) exhibited a higher percentage of protein concentration decreased (36.19 ± 0.80%). The fact that the percentage of protein concentration decreased was an intriguing discovery for evaluating the effectiveness of TAPzyme and NaCl in hydrolyzing and denaturing the protein structure into smaller peptides.

Table 1. Percentage of protein concentration decreased of treated BSFL meal

Treatment of BSFL meal	Protein concentration decreased (%)	p-value %
C	0.00 ± 0.00^{a}	0.000
T1	10.36 ± 0.84^{b}	0.000
T2	36.19 ± 0.80^{c}	0.001

Means with different superscript letters (a-f) within the same column differ significantly (Turkey test, $p < 0.05$), Control (C); 10% NaCl (T1); and 10% TAPzyme (T2)

Effect of salt, and protease on the percentage of protein concentration decreased of BSFL

In Treatment 2, 10% TAPzyme resulted in a higher percentage of protein concentration decreased, 36.19%. TAPzyme was previously employed in a previous study to hydrolyze the protein of BSFL at optimum circumstances as determined by Response Surface Methodology (RSM)(Mohd Zuki et al., 2020). Furthermore, the percentage of protein concentration decreased to 32.08%, which was nearly identical to the current study (Mohd Zuki et al., 2021). Also, existing research has also identified the importance of the enzymes bromelain and alcalase as catalysts in the production of protein hydrolysate from BSFL and lead tree seed (Firmansyah & Abduh, 2019).

Table 1 showed that Treatment 1, 10% NaCl, had the lowest percentage of protein concentration decreased. According to the table, protein denaturation was unable to partly unfold diverse protein structures into smaller peptides. A prior study found that NaCl denatured protein structure better than potassium chloride (KCl)(Mohd Zuki et al., 2021). However, NaCl was unable to outperform TAPzyme in performing protein hydrolysis in 24 hours.

CONCLUSION

Protease treatment revealed that the BSFL protein can be partially degraded into smaller peptides. These findings also indicated that protein hydrolysate from BSFL may be utilized to improve protein absorption in animal feed. More research is needed to completely understand the impact of feeding protein hydrolysate from insect meal, such as BSFL meal, to animals.

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FS10

Texture of High-Pressure Cooking Toli Shad (*Tenualosa toli*) Fish Marinated with Velvet Tamarind Paste

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ABSTRACT

Toli Shad (*Tenualosa Toli*) is a type of clupeid fish that possessed high amount of tiny bones and commonly used as dishes that contributes towards gastronomic ecosystem in Malaysia. In order to encounter the problem regarding of tiny bones existence while consuming toli shad, high pressure and thermal processing are two major treatments that effective in fishbone softening. Effect of these two treatments on fishbone softening could be enhanced by acidic catalyst. Velvet tamarind is a wild edible species fruits in Malaysia that contain high amount and versatile type of organic acid which suitable to be processed into paste for marinating purpose. This study focused on analysis of hardness values of toli shad treated with designated high pressure processing time with fix pressure value 2 atm and temperature within value of 115°C-120°C. Toli shad will be marinated with different amount of velvet tamarind paste for 24 hours before treated with high pressure processing with different designated processing period. Hardness value of fishbone decrease significantly as amount of marinated paste and high-pressure processing time increase. This is due to depleting and diffusion of calcium ion from fishbone structure.

Keywords: Toli shad, velvet tamarind, high pressure, paste, marinating

INTRODUCTION

Toli shad is a sub genus *Tenualosa* species that could be spotted in river and coastal region of east Malaysia (Sabah & Sarawak) locally known as *Terubok*. Aside from soft flesh and high in fat content that contribute towards sensory satisfaction, toli shad also possessed abundant amount of PUFA that are beneficial to avoid cardiovascular disease. However, due to existence of tiny bones within the flesh, toli shad usually regards and categorized under inconvenience food. Moreover, process of separating those bones from flesh considered unpractical and time wasting that also could produce food waste (Kasuma et al., 2019).

Meanwhile, velvet tamarind is a wild edible species fruit that usually consumed as sweet or processed into juice. Sour and savoury taste of this fruit are due to significant amounts of organic acid and other phytochemicals such as minerals, sugars, tartaric acid, citric acid, malic acid, ascorbic acid and Niacin (Okudu et al., 2017). Acidic condition is a significant factor in enhancing bone softening aside with high pressure & thermal treatments. Currently, Min et al. (2019) utilize acetic acid in promoting bone softening of conger eel.

Fishbone softening is an economic ways in reducing the waste produced from fisheries sector while establishing the convenience ways in optimizing the nutrition intake from this component (Nawaz et al.,

2020). High pressure processing is an effective method to enhance fishbone softening and acidic treatment (marinating) toward the fish will reduce the time taken to complete the softening process.

MATERIALS AND METHODS

Velvet tamarind paste preparation

Velvet tamarind will be prepared by discarding mesocarp and seed. Then, the collected endocarp of velvet tamarind will be weighed until reached 3 kg of weight and will be mixed with 3.5 kg of mixture on chilli paste, pepper and pepper powder, 500g of sugar and 60g of salt with estimated yield of 7kg velvet tamarind paste.

Fish samples preparation

Fish samples will be prepared by three samples for each amount of marinated paste and high-pressure processing time to indicate three replications (n=3). Fish samples will be preliminary processed by gutting and cleaning. The samples will be cut at dorsal part for marinating purpose. Then, the samples will be air dried for 45 minutes. The dried samples will be weighed and its length of will be measured. After that, samples will be marinated with different amount of velvet tamarind paste (0 [control], 50g, 100g, 150g & 200g) for 24 hours.

High pressure processing of marinated fish samples

Two liters of distilled water will be poured into pressure cooker for high pressure steaming process. Then, marinated fish will be arranged neatly on steamer rack and padded with high pressure resistant pad to avoid fish from attached within the samples. Then, stove will be ignited with moderate flame (blue flame) until steam constantly diffuse out from pressure regulator. Time will be recorded starting from the initial diffusion of steam from pressure regulator until reached desired time (30, 45 and 60 minutes). Upon reaching desired time, ignition of stove will be stop and remaining steam will be forced to diffuse out by removing the pressure regulator cap. Then, the samples will be allowed to cool for 40 minutes before being weighed and measured.

Texture profile analysis

Processed samples will be divided into three parts of fish which are head, vertebral and tail with measurement of 1/3 of fish length for each part. Then, hardness value of samples will be analysed followed the procedure from (Hemung & Sriuttha, 2014) with slight modification in types and number of samples used. Samples will be tested using Texture Analyzer (TA-XT2) using cylindrical probe. Diameter of the cylindrical probe is 25 mm and the pre- and post-speeds will be fixed at 5 mm.s-1 both. The samples will be compressed in two consecutive cycles until they reached 75% of the original sample height with a 2 s interval between cycles.

EXPECTED RESULTS AND DISCUSSION

Hardness values of Toli Shad Fish

Increasing in amount of marinated velvet tamarind paste will decrease high pressure processing period (Figure 1.). This could be represented by hardness values of fishbone structure at each high-pressure processing time interval. This occurs due to diffusion of calcium ion from fishbone structure.

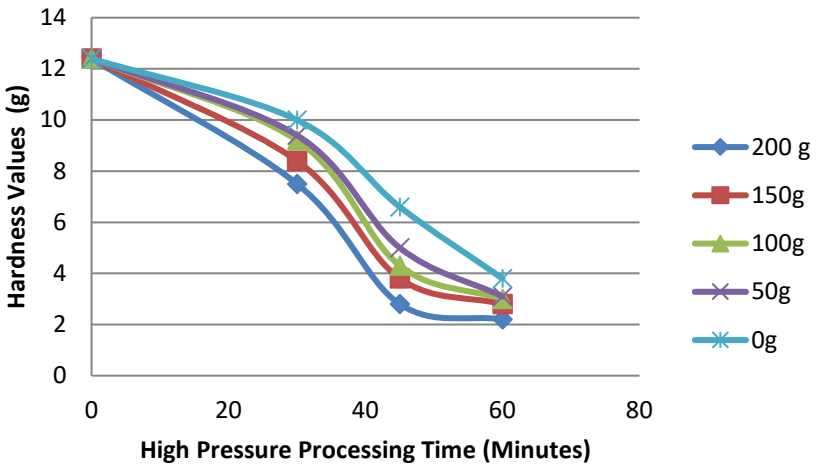

Figure 1. Expected Hardness Values of Toli Shad Bone as Affected by Amount of Marinated Velvet Tamarind Paste and High-Pressure Processing Time

CONCLUSION

Toli shad is a good source of food which contain significant amount of protein, calcium and Polyunsaturated Fatty Acid (PUFA) such as omega 3 and omega 6. Velvet tamarind possessed decent amount and versatile types of organic acid that could enhance fishbone softening. By marinating fish with velvet tamarind, high pressure processing time could be reduced during production of soft boned fish.

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Scope
Product Development (PD)

PD01

Total Phenolic Content of Stingless Bee Honey with Treated Cornsilk Extract

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ABSTRACT

Stingless bee honey is known for its nutritional and healing properties. Cornsilk is the yellowish thread found on top of corn fruits which usually been discarded as by product. This research is aimed to determine the total phenolic content of treated cornsilk extract added with stingless bee honey. The cornsilk either steam or hot water blanched with the CS/HS ratio of 5:0, 4:1, 3:2 and 0:5 were prepared. The different concentration of cornsilk extract with honey were analysed for the total phenolic content using Folin- Ciocalteau colorimeter method. The data was expressed in mg gallic acid equivalent (GAE) per 100 g dried cornsilk. The result showed the total phenolic content of cornsilk was increased after the blanching treatments. Before adding stingless bee honey, the cornsilk treated with steam blanching contained the highest total phenolic content, 55.917 ± 0.992 mg GAE/ 100 g dried cornsilk. Meanwhile, the cornsilk that treated by steam blanching with CS/HS ratio (3:2) has the highest total phenolic content, 62.727 ± 0.239 mg GAE/ 100g dried cornsilk. This showed the blanching treatment and the addition of stingless bee honey was enhanced the total phenolic content of treated cornsilk extract.

Keywords: Stingless bee honey, Cornsilk, Blanching, Total Phenolic Content

INTRODUCTION

Honey is the creation of the bee in the process of delivering nectar back to the hive. The quality and composition of honey is affected by the origins, environment and physical treatment. The common species of stingless bee that can be found in Malaysia, the *Trigona sp.*, is also known as "*lebah kelulut*". Compare to others honey with sweet taste, stingless bee honey brings a unique sourness. Honey is used as natural flavouring, preservation, and also in tradition therapy. The phenolic acids, flavonoids and others constituents of stingless bee honey have been contributed on its antioxidant properties (Moniruzzaman et al., 2013).

Cornsilk or *Zea mays* hairs is the purple yellowish stigmas from corn flower. As one of the by-products from corn production industry, cornsilk contains nutrients and bioactive compounds that benefit to human. The phytochemical compounds in cornsilks contribute to its strength of antioxidant. Phenolic compounds are one of the phytochemical compounds that with one or more hydroxyl groups. In the presence of phenolic compounds, the reduction of Folin-Ciocalteau (FC) reagent will occur and produce the molybdenumtungsten blue which can be measured under spectrophotometer (Malta & Liu, 2014). This research was aimed to determine the total phenolic content of treated cornsilk extract added with stingless bee honey.

MATERIALS AND METHODS

Cornsilk preparation

The baby corns (*Zea mays*) were purchased from local vegetable supplier in Tanah Merah, Kelantan. The fresh cornsilks were detached from the baby corn and subjected for three different treatments that were non-treated (NT), steam blanching (SB) and hot water blanching (HWB). Blanching was carried out for 2 mins. All cornsilks were then dried in an oven (Protech Model FAC-350H, USA) at 55 °C for 24 h. The dried cornsilks were grounded into powder form, sieved and stored in screwed cap bottle at 4 °C until used.

Cornsilk extraction

The non-treated (NT), steam blanching (SB) and hot water blanching (HWB) cornsilks (25.0 g each sample) were extracted in 250.0 mL distilled water using a homogenizer (IKA T25 Digital Ultra Turrax, Germany) at 10,000 rpm for 15 min. The extract was filtered with Whatman No 1. filter paper, centrifuged (10, 000 x g) for 15 min.

Preparation of cornsilk and honey extract sample

In a 10 ml centrifuge tube, cornsilk extract (CS) was mixed with stingless bee honey (HS) with the CS/HS ratio of 5:0, 4:1, 3:2, and 0:5 and mixed well using a vortex mixer.

Total phenolic content

The total phenolic content of treated cornsilk and honeys were determined by using Folin-Ciocalteau calorimetric method. A 1.0 mL of sample was pipetted into a 10.0 mL volumetric flask, followed with addition of 1.0 mL of FC reagent (0.2 N). The mixture was allowed to stand at room temperature for 6 min before added with 1.5 mL of sodium bicarbonate (20% w/v) and distilled water. The mixture was kept in the dark condition for 1h 30 min. The absorbance of samples was recorded at 765 nm against the blank. The total phenolic content was compared with the gallic acid standard curve covering the concentration of 5 - 80 µg/mL. Samples were measured in triplicate analysis.

Statistical analysis

The results were expressed as means ± standard deviation of triplicate analysis.

RESULTS AND DISCUSSION

The more electrons were been donated, the higher intensity of blue coloured complex, indicated the higher total phenolic content of sample (Vernon et al., 1999) The Folin-Ciocalteau (FC) reagent will be reduced and changes the colour from yellow into blue in alkaline solution. The total phenolic content of samples was analyzed by the modification method of Nurhanan et al. (2012). The total phenolic content was expressed in mg GAE/ 100g dry cornsilk powder (CSP). Without the additional of stingless bee honey., the SBCS with 55.917 ± 0.992 mg GAE/ 100g dried cornsilk has the highest total phenol of content which was slightly higher than HWBCS and NTCS with 55.609 ± 1.524 and 50.802 ± 1.316 mg GAE/ 100g dried cornsilk respectively. This may due to some of the phenolic compounds slightly dissolve in hot water during blanching treatment. The blanching treatment may help to break the cell wall which improve the absorption of bioactive compound during the extraction (Priecina et al., 2018). Based on Table 1, the total phenolic content of cornsilk increased by adding with different concentration of stingless bee honey. From the table, the total phenolic content of treated cornsilk can be increased up to similar with pure stingless bee honey with the CS/HS ratio 4:1 compared to the non-treated cornsilk.

Table 1. Total Phenolic Content of Treated Cornsilk Extract with Stingless Bee Honey.

Sample (CS／HS)	Total Phenolic Content (mg GAE/ 100g Dried Cornsilk)		
	Non-Treated (NT)	Steam Blanching (SB)	Hot Water Blanching (HWB)
5:0	50.802 ± 1.316	55.917 ± 0.992	55.609 ± 1.524
4:1	52.254 ± 1.927	59.174 ± 1.090	60.626 ± 1.278
3:2	54.751 ± 1.196	62.727 ± 0.239	62.628 ± 1.010
0:5	58.976 ± 3.400	58.976 ± 3.400	58.976 ± 3.400

CS – Cornsilk Extract; HS - Stingless Bee Honey.

CONCLUSION

In conclusion, the study showed that blanching treatment and the additional of stingless bee honey may help in improving the phenolic content of dried cornsilk. Hence, the combination of stingless bee honey with treated cornsilk extract has potential to enhance the scavenging ability of free radicals and antidiabetic activity.

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PD03

Optimization of Watermelon Rind Extraction Conditions by Sonication Extraction

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ABSTRACT

Watermelon white rind is considered the most underutilized watermelon fruit resource as it is usually discarded as waste due to unappealing in flavour. Watermelon rinds are rich in antioxidant compounds, carbohydrates, fibre and wax. Utilizing watermelon rind could decrease the amount of biological waste, thus recognizing the rind's potential economic value in various industries, mainly in food and cosmeceutical products. This study aims to determine the optimal extraction conditions in extracting watermelon rind using a sonicator extraction procedure. Two samples subjected to oven and dehydrator drying were evaluated using a one-factor-at-a-time (OFAT) experiment to identify the most significant factor for the sonicator extraction time (1 – 5 h) parameter. This method resulted in the optimal extraction time for the oven drying watermelon rind sample was 1 h, and the maximum extraction yield was 15 %. On the other hand, the optimal extraction time for the dehydrator drying sample was 1 h, and the maximum yield was 10%. Results indicated that the extraction time parameter plays a significant role in watermelon rind extraction.

Keywords: Watermelon rind, *Citrullus lanatus*, OFAT, optimization, sonicator extraction

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INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is an important crop that belongs to the family Cucurbitaceae. Generally, the three main parts of watermelon are flesh, rind and seed. Watermelon flesh contribute to 68% (w/w) of overall fruit mass, 30% (w/w) is watermelon rind, and the other 2% (w/w) is leftover (Ramakrishnan et al., 2020). The composition of watermelon rind mainly consists of 13% (w/w) pectin, 10% (w/w) lignin, 23% (w/w) hemicellulose and 20% (w/w) cellulose depending on watermelon genotype.

Watermelon rind waste is often utilized in food products such as pickles, stir-fried, stewed and high fibre flour for baked food (Adegunwa et al., 2019). In the pharmaceutical industry, watermelon rind extract in L-citrulline has been used as a dietary supplement to treat certain urea cycle disorders (UCD) (Johnson, 2017). Watermelon rind pectin is widely used as a food additive in the food industry and as a biopolymer in pharmaceutical industries. Other than that, watermelon rind can be converted into a bio sorbent material to remove heavy metals from wastewater (Lee & Choo, 2020; Ramakrishnan et al., 2020).

Watermelon rind has potential economic value in various applications such as food additives, anti-ageing ingredients in cosmeceutical products, and dietary supplements. Determining an optimal condition for

watermelon rind extraction is vital to optimizing the extract yield for industrial application. The one-factor-at-a-time (OFAT) design evaluation was applied to study one significant factor at a time instead of multiple factors simultaneously. The present study evaluates the effect of different extraction times for extraction yield maximization of two different sample conditions, oven drying and dehydrator drying sample. At the same time, the other variables were fixed during the experiment.

MATERIALS AND METHODS

Pre-extraction

Watermelon fruits were obtained from a local market in Kelantan, Malaysia. The red fleshy pulp and green peel were removed, the white rind was cut into a cube with 10 mm thickness. The sample was dried at 60 °C temperature for 48 h in two different drying conditions; a convection oven and a food dehydrator. The dried watermelon rind was refined using stainless steel grinder and sieve into a fine powder (500 μm). Ground watermelon powders were kept in the desiccator silica gel at room temperature before extraction.

Extraction

The watermelon rinds extraction was conducted using the sonicator extraction technique. The samples were extracted with 80 % ethanol with a ratio (1 g: 20 ml) in a flask placed in an ultrasonic bath sonicator at 40 kHz and then centrifuged at 2,800 xg for 10 min. The supernatant was filtered through 180 mm filter paper, and the solvents were removed at 60 °C via a rotary evaporation system.

One-factor-at-a-time (OFAT) evaluation

One-factor-at-a-time (OFAT) design evaluation was applied to study experimental factors of sonicator extraction time to five points: 1 h, 2 h, 3 h, 4 h, and 5 h. The fixed-parameter is a solvent-to-solid ratio (1 g: 20 ml), ethanol concentration (80 %) and sample drying time (48 h).

RESULTS AND DISCUSSION

One-factor-at-a-time (OFAT) was carried out to simplify the evaluation by analyzing a single factor, extraction time, to reduce the time and cost of the experiment. Table 1 shows the influence of sonicator extraction time on the yield of watermelon rind after 10 single factor runs. As seen, oven drying sample at 1 h extraction time had achieved the maximum extraction yield of watermelon rind followed by dehydrator drying at 1 h extraction time with yield 15 % and 10 %, respectively. The result was higher than Lee and Choo (2020), as their yield of watermelon rind pectin obtained using ultrasound-assisted extraction was 8.38 %. This might be due to the different ultrasonic frequencies applied during extraction to disrupt plant cell walls for solvent penetration.

Table 1. Influence of extraction time on extraction yield of watermelon rind extract using OFAT experiment.

Std	Sample	Run	Extraction time (h)	Extraction yield (%)
1	Dehydrator drying	1	1	10
3	Dehydrator drying	2	3	8
4	Dehydrator drying	3	4	9
2	Dehydrator drying	4	2	10
5	Dehydrator drying	5	5	8
7	Oven drying	6	2	13
9	Oven drying	7	4	8
6	Oven drying	8	1	15
10	Oven drying	9	5	9
8	Oven drying	10	3	9

The extraction yield of watermelon rind decreases from 1 h to 5 h extraction time, as shown in Figure 1. Longer extraction time resulted in the reduction of yields due to thermal degradation of the sample. This is because the ultrasonic process during extraction also acts to heat the fluid up to 42 °C. Abubakar and Haque (2020) claimed that the high sound and thermal energy might cause the degradation of plant active constituents by producing free radicals.

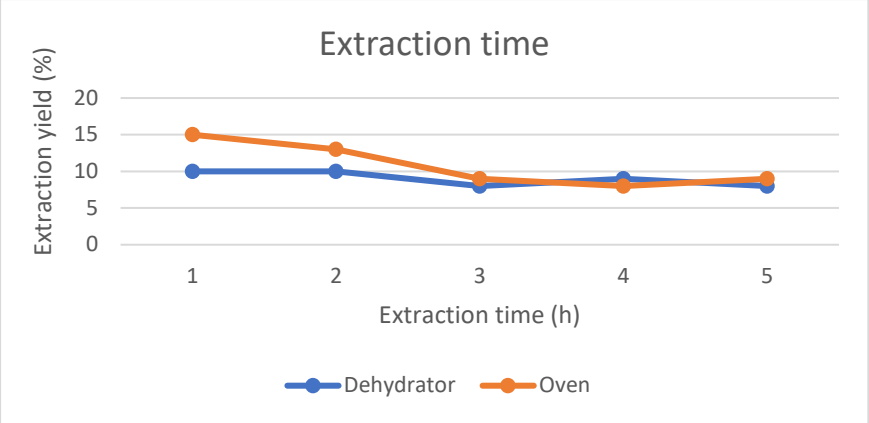

Figure 1. Extraction yield of watermelon rind at different extraction time parameter.

CONCLUSION

The extraction yield of the watermelon rind was optimized at a 1 h extraction time. Longer extraction times leads to degradation of sample and reduced the extraction efficiency. The oven-drying sample shows better yield recovery than the dehydrator drying sample. OFAT experiment was used to determine the most significant factors to be used before optimizing the variables using Response Surface Methodology (RSM).

ACKNOWLEDGEMENT

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PD04

Optimization of Extraction Conditions of Ultrasonic-Assisted Extraction (UAE) Technique for the Analysis of Antioxidant in *Solanum lycopersicum*

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ABSTRACT

Tomato is rich in bioactive compounds, primarily carotenoids and lycopene, respectively. Tomato plants contain vitamins A, C and E that help improve healthy skin, especially lips. Thus, it is crucial to extract these plants to be applied in cosmetic products. This study focuses on the extraction of tomato samples using ultrasonic-assisted extraction techniques and observation of the extract's antioxidant content. The process factors in extraction, including extraction time, solvent ratio and temperature, were utilized to find the optimum extraction conditions for each extraction method. Antioxidant content was observed using the Thin Layer Chromatography technique using DPPH as a reagent. Central Composite Design (CCD) was used to investigate process variables' effect on the extraction yield. The optimum condition for the extraction yield of tomato is 1.41 g. All of the samples contained antioxidants with an Rf value above 0.90.

Keywords: Extraction, ultrasonic-assisted extraction, antioxidant, response surface methodology, optimization

INTRODUCTION

It is increasing in popularity towards plant active compounds as cosmetic products ingredients because of its benefits in protecting and curing the skin (Saraf & Kaur, 2010). Fruits and vegetables contain phenolic compounds such as flavonoids, tocopherols, phenolic acids, alkaloids, chlorophyll derivatives, and carotenoids that serve high antioxidant content possess many health benefits towards human consumption. The phenolic compound of fruits or vegetables is based on plant origins. Extraction from different types of fruit or vegetables will result in extra capacities of phenolic compounds. Deep coloured fruits and vegetables were reported to have good sources of phenolic, including flavonoids, carotenoids and antioxidants. Several deep coloured vegetables, such as tomatoes, were said to have a high amount of bioactive compounds and strong antioxidant activity (Chi et al., 2012).

Tomatoes is a plant that mainly grows tropical vegetables worldwide and is processed in various ways of products. *Solanum lycopersicum* or commonly known as tomato, originated from the family of *Solanaceae*. Tomato fruit has a short shelf life under ambient storage conditions. The short post-harvest life of tomatoes will lead to an increase in post-harvest losses. Lycopene is the most abundant carotenoid found in the tomatoes flesh (Bashir et al., 2014). Tomatoes have many benefits to human health due to their bioactive compounds contain. Hence, this study aimed to optimize the ultrasonic-assisted extraction method and observation of antioxidants contained in tomatoes.

MATERIALS AND METHODS

Ultrasonic-assisted extraction (UAE)

Tomato samples were dried using a dehydrator before being grounded and sieved. 2 g of the samples were extracted using conditions listed in Table 1.

Table 1. Design of experiment by Design Expert Software.

Sample	Temperature (°C)	Solvent concentration (%)	Extraction time (h)
1	40	20	1
2	40	100	1
3	53	60	3.5
4	74	60	3.5

Antioxidant analysis

A DPPH reagent and solution for the mobile phase were prepared. A crude extract sample was used as a sample dot on the TLC paper. Rf value was observed and calculated using the formula:

Rf value = Distance travelled by the compound ÷ Distance travelled by the solvent front

RESULTS AND DISCUSSION

Investigation of extraction factor parameter influences

Table 2. Result of the extraction yield.

Sample	Temperature (°C)	Solvent concentration of ethanol (%)	Extraction time (h)	Crude extract (g)
1	40	20	1	0.36
2	40	100	1	0.83
3	53	60	3.5	1.41
4	74	60	3.5	1.07

The result in Table 2 showed that the optimum condition and the highest extraction yield of 1.41 g was on the tomatoes sample with a temperature of extraction of 53°C, the solvent concentration of ethanol of 60% and extraction time 3.5 hours. In contrast, the lowest was 0.36 g at 40°C, 20%, one hour for the extraction temperature, the solvent concentration of ethanol and extraction time, respectively. As stated by Eh and Teoh (2012), all of the displayed parameters influenced the extraction yield value. As the temperature, solvent concentration and time increase, the extraction yield increases until it reaches the optimum value. At a temperature of 74°C, the extraction yield result was low compared to 53°C. It might be due to the above optimum temperature value causing to degradation of the sample. As reported by Silva et al. (2018), the increase of extraction yield was related to the rise of temperature and time until it reached an optimum level and caused to degradation of the sample.

Antioxidant observation

Table 3. Rf value result of antioxidant

Sample	Crude extract (g)	Rf value for antioxidant
1	0.36	0.90
2	0.83	0.92
3	1.41	0.95
4	1.07	0.92

Table 3 presents the Rf value for each of the tomato samples from different parameter factors. The result showed that all of the crude extracts of samples contain antioxidants. The factors applied in the extraction did not cause the antioxidant to degrade.

CONCLUSION

In conclusion, the optimum condition for the tomatoes extraction using the UAE method was at 53°C, 60% and 3.5 hrs for temperature, solvent concentration and time factors, respectively. All of the samples treated by different parameters do not affect the antioxidants in the samples.

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This study was supported by the Ministry of Education Malaysia through the Fundamental Research Grant Scheme for Research Acculturation of Early Career Researchers (FRGS-RACER,R/FRGS/ A0700/01552A/003/2019/00665) and Universiti Malaysia Kelantan , UMK Prototype Research Grant (UMK-PRO 2020, R/PRO/A0700/01552A/004/2020/00872). This support is gratefully acknowledged.

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PD05

Total Phenolic and Flavonoid Content and Chemical Profiling Using GCMS of *Cocos nucifera* Sap (CNS) and its Extract

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ABSTRACT

Diabetes Mellitus (DM) is categorized under non-communicable diseases (NCDs) and become one of the prevailing diseases in Malaysia. There is growing attention to avail of natural products in replacing table sugar as an alternative remedy in therapy for diabetes and has uncovered a good status. *Cocos nucifera* sap (CNS) also known as 'neera' is the sweet, oyster-white colored sap collected from immature coconut spadix (inflorescence) and has been reported to have a low GI index. This study aims to profile the phytochemical compounds of the extract(s). The CNS was extracted using hexane and ethyl acetate. Total phenolic and total flavonoid content of ethyl acetate has higher total phenolic content (19.25 ± 1.24 mg GAE/g extract) and flavonoids (2.50 ± 0.11 mg RE/g extract) than hexane extract (phenolic: 12.46 ± 0.04 mg GAE/g extract, flavonoids: 1.52 ± 0.08 mg RE/g extract) and CNS (phenolic: 6.35 ± 0.81 mg GAE/g extract, flavonoids: 1.30 ± 0.11 mg RE/g extract). GC-MS analysis of the extract(s) identified seven volatiles compounds where the major volatile components were 2- Furancarboxaldehyde, 5-hydroxymethyl, and 4H Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-. These results suggest that CNS and its extracts would have higher potential as nutraceuticals and could serve as natural alternatives to anti-diabetic remedies.

Keywords: *Cocos nucifera* sap, GI index, phytochemicals compound

INTRODUCTION

Nowadays, many food products that contain sugar-free, low-sugar, and synthetic sugar are available in food markets. However, these sugars are considered unsafe and unhealthy, because they contain a high glycaemic index (GI) that can cause side effects including diabetes and weight gain. The consumption of natural products for preventing diabetes is supported in a fraction of traditional systems of medicine. CNS is a traditional beverage consumed in most ASEAN countries including Malaysia. CNS is known as 'Tuak' has the sweet taste and translucent juice derived from coconut inflorescence sap. It is popular in the East Coast of Malaysia (Kelantan, Terengganu, and Pahang); was reported to have a low GI index (35) compared to other tables sugar such as palm sugar and sugarcane at 42 and 58-82, respectively (Saputro et al., 2017). Thus, this study was to investigate the antidiabetic properties of CNS as the therapeutic potential for diabetic patients.

MATERIALS AND METHODS

Sap preparation and extraction

CNS were collected from Kampung Durian Pahit, Ketereh, Kelantan. The fresh sap was harvested from cutting the inflorescence spadix of the coconut tree. The sap was collected using a container that is tied up near the trunk and leaves it about 9-12 hours. CNS will be freeze-dried at -55 ℃ until it becomes a powder. Then, CNS was extracted using the liquid-liquid extraction method as described in the previous method (Othman et al., 2015). CNS powder (5 g) was dissolved in 200 mL of distilled water. Two different polarities of solvents (hexane and ethyl acetate) were used. Each partition was conducted in triplicate and the fraction was mixed and was dried using a rotary evaporator. The dry weight of each partition was recorded as a percentage (%) yield of the fraction. The fractions were kept in the freezer until further analyses.

Total phenolic content

The total phenolic content of the extract(s) was measured using a 96 well plate reader as described by the standard method in Yusof et al. (2015). Briefly, Folin–Ciocalteu reagents (50 μL) were diluted with distilled water in a ratio of 1:10. The mixture was incubated with the extract(s) (50 μL) at various concentrations (6.3, 12.5, 25.0 and 50.0 mg/mL) for 20 min in the dark. 7% of sodium carbonate (35 μL) was added later. Following another 20 min of incubation, the absorbance was measured at 750 nm using a spectrophotometer. Gallic acid standard at various concentrations (0–200 μg/mL) will be used to generate the calibration curve. The total phenolic content will be reported as mg gallic acid equivalent/g of the dry extract.

Total flavonoid content

The total flavonoid content of the extract(s) was determined according to the standard method described in Yusof et al., (2015). The extract(s) (50 μL) at various concentrations (6.3, 12.5, 25.0 and 50.0 mg/ mL) were mixed with 10% aluminium chloride hexahydrate solution (5 μL), 1 M potassium acetate solution (5 μL) and distilled water (140 μL). The mixture was incubated for 40 min in the dark. After incubation, the absorbance was measured at 415 nm using a microplate reader. The Rutin standard at various concentrations (0 – 200 μg/mL) will be used to generate a calibration curve. The total flavonoid content was represented as mg rutin equivalent/g of dry extract.

Gas chromatography-mass spectrum: chemical profiling of the extract(s)

Separations were accomplished with HP-5MS capillary column (0.25 mm i.d. x 30 m specification length x 0.25 μm film thickness, Agilent). The extract(s) (50 mL) were gently shaken with 20 mL of a 1:1 (v/v) mixture of diethyl ether: n-pentane in a separating funnel for 10 min and the aqueous phase was discarded. The organic phase was collected and mixed with anhydrous sodium sulfate (1.0 g) and was centrifuged at 9500 rpm for 20 min at 4ºC. The GC injector was set to the general splitless mode at a carrier gas (He) with a flow rate of 1.0 mL min–1. The oven temperature was set at 70ºC with a final temperature at 280 ºC after 35.50 min. The injection volume was 1 μL solution of extract (5 mg/mL). VOCs and non-VOCs were identified using library data in GCMS (NIST02).

Statistical analysis

Total phenolic and flavonoid contents were performed in triplicate and expressed as mean ± SEM. The results were analyzed using the one-way analysis of variance (ANOVA) followed by Dunnett's comparison test. Values with $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

Results in Table 1 showed that ethyl acetate extract has higher total phenolic content (19.25 ± 1.24 mg GAE/g extract) and flavonoids (2.50 ± 0.11 mg RE/g extract) than hexane extract (phenolic: 12.46 ± 0.04 mg GAE/g extract, flavonoids: 1.52 ± 0.08 mg RE/g extract) and CNS (phenolic: 6.35 ± 0.81 mg GAE/g

extract, flavonoids: 1.30 ± 0.11 mg RE/g extract). The effectiveness of phenolic compounds depended on the type, structure, number, and position of the hydroxyl group of the benzene ring. Flavonoids are more favored in more polar solvent extract where ethyl acetate extract shows the highest flavonoid content. There is a correlation between TPC and TFC where high phenolic content gives high flavonoid content.

Table 1. Total phenolic and flavonoid contents of CNS and its extract

Type of extract	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg RE/g)
CNS	6.35 ± 0.81	1.30 ± 0.11
Hexane	12.46 ± 0.04	1.52 ± 0.08
Ethyl acetate	19.25 ± 1.24	2.50 ± 0.11

The data represented as mean ± SEM of triplicate analysis.

Chemical composition of the extract(s)

The extract(s) were subjected to the chemical profiling analysis using GC-MS. The listed compounds in Table 2 are the compounds that match quality above 80% as compared with the NIST02 mass spectral database. Seven compounds were characterized in the extract(s), where hexane extract has the most compounds (6 compounds) followed by ethyl acetate extract (5 compounds) and CNS (3 compounds). All extract(s) showed highest total composition of 2- furancarboxaldehyde, 5-hydroxymethyl at 51.42% (hexane), 41.70% (ethyl acetate) and 41.03% (CNS) respectively. It possessed antioxidant and anti-diabetic properties. The differences in volatile components identified in the extract(s) are possible due to the different polarities of the solvent extract used.

Table 2. Chemical profile of the extract of CNS

SI No.	RT	Library/ID (NIST02)	Total Composition (%)		
			CNS	Hex	EA
1	3.386	3-Furaldehyde	nd	3.16	nd
2	3.400	Furfural	2.18	5.66	2.86
3	3.671	1-H-Imidazole, 1,5-dimethyl-	nd	3.13	nd
4	7.919	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.01	12.29	11.43
5	9.474	2- Furancarboxaldehyde, 5-hydroxymethyl	41.03	51.42	41.70
6	15.514	5-Methoxycarbonyl-2-thiophene carboxylic Acid Hydraxide	nd	0.10	nd
7	21.109	Cyclononasiloxane, octadecamethyl-	nd	nd	0.22

RT: Retention time, CNS: *Cocos nucifera* sap, Hex: Hexane extract, EA: Ethyl acetate extract, nd: Not detected

CONCLUSION

The total phenolic and flavonoid content of ethyl extract showed high composition at 19.25 ± 1.24 mg GAE/g extract and 2.50 ± 0.11 mg RE/g extract compared to other extracts. GC-MS analysis of the extract(s) exposed some medicinally important compounds (Table 2) along with the compounds responsible for the antidiabetic properties were the important findings in this study. The further pharmacological investigation should be done as it has numerous un-revealed aspects left behind which are still waiting to be discovered.

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PD07

Optimization of Turbidity Removal from Groundwater using Nanomagnetic Adsorbent Composite

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ABSTRACT

The widespread use of alternative water sources in Kelantan leads to the development of cost-effective methods for the purification of water. A simple and straightforward adsorption process using a nanomagnetic adsorption composite (NMAC) was introduced in this study as a new adsorbent for the treatment of turbid polluted groundwater. A 3^k maximum factor configuration of four factors; adsorbent dosage (0.02, 0.04, and 0.06 g), agitation time (15, 30, and 60 min), agitation rate (150, 200, and 250 rpm), and adsorbent scale (>45 µm and > 300 µm) was used for NMAC turbidity removal. The study of variance (ANOVA) and surface response methodology reveals that the turbidity removal efficiency of NMAC is affected by the four factors investigated. Of all the samples, 0.04g NMAC, 48 minutes agitation, 150 rpm, and size > 45 µm showed maximum adsorption at 98.96 percent efficiency. The findings showed that NMAC is a strong adsorbent for the treatment of raw water.

Keywords: Kelantan, nanomagnetic, turbidity, water

INTRODUCTION

Kelantan is one of the east coast states in Malaysia. The state was blessed with alluvial soil and groundwater. People in Kelantan were using groundwater via well or ages. The groundwater was important for their domestic usages, agricultural, and industrial activities. The advantage of groundwater was natural filtration as the water through soil and rock that filtrated the pollutants (Hamzah et al., 2014). Despite the advantage, the natural filters were highly affected by the weather. Heavy rain led to rain filtration through soil and rocks, therefore, polluting groundwater. The polluted groundwater would change aesthetically which later results in turbidity of the water (Abdul Aziz et al., 2020).

Adsorption method was suggested to overcome the turbidity problem as well as an alternative affordable water treatment method. In this study, nanomagnetic adsorbent composite (NMAC) was applied to purify the groundwater. The NMAC was biocarbon derived from agriculture waste and impregnated with iron oxide nanoparticles. The presence of iron oxide nanoparticles improved surface area for adsorption, so, providing efficient removal of turbidity from groundwater (Wannahari et al., 2018). Therefore, the primary aims for this study comprised of generating a model for the adsorption process by NMAC and determining optimized variables in the process.

MATERIALS AND METHODS

Batch adsorption

The adsorption was carried out with 10% adsorbent in the working volume. The percentage of turbidity removal was calculated as in Eq. 1.

$$\%\ Turbidity\ removal\ efficiency\ = \frac{Ci-Ce}{Ci} * 100 \quad (1)$$

Where initial concentration and residual concentration (NTU) of water sample (Ci) and (Ce) respectively.

Experimental design

The 3-Factorial design was adopted in the study using Design Expert v.11 software.

Table 1. Input factors for 3^k factorial design and their levels.

Types of factors	Factors	Symbol	Unit	Levels		
				-1	0	1
Numerical	Dosage of adsorbent	g	A	0.02	0.04	0.06
	Time of agitation	min	B	15	30	60
	Agitation rate	rpm	C	150	200	250
Categorical	Size of adsorbent	μm	D	< 45		>300

RESULTS AD DISCUSSION

Second-order polynomial model

The analysis of responses for each variable (Table 1) generated a second-order polynomial model (Equation 2). The regression of the model (R^2) was 0.9905 with a standard deviation of 0.3803. The Adjusted and predicted R^2 were 0.9878 and 0.9821 respectively. The lack of fit (Table 2) of the model was 0.3377 means that the model was adequate to optimize the variables.

$$Y_{\text{turbidity removal efficiency}} = +94.92-0.1561A+1.87B+1.58C+2.30D+0.0626AB-0.3449AC-0.5153AD -1.12BC-0.7042BD-0.9864\ CD-0.4321\ A^2-1.89\ B^2+1.37\ C^2$$

Analysis of the effects of the variables towards turbidity removal efficiency

Figure 1(a) shows that turbidity removal efficiency increased as the time of agitation and dosage of adsorbent increased. However, during 51 to 60 minutes, the turbidity removal became a plateau. Based on Figure 1(b), the increment rate of agitation and dosage of adsorbent is proportional to turbidity removal efficiency.

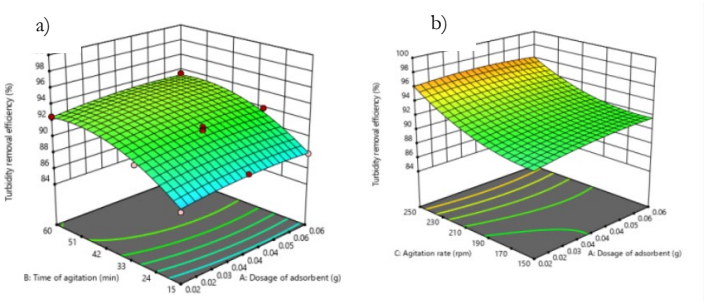

Figure 1. Interaction (a) between the dosage of adsorbent (g) and time of agitation (min); (b) dosage of adsorbent (g) and rate of agitation (rpm) for removing turbidity from groundwater sample by NMAC.

The verification test showed that the difference between experimental and predicted values based on the generated model for turbidity removal efficiency was 0.47%.

CONCLUSION

As a conclusion, the model generated by 3^k factorial design determined that the adsorption process was optimum at 0.04g NMAC, 48 minutes agitation, 150 rpm, and size > 45 µm. Thus, it showed that NMAC is a strong adsorbent with a high turbidity removal efficiency of 98.96%.

ACKNOWLEDGEMENT

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PD08

The Efficiency of Dry Leaf-based Biocarbon for Ammonia Removal in Aquaculture Wastewater

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ABSTRACT

Ammonia must always be measured in fish hatcheries. Higher ammonia content in aquaculture will cause severe fish stress and results in unexplained losses. The aim of this work is to study the efficiency of leaf biomass from local *Macaranga gigantea* as low cost bioadsorbent for ammonia (NH_3-N) removal. To ascertain NH_3-N removal capability, the initial ammonia concentration (10-100 mg/L), biocarbon dosage (0.125-0.500 g) and contact time (0-100 min) were investigated by batch sorption experiment. From this study, it was revealed that 0.250 g of biocarbon material able to remove NH_3-N as much as 42.55% to 79.53% at initial concentration of 10-100 mg/L and 60 min contact time ($p<0.05$). The SEM examination revealed that well developed and accessible internal pores were observed in the biocarbon structures that can absorb NH_3-N. Thus, this study affirms that the new biocarbon material derived from *Macaranga gigantea* had considerable potential for the removal of NH_3-N from aquaculture wastewater.

Keywords: Adsorption, leaf-based biocarbon, salicylate method, total ammonia nitrogen (TAN), carbonization.

INTRODUCTION

In the aquaculture industry, ammonia nitrogen becomes major problems for the fish farmers because it may cause poor growth rates, stress, damage gills, and death. The unionized NH_3-N is very toxic to fish while presence of NH^{4+} is harmless and more tolerant (Sichula et al., 2011).The interest in low cost and environmental friendly technology for water treatment are continuously increase nowadays. Biocarbon is an inexpensive material with a broad range of porous forms, resulting stronger adsorbing properties. Many research works have been conducted on biocarbon as absorbents such as for removal of antibiotics (Peng et al., 2016), dye and also heavy metal (Duan et al., 2016). The aim of this study is to explore the capability of leaf biomass of under-utilized tree species *Macaranga gigantea* as a new biocarbon precursor for removal of ammonia from aquaculture wastewater.

MATERIALS AND METHODS

Preparation of adsorbent

Dry *Macaranga gigantea* leave biomass was collected on the forest floor along the roadside in Jeli, Kelantan. They were washed with plenty of water to remove dirt and surface impurities before dried under the sun followed by oven drying at 110 ºC overnight to remove excess moisture. The dried leaves were grinded into fine powders and sieved to ≤150 μm particle size by using auto sieve shaker (Model: A060-01). Finally, they were introduced into the muffle furnace (Carbolite ELF 11/6B) at a specific temperature and holding time for pyrolysis process.

Preparation of real aquaculture wastewater

The stock solution of real aquaculture wastewater was collected from 30 L aquarium that stocked with twenty-five (25) fish of *Oreochromis sp.* with average length 8.0 ± 2 cm. The concentration of ammonia stock solution was tested before used. Different initial concentrations of ammonia (10-100 mg/L) were prepared from this stock solution by appropriate dilutions.

Test method

Batch adsorption studies were conducted at room temperature. Each experiment was carried out in Erlenmeyer flask where accurately weighed amounts of biocarbon was added to 25 ml of different initial concentration of ammonia solution. The mixture was shaken in an orbital shaker at 200 rpm for a given length of time intervals. Finally, the adsorbents were separated from the solution by filter paper and the filtrate was pipetted into the cuvette for analysis using spectrophotometer (DR6000) at 665 nm. Meanwhile, the surface morphology of the biocarbon was observed using a JEOL scanning electron microscopy instrument (Jeol JSM-IT100).

RESULTS AND DISCUSSION

Effect of contact time towards NH_3-N removal

Figure 1 (a) shows the percentage removal of NH_3-N at 10 mg/L carry out by varying the contact time from 10 to 100 min using 0.125 g biocarbon. The highest mean percentage removal of NH_3-N was recorded at 60 min with 38.35 % removal, significantly ($p<0.05$) higher due to increasing of contact collisions between adsorbent and wastewater. By prolonging the time duration, the removal rate was insignificant due to fully surface coverage by ammonia ions.

Effect of biocarbon dosage towards NH_3-N removal

The dosage of the biocarbon used was varied from 0.125 to 0.500 g. The adsorption study was conducted at an initial concentration of 10 mg/L and fixed contact time of 60 minutes. Figure 1(b) shows that the percentage removal were increased from 38.35% to 79.53% with further increasing of biocarbon dose to 0.250 g, which was significantly higher ($p<0.05$) than using 0.125 and 0.500 g, respectively. It was believed that 0.250 g biocarbon used will provide sufficient number of active surface sites for ammonia sorption per the unit area, while further increased to 0.500 g reduced the percentage removal of NH_3-N due to the aggregation of the biocarbon material in the solution.

Effect of initial concentration towards NH_3-N removal

The effect of varying initial concentration of ammonia solution (10-100 mg/L) towards NH_3-N removal was shown in Figure 1 (c). The highest mean percentage removal of NH_3-N was observed at initial ammonia concentration 10 mg/L (79.53%), significantly higher ($p<0.05$) than 20-100 mg/L. The percentage removal were decreased with increasing of their initial concentration, after 60 min agitation time. Lower initial concentration providing a better driving force for adsorption to occur due to many vacant sites available

on the biocarbon. However, since the amount of biocarbon used is similar for higher initial concentration, the percentage removal showed a decreasing trend due to finite active sites incomparable to the available unadsorbed NH_3-N.

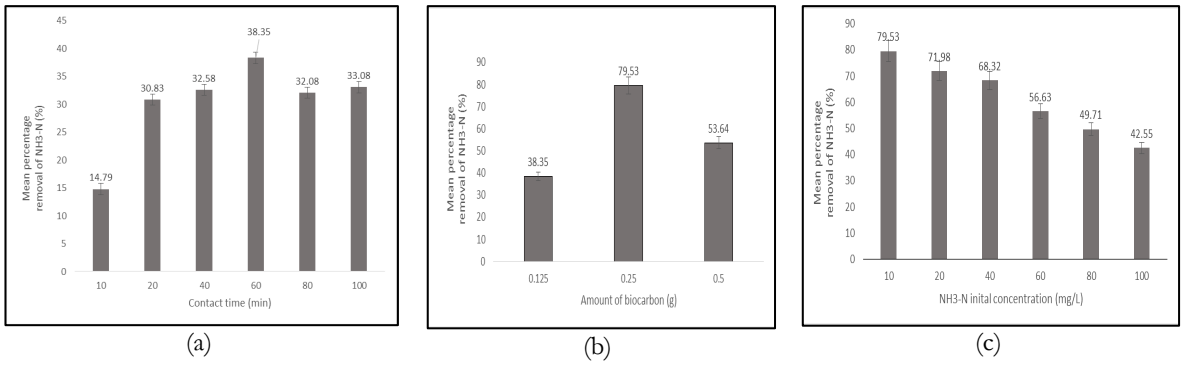

Figure 1. Mean percentage removal of NH_3-N against (a) contact time, (b) biocarbon dose, (c)initial concentration of NH_3-N.

Scanning electron microscopy (SEM)

The SEM micrograph of a newly prepared biocarbon exhibit a rough surface, non-compact with more openings and cavities due to evaporation of moisture and thermal degradation of volatile matters during pyrolysis process (Figure 2.).

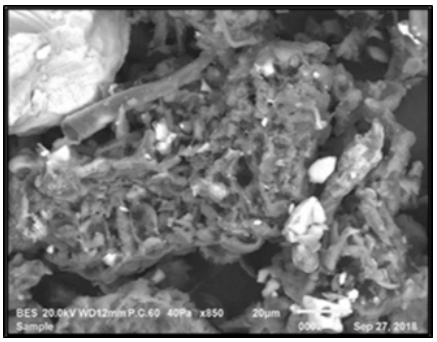

Figure 2. Scanning Electron Microscopy (SEM) micrograph

CONCLUSION

The percentage removal of NH_3-N was found to be dependent on the contact time, biocarbon dosage and initial ammonia concentration. The revealed pore like structure supported the capability of the biocarbon prepared from *Macaranga gigantea* had considerable potential for removal of ammonia from aquaculture wastewater.

ACKNOWLEDGEMENT

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PD09

Inhibition of Pro-inflammatory Mediators by Methanolic Extract of *Opuntia monacantha* Haw. (Cactaceae) in RAW 264.7 macrophages cells.

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ABSTRACT

Opuntia monacantha (Cactaceae), a cochineal prickly pear with various medicinal uses and consumed widely by local community in Mexico due its nutritional value and therapeutics effects, and known as an ornamental plant in Malaysia, with limited numbers of scientific studies. The objective of this study is **t**o clarify the anti-inflammatory activity and mechanisms of action of the cladodes of methanolic crude extract of *O. monacantha* (MEOM) and, phytocontituents profiling. The cladodes were extracted using methanol-maceration method and obtained the crude extract. The MEOM (12.5, 25, 50 and 100µg/mL) was tested using *in-vitro* models of inflammation in RAW 264.7. The MTT assay was used to evaluate cell viability and cytotoxicity, whereby Griess assay was used to determine NO concentration inhibition activity. Furthermore, to test the inhibitory effects pro-inflammatory mediators by MEOM, we were performed the ELISA assays for PGE_2, iNOs, COX-2 and TNF-α, and enzymatic assay for LOX in LPS/IFN-**γ** (inflammatory inducers). Finally, the chemical compounds were identified using UHPLC-Q-TOF/MS. MEOM contained anti-inflammatory properties; i) significantly did not affect the cells viability on the cytotoxicity test in dose-dependent manner, whereby, significantly reduced the NO production on the percentage of NO concentration inhibition at the all ranging of concentration of extract, respectively., ii) inhibition by MEOM, significantly in a dose-dependent manner in the LPS/IFN-**γ**-induced nitric oxide (NO), -PGE_2, -iNOs, -COX-2, -TNF-α and -LOX production levels in RAW 264.7, respectively. According to the library of UHPLC-MS spectra, it has been identified the MEOM contain 22 potential active compounds. The strongest peak identified as isoliquiritin, a flavonoid glycoside compound revealed the anti-inflammatory activity. The findings are in agreements to the traditional uses that consumed safely to treat of pain and inflammation with minimum and/or no side effects.

Keywords: *Opuntia monacantha*, inflammatory inducers, pro-inflammatory mediators, UHPLC-Q-TOF/MS.

INTRODUCTION

Inflammation is a type of ailments (Xueqin et al., 2016), that triggered by several harmful stimuli, either endogenous or exogenous inducers directly involved in several synthesis pathways of pro-inflammatory mediators such as prostaglandin, tumors necrosis factor that produced by macrophage (cell types) and alter the normal function of tissues and organ. Subsequently, several drugs currently used to suppress inflammation e.g., steroids, NSAIDs associated with several adverse effects. Therefore, *Opuntia monacantha* Haw (Cactaceae) known as cochineal prickly pear, is an important of medicinal plants is being study to evaluate the potential candidates as anti-inflammatory agents. In addition, cactus plants scientifically

revealed remarkable source of phytoconstituents (El Mostafa et al., 2014), possess of medical properties such as neuro-protector, anti-inflammatory, anti-cancer, etc.

MATERIALS AND METHODS

Sample preparation and extraction of MEOM

The cladodes of *O. monacantha* plant (voucher no: SK 2881/15) were collected from the coastal area of Tok Bali, Kelantan, Malaysia and certified by Dr. Syamsul Khamis, IBS, UPM, Selangor, Malaysia, and the procedure of extraction was carried out as described in details by Jaios ES et al. (2016), with slight modifications.

Inhibitory of the pro-inflammatory mediators by MEOM

Assessment on LPS/IFN-γ-induced NO production

The NO production was determined using the Griess reaction according to the method described in detailed by Zakaria et al. (2015) with slight modifications.

Assessment on LPS/IFN-γ-induced PGE_2, iNOs, COX-2, TNF-α and LOX production

The level of PGE_2, iNOs and COX-2, TNF-α, and LOX in the supernatants were measured using ELISA kits according to the manufacturer's instructions and method described in detailed with slight modifications.

UHPLC-Q-TOF/MS acquisition analysis

The analysis method and mass detection of synthetic compounds of MEOM was done by LCMS Unit (1290 UHPLC and 6520 Q-TOF mass spectrometer, Agilent Corp, USA), iPROMISE, UiTM, Selangor, Malaysia.

Statistical analysis

The one-way ANOVA-Dunetts's post hoc tests were used to determine the statistical significance of differences between the values for the various experimental and control. Data are expressed as means ± S.E.M (at least three independent experiments performed in triplicate). *P*-Values of 0.05 or less were considered statistically significant.

RESULTS AND DISCUSSION

The present study aimed to evaluate the potential inhibitors of MEOM-plants' extract of the pathways that acts as the inhibitors of pro-inflammatory mediators e.g., NO, PGE_2, COX-2, iNOs, TNF-α and LOX induced by LPS/IFN-γ stimulated RAW 264.7 macrophage cells that measured by the ELISA according to the manufacturer's procedures and enzymatic assay. Effects of MEOM on LPS/IFN-γ induced NO production and cell viability, there was no basal NO production when cells were incubated with only the crude extract, 12.5-100 μg/mL without LPS/IFN-γ or >80% cells growth, which was considered noncytotoxic. However, the MEOM significantly, inhibited NO production between (32% and 78%, and 29% and 71%) with LPS and/or IFN-γ induced RAW 264.7 for 24 hrs in a dose-dependent manner, respectively, indicating the anti-inflammatory effect and enough to inhibit production of NO in the cells.

Meanwhile, inducible nitric oxide synthase (iNOs) is involved in the pathogenesis of inflammation (Hins B and Brune K, 1999). During the inflammatory process, iNOS and COX-2 are specifically expressed in the stimulation with LPS, IFN-γ and others pro-inflammatory cytokines (Yang *et al*., 2012), that produce pro-inflammatory mediators such as NO and PGE_2 (Posadas I et al., 2000). In addition, COX-2 is the more

important source of prostanoid formation in the inflammation and proliferative diseases induced by inflammatory stimuli and growth factors. Results showed that, the extract possesses anti-inflammatory effect by reducing the LPS and/or IFN-γ induced inflammation mediated-PGE_2 production between (19.0% and 70.8%, and 23.5% and 76.2%), significantly in a dose-dependent manner, suggesting could block the expression of iNOs and COX-2, significantly that stimulated by LPS and/or IFN-γ mediated pro-inflammatory to produce inflammation of macrophages, respectively.

Subsequently, tumor necrosis factor (TNF-α) is an important inflammatory cytokine that produced from macrophages corresponding to inflammation such as arthritis and the level of cytokine production are used as an indicator of macrophage responses. Results showed that, inhibition of TNF-α release by LPS and/or IFN-γ stimulated RAW 264.7 elicited by MEOM, significantly in a dose-dependent manner between (26.0% and, 28.7% and 82.0%). Suggesting that, *O. monacantha* may acts as antagonise of this cytokine or potential TNF-α inhibitors.

Lastly, lipoxygenase (LOX) is mainly involved in the oxidation process of arachidonic acid (AA) into inflammatory mediators known as leukotrienes (LT's), which mediates the occurrence of inflammation. Result obtained that, LOX inhibitory effect of MEOM significantly was found to be in a dose-dependent manner between (41.7% and 11.7%, 40.0% and 11.0%), respectively. Thus, inhibition of LOX by MEOM is believed to be a major in attenuation of the formation of gastric ulcer.

UHPLC-Q-TOF/MS chromatogram in the Figure 1, showed 22 proposed compounds have been identified are classified as; 3 alkoloids, 5 polyphenols, 3 flavanones, 8 flavanoids, 2 nitrogen-containing compounds, 1 isoflavanoid & 1 flavanoid glycoside. Based on the chromatogram, the strongest peak is identified as isoliquiritin, a flavonoid glycoside compound that has been reported to exhibit several pharmacological activities including antioxidant, anti-inflammatory, and anti-depression activities.

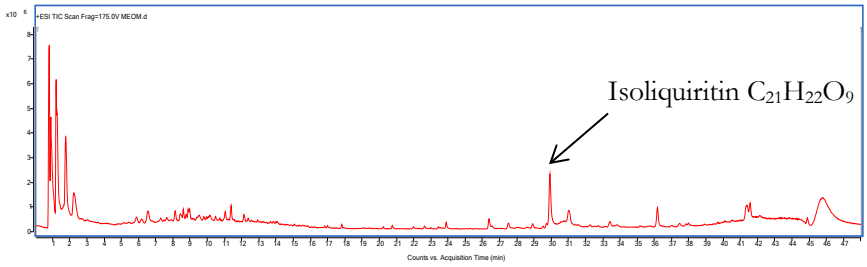

Figure 1. LC-MS/MS chromatogram of MEOM by UHPLC-Q-TOF/MS

CONCLUSION

In conclusion, the MEOM demonstrates the potential anti-inflammatory activity against LPS/IFN-γ-induced inflammation models, which could be attributed to the extract's; i) anti-inflammatory activities, ii) potential to regulate the PGE_2, COX-2, TNF-α, iNOs and LOX synthesis and, iii) ability to work via pathways involving the NO. Moreover, this activity could be plausibly linked to the presence of inflammatory agents such as a flavonoid glycoside bio-compounds, which might act synergistically to produce the observed activity.

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PD11

Production of Gelable Exopolysaccharides from *Agrobacteria sp.*

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ABSTRACT

Microbial exopolysaccharides (EPS) gain intensive demand due to their distinct characteristics of producing a higher yield of EPS. Curdlan is one of the microbial EPS produced by bacteria that has been commercialized in various applications such as bioadhesives, stabilizer thickening, gelling agents in food and pharmaceutical industries. However, the usage of curdlan polymer in these industries is limited because of its costly production process. In this study, the gelable EPS is produced by β-(1-3)-glucan EPS-producing strain, *Agrobacterium sp.* ATCC 31749 was investigated as a substitute for a commonly used sugar source. The *Agrobacterium sp.* ATCC 31749 was grown in flasks containing a production medium supplemented with common sugar and sugarcane. The yield of curdlan from these different carbon sources was monitored based on the dry weight of EPS. The extraction was performed by the following Sodium hydroxide: Acetic acid precipitation strategy. For sugar cane extract, the β-(1-3)-glucan -producing strain produced more curdlan, which is 0.8867 g/mL higher compared to the common sugar extract, 0.7867 g/mL. The result suggests that the suitability of sugar cane extract as a carbon source from β-(1-3)-glucan-producing strain, *Agrobacterium sp.* ATCC 31749 has the potential to be utilized in producing a new value-added product and minimizing the cost of production for the raw material, including carbon and nitrogen sources which relate to the limitation in EPS production.

Keywords: Exopolysaccharides (EPS), β-glucan, *Agrobacterium sp.* ATCC 31749, Curdlan

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INTRODUCTION

β-glucans can be extracted from a variety of sources, including cereal, mushrooms, seaweed, yeast, and bacteria (McIntosh et al., 2005; Laroche & Michaud, 2007; Zhang & Edgar, 2014) β-glucans are a type of β-D-glucose polysaccharide present in eukaryotes and (McIntosh et al., 2005; Laroche & Michaud, 2007). β-(1,3)-D-glucans and β-(1,3) (1,6)-D-glucans are the most study of the naturally occurring β-glucans. A variety of bacteria produce extracellular exopolysaccharides (EPS). In many cases, EPS play a role in the association between bacteria and surfaces (biotic & abiotic) around them (Laroche & Michaud, 2007). EPS are also major matrix components of bacterial biofilm (Sutherland, 2001). Bacteria strains such as *Agrobacterium sp.* produce β-glucan (curdlan) as one of their EPS under appropriate physiological conditions. Production of EPS are affected by the availability of nutrients in the culture broth, particularly the availability of carbon sources (glucose or sucrose for example) and also nitrogen sources. The optimum curdlan production by *Agrobacterium sp.,* for example, is dependent on biomass formation at the growth phase and nitrogen sources starvation at the stationary phase (Laroche & Michaud, 2007). Other factors that might affect β-glucan production by bacterial strains may include temperature, pH, phosphate concentration and dissolved oxygen level (Laroche & Michaud, 2007).

MATERIALS AND METHODS

Inoculation of β-glucans producing strains and media

The tested, β-glucan -producing *Agrobacterium* ATCC 31749 strain was obtained from the American Type Culture Collection as a freeze-dried culture in ampoules. The ATCC 31749 strain was cultured in Luria-Bertani (LB) broth in the test tubes containing 1% (w/v) peptone, 1% (w/v) sodium chloride, 0.5% (w/v) yeast extract and 1.6% (w/v) agar supplemented with 20% (w/v) sucrose. The experiments were performed in 250 mL conical flasks containing production medium of the following components (g/L): $(NH_4)_2HPO_4$, 2.3; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.4; with 1% (w/v) trace element solution, 4% (w/v) sodium citrate and 16% (w/v) carbon sources.

Curdlan extraction

The bacteria strain was first cultured in an orbital shaker at 30°C with an agitation speed of 150 rpm for 72 hours. The experiments were carried out in duplicate to 4 flasks by which the 2 flasks for 16% (w/v) sugar cane and another two flasks for 16% (w/v) as carbon sources with 4% (w/v) sodium citrate as a buffer. The production of curdlan was monitored after three days of incubation by extracting 20 mL of culture via centrifugation at 5500 rpm for 40 minutes at 4°C to separate the pellet cell material. The supernatant was discarded, and the cell pellets were incubated for 3 hours at 30°C of 150 rpm. The samples were once again centrifuged at 5500 rpm at 4°C for 40 minutes to collect the supernatant. The supernatant was precipitated with 15% acetic acid between the range 2 and 3 before centrifuging again at 5500 rpm for 40 minutes at 4°C before being dried in an oven at 65 at 5500 rpm for 40 minutes at 4°C for 24 hours to proceed curdlan dried weight.

RESULTS AND DISCUSSION

The dry weight of curdlan

Production of curdlan demonstrated by β-glucan -producing *Agrobacterium* strain ATCC 31749 was evaluated in a production medium supplemented with two different carbon sources of sugar cane and common sugar extract (Table 1).

Table 1. Type of carbon sources and dry weight of curdlan.

Type of carbon sources	Average dry weight of curdlan (g/mL)
Sugar cane extract (t1)	0.88
Sugar cane extract (t2)	0.89
Sugar (cs1)	0.79
Sugar (cs2)	0.81

The β-glucan -producing *Agrobacterium* strain ATCC 31749 when incubated in a production medium supplemented with sugar cane extract showed the highest average dry weight of both t1 (0.88 g/mL) and t2 (0.89 g/mL) when compared to the common sugar extract, cs1 (0.79 g/mL) and cs2 (0.81 g/mL) (Table 1). It was previously reported that the production of curdlan from sugar cane extract is proven to be an effective carbon source; thus, the β-glucan -producing *Agrobacterium* strain ATCC 31749 can be a good candidate to promote yield of curdlan when replacing sugar cane extract as their carbon source to generate energy (Jung et al., 2001). The production of curdlan by β-glucan -producing *Agrobacterium* strain ATCC 31749 could be due to the starvation for limited nutrients such as nitrogen, oxygen and phosphate salts in the production medium in order to survive in harsh fermentation conditions.

Yield of curdlan

To evaluate the yield of curdlan of β-glucan -producing *Agrobacterium* strain ATCC 31749, the average dry weight was divided by the initial volume sample taken (20 mL) after three days of fermentation.

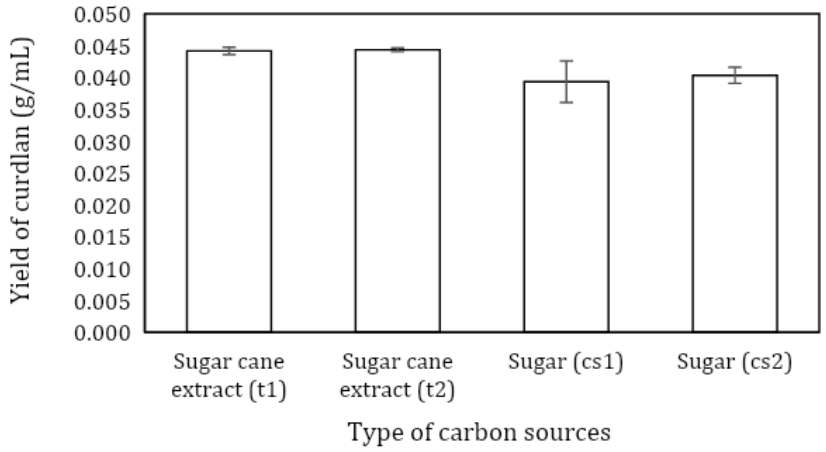

Figure 1. Type of carbon sources versus yield of curdlan (g/mL) for t1, t2, cs1, cs2 for all extraction.

For curdlan yield (Figure 1) by β-glucan -producing *Agrobacterium* strain ATCC 31749 containing sugar cane extract demonstrated the highest yield of curdlan, which was identical in both t1 and t2 (0.044 g/mL). As for common sugar extract, the yield of curdlan was two folds lower in both cs1 (0.038 g/mL) and cs2 (0.040 g/mL). This indicated that the dry weight of curdlan is corresponded to the yield of curdlan by β-glucan -producing *Agrobacterium* strain ATCC 31749.

CONCLUSION

β-glucan -producing *Agrobacterium* strain ATCC 31749 was able to produce a higher yield of gelable exopolysaccharides (curdlan) when supplemented with sugar cane extract as their carbon source. The *Agrobacterium* strain ATCC 31749 demonstrated a remarkable efficiency curdlan yield when compared to the common sugar extract in the fermentation process after three days. Insight from this study, it could expand the potential of β-glucan -producing *Agrobacterium* strain ATCC 31749 in cutting down the cost of fermentation process and time on industrial scale.

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PD12

Phytochemical Analysis of *Piper sarmentosum* Leaves Extract

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ABSTRACT

Kaduk (*P. sarmentosum*) is a cultivated plant that grows wildly in the tropical and subtropical region including in Malaysia. It is not only as a condiment in local cuisine but also used widely to treat various ailments and discomfort among local people that contributed by its wide range of phytochemicals. The aim of this study is to identify the chemical constituents that contain in the aqueous extracts of leaves of *P. sarmentosum*. The fresh leaves *P. sarmentosum* were collected in Bachok and given a voucher number of UKMB40387. The extraction procedure was performed at Craniofacial Laboratory in Universiti Sains Malaysia (USM) and the extract was then sent to Integrative Pharmacogenomics Institute (iPROMISE), Universiti Teknologi Mara (UiTM) for Liquid Chromatography Mass Spectrometry (LC-MS) analysis. Sixteen compounds were identified from the aqueous extract namely (1) 1-allyl-2-methoxy-4-5-methylenedioxybenzene, (2) sarmentine, (3) sarmentosine, (4) pellitorine, (5) (sesamin, (6) sarmentamide A, (7) sarmentamide B, (8) sarmentamide C, (9) piperine, (10) methyl piperate, (11) 2-hydroxypropanoic acid, (12) benzaldehyde, (13) rutin, (14) vitexin, (15) naringin, and (16) geneistein. From the result, it showed that the *P. sarmentosum* extract contains various compounds that could contribute to various biological activities and possess therapeutic effects.

Keywords: *Piper sarmentosum*, Liquid- chromatography Mass Spectrometry (LC-MS) analysis, piperine, rutin, wound healing

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INTRODUCTION

P. sarmentosum belongs to Piperaceae family which is known as "Kaduk" in Malaysia. It is a herbal plant that is used widely as traditional medicine whereby the leaves and roots were applied to the forehead to comfort headache, while its decoction is known to relieve muscle weakness and pain in the bones. Ethnophamacologically, the aqueous extract of the leaves has been reported to reduce inflammatory activity, anti-nociceptive and act as an anti-hypertensive agent. In this present work, the phytochemical screening using LC-MS was perform to identify phytoconstituents in the aqueous extract of *P. sarmentosum* that could responsible for its wide range of therapeutic effects.

MATERIALS AND METHODS

Preparation of Aqueous Extract of *P. sarmentosum*

Fresh leaves of the plants were cleaned with tap water and left dried in the oven for 36 hours at 50°C. Then, 50 g leaves were cut and ground into small pieces, followed by extracting in 1000 ml distilled water for 3

hours at 80°C. The extract was then filtered and sent for a freeze-drying to obtain powder that later been kept at 4°C until further use.

Phytochemical Studies

UHPLC-Q-TOF/MS system was composed of Agilent 1290 UHPLC instrument (Agilent Technologies, Waldbronn, Germany) and Agilent 6520 Q-TOF mass spectrometer (Agilent Corporation, Santa Clara, CA, USA). The mass spectra data was acquired in the negative electrospray ion (ESI) mode. The chromatographic peaks were separated on a ZORAX Eclipse Plus c18 Rapid Resolution HT (2.1X100 mm, Agilent) at a flow rate of 0.25 ml/min. The temperature of column was at room temperature (40°C). Mobile phase consisted of 1% formic acid + water (A) and 1% formic acid + acetonitrile (B). The binary gradient elution program was set as follows: 0-5 min, 0%-5% B; 5-36 min, 5%-95% B; 36-41 min, 95% B; 41.1 min, 5% B; 41.1-48 min, 5% B. The post time run was 5 minutes. The injection was 2 µl. The related Q-TOF/MS parameters were listed as follows: drying gas, N2; gas flow rate, 11 L/min; drying gas temperature, 330°C; nebulizer gas pressure, 40 psig; capillary voltage, 3500 V; fragmentor voltage, 120 V; skimmer voltage, 65 V; octopole RF, 750 V; collision energy (CE), 20 and 30 V. The scan range of mass spectra was m/z 100-1500.

RESULTS AND DISCUSSION

The LC-MS chromatogram of the aqueous extract showed five vital peaks which indicate the presence of five major constituents (Figure 1). The compounds were further analysed using their molecular weight. Previous phytochemical studies on this plant, including leaves resulted in the isolation of a number of flavonoid and phenolic compounds. Azizah et al. (2012) reported that vitexin is a main flavonoid in aqueous extract of *P. sarmentosum*. Since there is no research conducted on wound healing activity, this flavonoid can be exploited to study its wound healing effects. *Piper hayneanum* which contained of vitexin proved that animal treated with the fractions showed better wound healing compared to those treated with the gentamicin (*S. aures*) and miconazole (*C. albicans*). Besides, vitexin also act as antioxidant, anti-inflammatory, anticancer, antidiabetic, and antinociceptive activities.

Sesamin has been used as a dietary fat-reduction as it can accelerate fat loss and helps to prevent the body from storing fat by helping to break down essential fatty acids. Since it has no stimulant, it helps preserve lean muscle mass. It also possesses anti-oxidant and anti-inflammatory properties. Studied showed in *Piper betle* that sesamin may help to reduce cholesterol, support the liver, help maintain healthy lipid profiles and have anti-hypertensive agents. Hartati et al. (2021) reported that piperine exhibit anti-oxidant, anti-inflammatory, immunomodulatory, anti-asthmatic, anti-convulsant, anti-mutagenic, anti-mycobacterial, anti-amoebic and anti-cancer activities. It also has a significant effect on the drug metabolizing enzyme (DME) system and acts as bioavailability enhancer for many chemotherapeutic agents.

Naringin is a type of glycoside flavonoid found in most plant and it plays a critical role in the treatment of Metabolic Syndrome (MetS) due to its antioxidant activity and ability to regulate cytokines. MetS consists of cluster of conditions, hypertension, hyperlipidemia, hyperglycemia, and visceral obesity which is affecting population worldwide. According to Pittaya et al., (2006), *P. sarmetosum* and *P. betle* also showed anti-bacterial activitiy which is the main reason for wound treated.

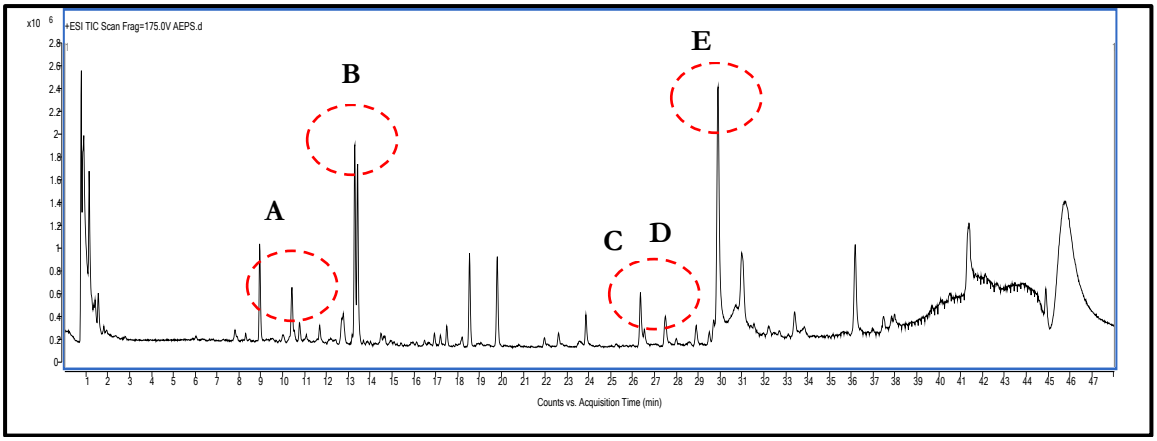

Figure 1. LC-MS chromatogram of *P. sarmentosum* aqueous extract showed the presence of compounds identified as (A) vitexin, (B) (sesamin, (C) piperine, (D) naringin, and (E) sarmentamide B.

Sarmentamide B is a new natural product that were found in this extract which function is unclear and still being studied. However, according to Azizah et al. (2012), the compound was being tested for antiplasmodial, antimycobacterial and antifungal activities and exhibited anti-bacterial activity. As a conclusion, it is evident that *P. sarmentosum* contains various potential compounds such asvitexin, (+)-sesamin, piperine, naringin and sarmentamide B that had been proven to have therapeutic effects. The effects also justify the ethnopharmacological claim of wound healing effects shown by *Piper* species.

CONCLUSION

Further studies on opportunities of *P. sarmentosum* as wound healing treatment are warranted based on the present's compounds which can be positively exploited as shown in this study. The LC-MS chromatogram of the *P. sarmentosum* extract showed five peaks that belong to compounds identified as vitexin, sesamin, piperine, naringin, and sarmentamide B. The compounds could lead and responsible to various therapeutic effects as claimed by local community.

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PD13

Isolation and Morphological characteristic of Acetic Acid Bacteria (AAB) from *Lansium domesticum* (Dokong) and *Nephelium lappaceum* (Rambutan) Vinegars.

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ABSTRACT

Acetic acid bacteria (AAB) are a group of gram-negative bacteria that oxidise ethanol to acetic acid bacteria (AAB) in industrial production of vinegar. The present study aims to isolate the acetic acid bacteria from both *Lansium domesticum* (Dokong) and *Nephelium lappaceum* (Rambutan) mother of vinegars and vinegars as a potential starter culture for vinegar production. Mother of vinegars and vinegars were collected from 6-8 weeks fermented Dokong and Rambutan vinegars in the Food Laboratory of UMK Jeli. Sample collected were enriched with sterile distilled water supplemented with 2.0% ethanol (v/v) and yeast for isolation of AAB for 3 days in a in a rotary shaker at 30℃ agitated at 150 rpm. The enriched sample were then inoculated on selective Carr medium supplemented with bromocresol green and standard glucose, yeast extract, Calcium Carbonate (GYC) solid media. 37 isolates having a yellow formation zone from Carr medium and 78 isolates having a halo clear zone from GYC medium were selected. Each isolate was further enriched in a 2.0% alcohol broth medium. All 115 isolates were tested for its total acetic acid content (TCA), total solid content (TSS) and pH. 62 isolates which produced higher acetic acid content (>6%) were kept for Gram stain and catalase test for identification. From 62 isolates, 8 shown to be Gram negative, rod-shaped and catalase positive having to produce acetic acid ranging from 6.0% to 10.2% of TCA, 8.5% to 10.8% °Brix TSS and pH of 2.3 to 3.2.

Keywords: Isolation, acetic acid bacteria (AAB), vinegar, rambutan, dokong

INTRODUCTION

Acetic acid bacteria (AAB) belong to the *Acetobacteraceae* family. The main species responsible; for the production of vinegar belongs to the genera *Acetobacter, Gluconacetobacter, Gluconobacter* and *Komagataeibacter* because of their high capacity to oxidise ethanol to acetic acid and high resistance to acetic acid release into the fermentative medium (Gomes et. al., 2018). These are obligate aerobic Gram negative or Gram variable, ellipsoidal to rod-shaped, straight or slightly curved, 0.6 - 0.8μm x 1.0 – 0.4μm, occurring singly, in pairs or chains. Pleomorphic forms occur which may be spherical, elongated, swollen, club-shaped, curved or filamentous. In liquid media Acetobacter forms a film or pellicle made of cellulose. The AAB and yeasts present in the fermentation broth get entangled in the cellulose pellicle to form a mat-like structure called the 'mother of vinegar'. (Matthew et. al., 2019). The aim of the study is to isolate the AAB from naturally fermented *Lansium domesticum* (Dokong) and *Nephelium lappaceum* (Rambutan) vinegars and mother of vinegars of both fruits. Gram negative AAB was identified morphologically using Gram stain and catalase test for biochemical identification. The amount of acetic acid production was measured for each isolate.

MATERIALS AND METHODS

Sample collection

Four samples were taken from 6-8 weeks of oxidative and traditionally fermented vinegars of Dokong and Rambutan fruits in the Food Laboratory of UMK Jeli. The samples were labelled as Dokong Mother of Vinegar (DMV), Dokong Vinegar (DV), Rambutan Mother of Vinegar (RMV) and Rambutan Vinegar (RV) and kept refrigerated at 11°C.

Enrichment media

Yeast extract (5%) broth was prepared and enriched with 2%(v/v) of ethanol. 1 ml of each sample DMV, DV, RMV and RV was individually inoculated in 200ml enriched media in a 250ml of conical flask aseptically. For control 1ml of sterile distilled water was used. Cultures were incubated for 3 days in a rotary shaker at 30℃ agitated at 150 rpm. The presence of turbidity of the AAB growth was monitored using a spectrophotometer at 660nm wavelength.

Preparation of Carr and GYC media

A litre of distilled water, 30g of Yeast extract, 20g of Agar, 0.02g of Bromocresol green was autoclaved and 20ml of Ethanol (99.8%) was added subsequently for Carr medium preparation. A litre of distilled water, 20g of glucose, 10g of Yeast extract, 20g of Agar and 20g of Calcium Carbonate was autoclaved for GYC medium preparation. Both media were poured onto sterilised plates. 100µl of 10^{-6} sample was inoculated onto Carr and GYC media in triplicates. A serial dilution of enriched sample was conducted. 100µl of sample 10^{-8} serial dilution were inoculated onto Carr and GYC media in triplicates. All the plates were incubated at 30℃ and growth of colonies were observed after 1 to 3 days.

Isolation of Acetic Acid Bacteria

Selected colonies were first transferred into a partition of either Carr or GYC medium to further observe the yellow and halo clear zones. Pure colonies confirmed to have halo zone were then streak on Carr or GYC medium to obtained pure culture and incubated at 30℃. Growth of colonies was observed after 1 to 3 days. Pure culture was later inoculated in a slant agar for preservation.

Screening for Acetic Acid Bacteria Producer

115 colony isolated was inoculated in an enriched 2%(v/v) of ethanol and Yeast extract (5%) broth medium for 5 days at 30℃ in a rotary shaker at 150rpm. The amount of acetic acid produced, total solid content and pH were measured. Based on TCA production (%) 62 isolates were selected for morphological identification by Gram staining. Morphology of isolates were examined under light microscope at 40x and 100x magnifications. The Gram-negative isolates were selected for catalase test for biochemical characterization according to the standard guideline of Bergey's manual determinative bacteriology (Goodfellow, et. al., 2012).

RESULTS AND DISCUSSION

The aim of this study was to isolate acetic acid bacteria from the mother of vinegar and vinegar of Dokong and Rambutan. 115 isolates were isolated from Carr and GYC media plates (Table 1). Isolates were distinguished by their capability in producing a clear halo around the colonies grown on the GYC medium, indicating that the isolates produced acetic acid that dissolved the Calcium Carbonate ($CaCO_3$) (Mansor et.al., 2012). In Carr medium a yellow zone was observed converting the green colour of bromocresol green ethanol agar showing that the colonies were able to produce acetic acid (Figure 1).

Table 1. Isolates on Carr and GYC media having yellow and clearing zones.

Samples	Carr	GYC	Total of Isolates
DMV	-	24	24
DV	6	11	17
RMV	7	40	47
RV	24	3	27
Total	37	78	115

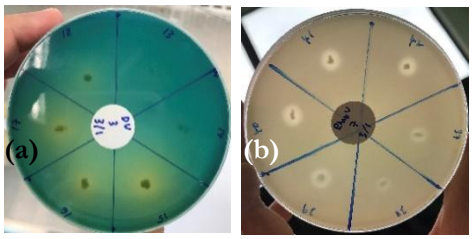

Figure 1. Screening of acetic acid producing microorganisms on Carr (a) and GYC (b) solid plates. Sample collection and screening procedures are explained in detail in Materials and Method. Yellow formation and clear zones around the colonies of isolates.

Table 2 shows eight isolates produced high acetic 6.0% to 10.2% when grown on enriched 2%(v/v) of ethanol and Yeast extract (5%) broth medium for 5 days at 30℃ in a rotary shaker at 150rpm and catalase positive. Figure 2 shows the eight isolates Gram negative and rod-shaped under light microscope at 40x or 100x oil immersion magnification.

Table 2. Eight Gram-negative isolates from DMV, DV, RMV and RV.

Isolate	Gram stain	Acetic Acid (%)	Total Soluble Solid (°Brix)	pH	Catalase
DMV2	-ve	9.0	9.8	2.6	+
DMV6	-ve	8.0	8.5	3.0	+
DMV24	-ve	9.2	10.2	2.4	+
DV1	-ve	10.2	10.8	2.3	+
RMV4	-ve	6.8	10.1	3.1	+
RV3	-ve	6.0	10.6	3.2	+
RV9	-ve	7.8	9.2	3.0	+
RV14	-ve	7.4	8.7	3.1	+

Note: -ve = negative, +=positive

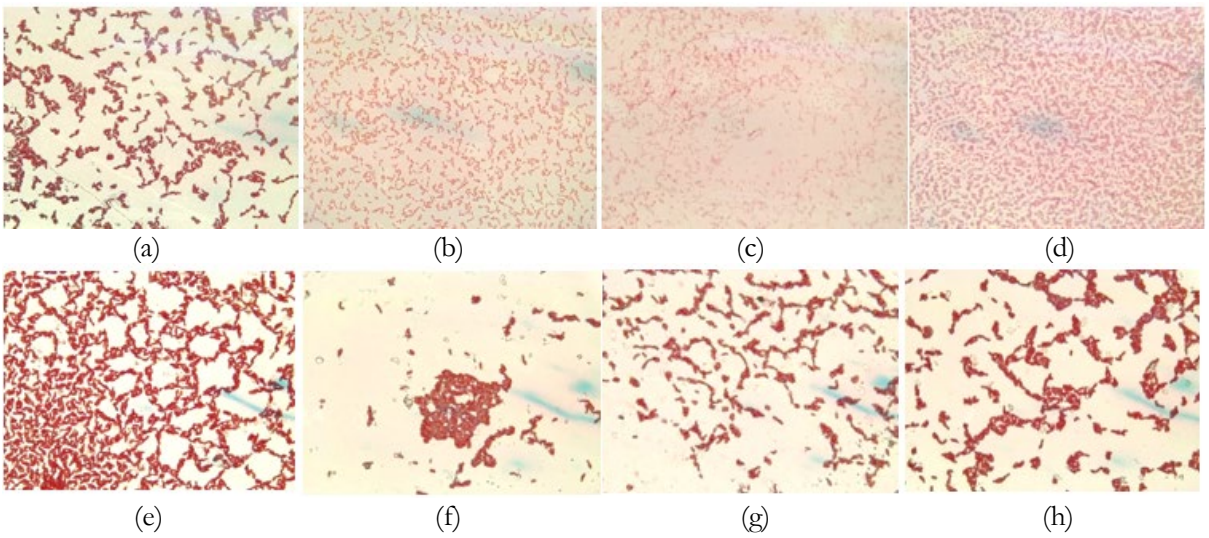

Figure 2. Microscopic observation of 8 Gram-negative isolates. (a) DMV2 (b) DMV6 (c) DMV24 (d) DV1 (e)RMV4 (f) RV3 (g) RV9 and (h) RV14

CONCLUSION

Eight isolates were having produced acetic acid at 6.0% to 10.2%, Gram stain negative, rod-shaped and catalase positive which presumptive belong to the family of the AAB. Oxidase and alcohol tolerance tests on the isolates will be performed in future. The isolates will be identified by molecular identification method.

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