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EDITORIAL TEAM

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Welcome message from Editor-in-Chief

I am pleased to present the second issue of the Journal of Science and Agricultural Technology (JSAT), the official journal of the Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna (RMUTL), Thailand. This issue includes five research articles from various institutions, both domestic and abroad. JSAT also publishes the selected manuscripts as the educational institution's partnership and proceedings to strengthen research collaboration. The journal promises to significantly impact the field of sciences and agricultural technology from Thai and international researchers around the globe. The JSAT is now indexed in Google Scholar and Digital Object Identifier (DOI) under the National Research Council of Thailand. As an Editor-in-Chief, I am working intensely with all authors, reviewers, editors, and friends to make a significant impact and move forwards to gain a higher index ranking. Besides, I strongly encourage researchers to submit manuscripts to share knowledge and promote the growing field of science and agricultural technology.

Assoc. Prof. Dr. Suntorn Wittayakun
Editor-in-Chief Journal of Science and Agricultural Technology
Dean of the Faculty of Science and Agricultural Technology
Rajamangala University of Technology Lanna, Thailand.

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ABOUT THE JOURNAL

Journal of Science and Agricultural Technology (JSAT) publishes original research contributions covering science and agricultural technology such as:

• Natural and applied sciences: biology, chemistry, computer science, physics, material science and related fields. Papers in mathematics and statistics are also welcomed, but should be of an applied nature rather than purely theoretical.

• Agricultural technology: plant science, animal science, aquatic science, food science, biotechnology, applied microbiology, agricultural machinery, agricultural engineering and related fields.

Furthermore, the JSAT journal aims to span the whole range of researches from local to global application.

The JSAT is published two issues a year.
Issue 1: January - June
Issue 2: July - December

Submissions are welcomed from international and Thai institutions. All submissions must be original research not previously published or simultaneously submitted for publication or submitted to other journals. Manuscripts are peer reviewed using the double-blinded review system by at least 2 reviewers before acceptance. There is no publication or processing fee.

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Editorial office:

Faculty of Science and Agricultural Technology,
Rajamangala University of Technology Lanna,
128 Huaykaew Rd., Changphuek, Muang, Chiang Mai, 50300 Thailand.
Tel/Fax: +665392 1444 Ext. 1506

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Research Article
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Energy management study of aeration system for Nile tilapia fish pond using solar photovoltaic together with utility grid system

Preeyanuch Numpha and Sarawut Polvongsri*

School of Renewable Energy, Maejo University, Chaing Mai 50290, Thailand.

***Corresponding author**: saravooth@hotmail.com*

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ABSTRACT

This paper presents the energy management models of the aeration system for the Nile tilapia pond using the electrical energy from solar photovoltaic (PV) and the utility grid system (Grid). The solar photovoltaic system is installed on nine panels in which each panel has a dimension of 2×1 m^2 with 325 Wp of polycrystalline silicon solar cell type to produce the electricity for the aeration motor of 2.05 kW. In addition to using electric power, things to keep in mind are the Dissolved Oxygen (DO) that affects fish survival. In Nile tilapia aquaculture, the aeration phase is divided into 4 phases, in which each phase is determined into 4 models of energy management. The results for the period time of Phase 1, Phase 2, Phase 3, and Phase 4 are 66, 48, 14, and 20 days, respectively. Phase 1 uses the energy management model PV; electricity is used only during the daytime, and no use of the aerator at night time, so there is no effect on electricity saving. While other three Phases 2^{nd} - 4^{th}, the optimize energy management model is model 3, which uses the least electrical energy during the daytime from solar PV together with the utility grid system (solar PV&Grid) and at nighttime by using Grid system which the total energy consumptions are 19.21, 21.70, and 24.34 kWh/day, respectively. For one crop by choosing the energy management model can reduce the electrical energy consumption of 1,018.82 kWh/crop, and a total saving is 4,411.49 Baht/crop.

Keywords: energy management, aerator, solar photovoltaic system, dissolved oxygen, Nile tilapia

INTRODUCTION

According to the Alternative Energy Development Plan 2015-2036, Thailand needs to promote the proportion of renewable energy, increasing by about 30% (AEDP, 2015). The interesting renewable energy that has been promoted is solar energy in the form of both heat and electricity. The government sets a target to increase the proportion of solar energy from 3.10-10.24% in all sectors such as industries, commercial buildings, households, and including the agriculture sector in farming, gardening, and aquaculture.

Moo Ban Thung Yao is an agricultural community locates in San Sai District, Chiang Mai Province. The total area of the community, about 70-80% have fish farming, such as Nile Tilapia and catfish in natural ponds. The fish growth period is about 4 months per crop. During culture, fish needs to use oxygen for breath and decomposition of organic material in the pond. During the daytime, phytoplankton will photosynthesize and releases oxygen, increasing the amount of Dissolved Oxygen (DO) that is sufficient for fish. On the other hand, during night time, there is no photosynthesis process; therefore, DO is continually decreasing. Typically, the standard of DO in the fish pond is not less than 3 mg/L (Choochote, 1993). If the DO is lower, the fish may have stress conditions, reduced food intake also results in reduced growth rate as well, and finally, it causes the fish to lack oxygen. Therefore, using the aerator in the fish pond is necessary to use the oxygen increasing in the water. Most of the farmers in Moo Ban Thung Yao use the surface propeller aerator. However, the motor of the aerator is big and consumes much electric power for motor-driven, then it causes the fish costs to increase. Therefore, a sand head aerator is used; instead, the advantage is that the motor runs at low pressure, so it uses less electricity and to reduce production costs. For the previous researches about energy management in the fish pond, Phan-Van et al. (2008) studied the assessed impact of 3 factors affecting water temperature and DO, including water depth, seasons, and fish bioturbation. Statistical data analyzed by Multifactor-ANOVA, and it was found that fish bioturbation and water depth affected the DO. Due to the activities of fish during the summer 8:30-10:00 a.m., when the depth decreased, the DO would decrease, accordingly by about 2.38-4.77, 2.08-4, and 0.62-1.08 mg/L, respectively. Tran-Duy et al. (2008) studied the DO for the Nile tilapia growth. The water flow rate was pumped at 6 L/min to gradually decrease at a continuous-time by setting weight classes (37 g small size, 90 g big size) and two DO levels (DO≤ 3.5 mg/L, low and DO ≥ 5.0 mg/L, high). From the study,

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it was found that when the amount of DO increasing the growth rate of the small fish was increased more than the big fish. Nalinanon et al. (2017) studied Nile tilapia in the conventional cage in which water circulated by the aerator about 22 hr/day and turn off 2 hr from the average initial body weight was 0.25±0.01 g. When feeding about 12 weeks, the average weight of the last fish was increased approximately 23±0.47 g resulting in weight gain, specific growth rate (SGR) was about 19 %/day. For the normal systems, the amount of DO was between 1.9-5.8 mg/L, which was inferior in quality to the aeration in the system resulting in DO increased about 7.90±0.84 5.8 mg/L. Aziz et al. (2020) mentioned the utilization of the PV-grid connected system had proven to be an effective energy supply option and had gained favor where it was accessible and that had a suitable amount of solar radiation. While the installation of off-grid PV systems is increasing dramatically around the world, solar PV systems connected to the electrical network are growing more rapidly and continue to account for the majority of global PV power.

This research provides an idea to reduce the dependence on electricity from the utility grid system. The objective of this study was to study the energy management model of the aeration system for Nile tilapia by using the solar PV system together with the utility grid system.

MATERIALS AND METHODS

Solar Photovoltaic System combined with Utility Grid

A schematic diagram of the solar photovoltaic (PV) system combined with the utility grid for the fish pond aerator is shown in Figure 1(a). The total power of solar panels is 2.925 kW, installed 9 panels of poly crystalline type in which each panel has dimension 2×1 m^2 with a maximum power of 325 Wp. The generated electricity will pass through 2.2 kW inverter that uses to convert direct current (DC) into alternating current (AC) (Model: SINAMICS V20, voltage range 200-240 V) to the sand head aeration motor of 2.05 kW size and 3,450 rpm/min. In the case of the electricity from solar PV system is not enough, the utility grid electricity will be used to supply the aerator when following the demand load of the aerator. The experiment solar PV and the sand head aerator are shown in Figure 1(b) and 1(c).

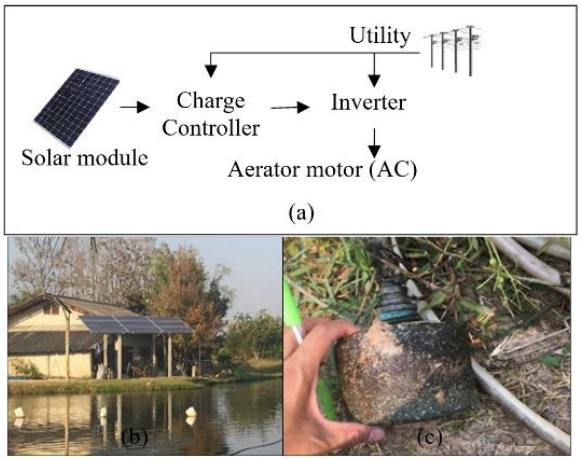

Figure 1. (a) Schematic diagram of the solar photovoltaic (PV) system combined with the utility grid system. (b) Solar PV system installation and (c) Sand head Aerator

Nile Tilapia fish farming

The experiment Nile Tilapia fish pond is located at Moo Ban Thung Yao. The fish pond area is approximately 5,000 m^2, the depth of the pond is 4 m. The farming density is 2 fish/m^2; therefore, the farmer chooses Nile Tilapia fish size by the bodyweight of fish is not less than 30 fish/kg, with a rate of reduced to 11,900 fish before harvesting. The fish culture divides into 4 periods of time (Phase 1, Phase 2, Phase 3, and Phase 4), by the average weight of fish are 0.27, 0.44, 0.47, and 0.51 kg/fish, respectively, as shown in Figure 2. Feeding time conducts two times in the morning and evening at 10:30 a.m. and 5:00 p.m.-5:20 p.m., respectively. The total electricity usage is 2,449.00 kWh/crop or totaling 10,615.60 Baht/crop with an average electricity cost of 4.33 Baht/kWh.

Figure 2. Size of Nile Tilapia fish in 1 crop.

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Energy management model for DO analysis

Normally, the aerator motor is turned on during the daytime and nighttime between 9:00 a.m.-5:00 p.m. and 11:00 p.m.-9:00 a.m. In this condition, define it as a control system. In Table 1, five types of energy management models of aeration systems are being studied using a solar PV system combined with a utility grid system, and there are determined for DO changing.

Table 1. Energy management models for the aeration system.

Model	Aeration system	
	Daytime*a	Nighttime
PV*b	Solar PV system	No use aerator
1	Solar PV system	Grid system*c
2	Solar PV system	Grid system (DO<3mg/L)*d
3	Solar PV&Grid systems	Grid system
4	Solar PV&Grid systems	Grid system (DO<3mg/L)

*a At daytime turns on the aerator motor between 9:00 a.m.-5:00 p.m.
*b Mode PV l uses only the first phase of fish culture.
*c Nighttime, modify the time to turn on all 4 phase motors, 1st – 4th (3:00 a.m.-9:00 a.m., 1:00 a.m.-9:00 a.m., 0:00 a.m.-9:00 a.m. and 11:00 p.m.-9:00 a.m., respectively.)
*d Nighttime, minimum time period is set for turning on the motor at 11:00 p.m.

Data collection for energy management analysis is divided into 2 parts as follows;

1) Collects data and analysis the electrical energy

The electrical data is measured by a clamp meter. The maximum voltage (V_m) and maximum current (I_m) of direct current (DC) will be calculated the maximum electrical power by using Equation (1) and calculates the electrical energy by using Equation (2).

$$P_m = I_m V_m \quad (1)$$

Where P_m is maximum power (W), I_m is maximum current (A) and V_m is maximum Voltage (V)

$$E = P_m \times t \quad (2)$$

Where E is maximum energy (Wh) and t is a time of operating (h)

Solar radiation intensity data (I_T) was collected using Apogee measuring instrument (Model: SP-100-L, Accuracy ±5%), and the input and output power consumption data of the inverter was collected using clamp meter (AC/DC) (Model: UNI-T UT204) measuring instruments. The diagram of measurement and data collection is shown in Figure 3.

2) Collects data and analysis the DO

The DO is measured by the DO meter (Model: Lutron DO-5512SD). Used as an indicator to compare DO with the control system for energy management in the aeration of each phase model.

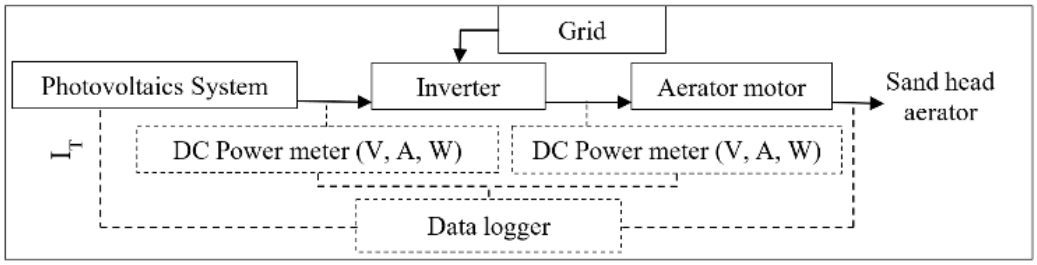

Figure 3. Measurement and data collection.

RESULTS AND DISCUSSION

The results from the energy management model of the aeration system are divided into 4 phases as follows:

Aeration of Phase 1

In Phase 1 of Nile Tilapia culture, the size fishes are small, so they do not need much oxygen for breathing. In addition, the behavior of non-breathing fish on the surface will observe in the morning so that the DO is sufficient for fish breathing. From Figure 4(a), it can be seen that in

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the control system (without aerator using), the DO will increase from the morning time since 6 a.m. to 4 p.m. with the highest value of 5.70 mg/L due to many aquatic plants photosynthesis and release oxygen, however after the sun sets, the DO will begin to decrease. The lowest DO is 1.00 mg/L, and the average daily DO is 2.05 mg/L. It can be seen that the DO is lower than the standard; when there is no aeration, the oxygen content decreases rapidly. But with continuous aeration, the DO slowly decreases, and the fish can still survive. In the first phase, fish are still small, less density, and therefore have a low oxygen demand than Phases 2nd - 4th.

When the aerator is turned on by all energy management models, it is found that all models reach higher DO values than the control case. The highest DO is occurred by Model 4 followed by Model 3, Model 2, and Model 1, respectively. This investigation can be concluded that every model of the aerator pattern can operate without the DO affecting in accordance with the conventional pattern (control system). Therefore, the quantity of electricity from the grid system is a decision for electricity choosing. The result of electrical energy using in Phase 1 is shown in Table 2.

Table 2 (Phase 1) shows the electrical energy that produces from the solar PV system and grid system. During the experiment, all days have similar, with an average value of 12.91 MJ/day. The results show that Model PV is most suitable for use during the Phase 1 cultured period because there is no electricity from Grid. While other models have to use electricity from Grid such as Model 1, and Model 3 which the electricity is used about 7.57 kWh/day, and 7.66 kWh/day, in addition, these results are less than Model 2 and Model 4, which uses electricity from Grid about 12.70 kWh/day and 12.78 kWh/day, respectively.

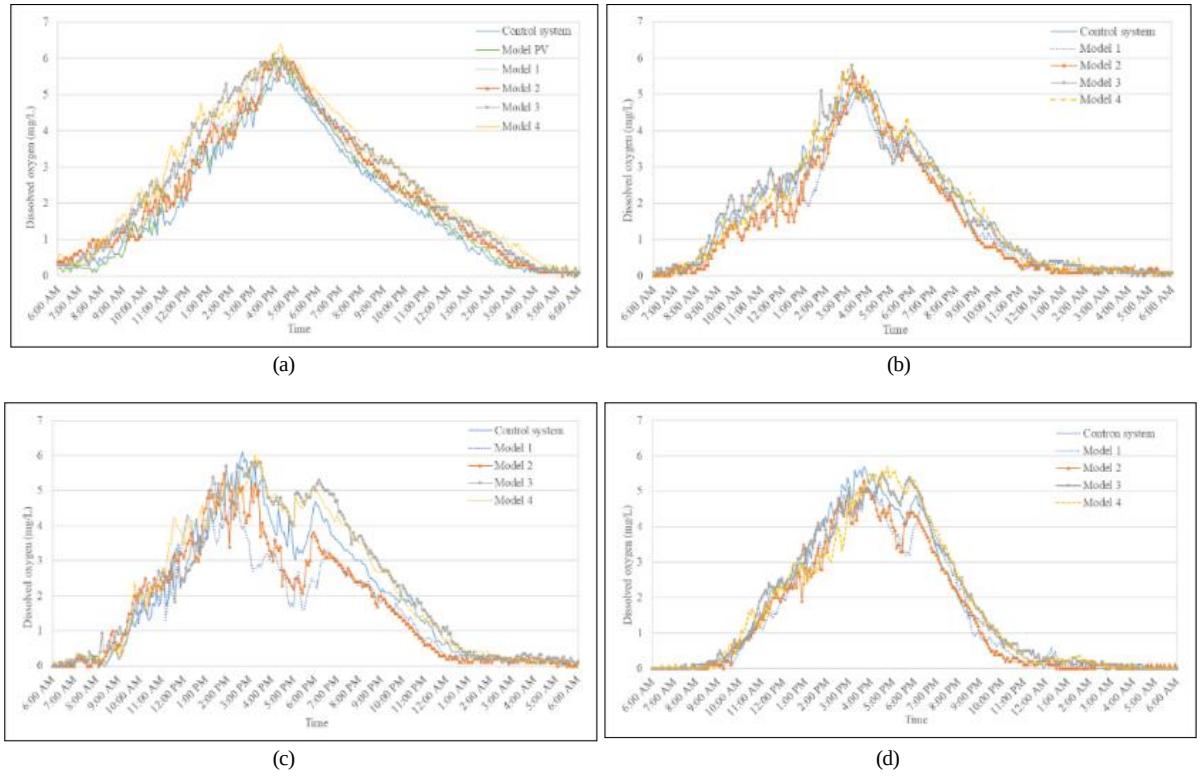

Figure 4. The amount of dissolved oxygen in all Phases.

Aeration of Phase 2

Phase 2, Nile tilapia fish is cultured around 48 days. After raising, it can be seen that the fish has increased growth and therefore needs more oxygen as well. On daytime and night time, if the aeration system operates with only a Model PV pattern in the early morning before the sun rises, fishes breathe on the water surface in order to find oxygen, which means DO in the water is not enough for fish. Therefore, Model PV is not considered in Phase 2 culture.

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Table 2. The energy consumption in the aeration.

period	Model	Total Solar radiation (MJ/m^2day)	$DO_{average}$ (mg/L)	Energy			
				$PV_{daytime}$ (kWh)	$Grid_{daytime}$ (kWh)	$Grid_{nighttime}$ (kWh)	Total $Grid_{all-day}$ (kWh)
Phase 1	Control	12.78	2.05	-	-	-	-
	Model PV	12.99	2.19	10.77	-	-	-
	Model 1	13.06	2.39	10.97	-	7.57	7.57
	Model 2	12.96	2.36	10.78	-	12.70	12.70
	Model 3	12.81	2.61	10.63	9.41	7.66	17.07
	Model 4	12.85	2.73	10.56	9.42	12.78	22.20
Phase 2	Control	14.08	1.77	-	20.68	13.05	33.73
	Model 1	13.99	1.52	11.40	-	10.12	10.12
	Model 2	14.13	1.53	11.44	-	12.53	12.53
	Model 3	14.13	1.79	11.48	8.94	10.18	19.12
	Model 4	14.11	1.77	11.46	8.88	12.69	21.57
Phase 3	Control	11.88	1.96	-	19.85	12.66	31.51
	Model 1	11.90	1.59	9.78	-	11.22	11.22
	Model 2	11.95	1.73	9.85	-	12.50	12.50
	Model 3	12.00	2.25	9.99	10.41	11.29	21.70
	Model 4	11.99	2.24	9.99	10.40	12.60	23.00
Phase 4	Control	11.27	1.67	-	19.77	12.88	32.65
	Model 1	10.97	1.49	8.83	-	12.55	12.55
	Model 2	10.95	1.47	8.80	-	12.83	12.83
	Model 3	11.01	1.75	8.83	11.60	12.74	24.34
	Model 4	11.09	1.70	8.92	11.40	12.72	24.12

Table 2 (Phase 2) shows energy consumption. The control system consumes electricity around 33.73 kWh/day from the grid system (Daytime: 20.68 kWh/day, Nighttime: 13.05 kWh/day). When considering all models, Model 1 and Model 2 have minimum electrical energy consumption at 10.12 kWh/day and 12.53 kWh/day followed by Model 3 and Model 4. The Model 3 has an operation time at 9:00 a.m.-5:00 p.m. and 1:00 a.m.-9:00 a.m. with total electricity consumption of 19.12 kWh/day (Daytime: 8.94 kWh/day, Nighttime: 10.18 kWh/day). For Model 4, total electricity consumption is 21.57 kWh/day (Daytime: 8.88 kWh/day, Nighttime: 12.69 kWh/day), respectively. However, when considering the DO as shown in Figure 4(b), it is found that the increase of DO is similar to the Phase 1 range, with the control system has the highest DO values 5.20 mg/L at 4:15 p.m. and the daily average DO is 1.77 mg/L.

Model 1, and Model 2 have DO lower than control systems all-time with an average of 1.52 mg/L and 1.53 mg/L, respectively; therefore, these models are not suitable for aeration system because the effect on fish growth may happen. While Model 3 and Model 4 has the DO higher than the control system with 1.79 mg/L and 1.77 mg/L, respectively. In conclusion, both the DO and electricity consumption must be considered simultaneously, so the results from Model 3 Model 4 are more suitable for aeration use, which can reduce the electricity consumption from the grid system by 14.61 kWh/day or 701.28 kWh during the Phase 2 culture.

Aeration of Phase 3

Phase 3, Nile tilapia fish is cultured around 14 days. Fish has a more increased growth rate, so the aeration system is necessarily similar to Phase 2 during the daytime. Figure 4(c), it is found that Model 1 and Model 2 are not suitable because the DO is lower than the control system, while the DO of Model 3 and Model 4 result obviously. When considering the electricity consumption, as shown in Table 2 (Phase 3), it is found that the control system consumes higher electrical energy about 32.51 kWh/day (Daytime: 19.85 kWh/day, Nighttime: 12.66 kWh/day) than other models. In addition, Model 3 activates at 9:00 a.m.-5:00 p.m. and 0:00 a.m.-9:00 a.m. is most suitable for use because it uses less energy than Model 4, with electricity from grid system about 21.70 kWh/day (Daytime: 10.41 kWh/day, Nighttime: 11.29 kWh/day) and 23.00 kWh/day (Daytime: 10.40 kWh/day, Nighttime: 12.60 kWh/day), respectively. If considering only the electricity usage from the grid system, every model would increase electricity consumption because, during the rainy season, the solar intensity fluctuates. In the case of Model 3, it can reduce electricity consumption from the grid system by about 10.81. kWh/day or 151.31 kWh.

Aeration of Phase 4

Phase 4, Nile tilapia fish, has a culture day of 20 days. The suitable model in Phase 4 is Model 3 that has been proposed to use electricity during the daytime with a solar PV&grid system at 9:00 a.m.-5:00 p.m. and at 11:00 p.m.-9:00 a.m. with the only Grid system. The energy consumption of many models, as shown in Table 2 (Phase 4), the control system consumes electricity from a grid system about 32.65 kW/day (Daytime: 19.77 kWh/day, Nighttime: 12.88 kWh/day). The results of Model 4 obtain has the least grid consumption about 24.12 kWh/day (Daytime: 10.40 kWh/day, Nighttime: 12.72 kWh/day); moreover, it is less than the results of Model 3, which consumes electricity from grid system about 24.34 kWh/day (Daytime: 11.60 kWh/day, Nighttime: 12.74 kWh/day). Anyway, it is found that the DO of Model 4, especially during the daytime, is lower than the DO of the control system, as shown in Figure 4(d), while The DO of Model 3 is higher than the control system. Therefore in this period culture, Model 3 is most suitable for aeration system use, which can reduce electricity consumption from the grid by about 8.31 kWh/day or 166.20 kWh.

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When considering the one crop as shown in Table 3, in the case of the control system consumes electricity of 2,727.18 kWh/crop while the adjustment of the aerator operation according to the above scheme (Phase 1 use Model PV and Phase 2, Phase 3, and Phase 4 use Model 3) will consume electricity from Grid 1,708.36. kWh/crop, so the electricity-saving is equal to 1,018.82 kWh/crop, representing an electricity saving of 4,411.49 Baht/crop (the average electricity charge is 4.33 Baht/kWh).

Table 3. Energy consumption of the aeration system from the utility grid system.

Phase	Period time (day)	Energy consumption from the utility grid system					
		Control system		Model 3		Saving	
		kWh/day	kWh/Phase	kWh/day	kWh/Phase	kWh/day	kWh/Phase
1	66	-	-	-	-	-	-
2	48	33.73	1,619.04	19.12	917.76	14.61	701.28
3	14	32.51	455.14	21.70	303.80	10.81	151.34
4	20	32.65	653.00	24.34	486.80	8.31	166.20
Sum (kWh/crop)			**2,727.18**		**1,708.36**		**1,018.82**

CONCLUSIONS

In the experiment, five models will test the usage of aerators and choose the best model which consumes the least electricity; moreover, the DO value in the fish pond must be not lower than the control system that farmers use. The results can be summarized according to the fish culture period as follows;

During the phase 1 culture period, the suitable model of the aeration system is Model PV. During the day, the aerator is turned on from 9:00 a.m.-5:00 p.m. using only electricity from the solar PV system. But since the control system had no aeration, so there is no effect on electricity saving. While the other three Phases 2^{nd} - 4^{th}, the control system uses 33.73, 32.51, and 32.65 kWh/day of electricity from the grid system, respectively. The optimize energy management model is model 3, which uses the least electrical energy during the daytime from solar PV together with the utility grid system (solar PV&Grid) and at night time by using Grid system which the total energy consumptions are 19.12, 21.70 and 24.34 kWh/day, respectively, resulted in a reduction in electricity consumption by about 14.61 kWh/day (701.28 kWh), 10.81 kWh/day (151.34 kWh) and 8.31 kWh/day (166.20 kWh), respectively.

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Research Article

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The extraction of estrogen-like compounds from *Pueraria mirifica*, the properties characterization and the preparation of nucleated nanoparticles

Wachira Yodthong[1], Saransiri Nuanmanee[2], Phenphichar Wanachantararak[3], Nattakanwadee Khumpirapang[4], and Tanongsak Sassa-deepaeng[1*]

[1]Graduate School, Faculty of Sciences and Agricultural Technology, Rajamangala University of Technology Lanna Lampang, Lampang 5200, Thailand

[2]Songkhla Aquatic Animal Health Research Center (SAAHRC), Aquatic Animal Health Research and Development Division, Department of Fisheries, Ministry of Agriculture and Cooperatives, Songkhla 90100, Thailand

[3]Dental Research Center, Faculty of Dentistry, Chiang Mai University, Chiang Mai 50200, Thailand

[4]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand

***Corresponding author**: tanongsaks@rmutl.ac.th*

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ABSTRACT

Pueraria mirifica (PM), also known as Kwao Krua Khaw, has been used as an herbal plant for many decades in the Northern part of Thailand. The root of PM contains estrogen-like compounds that are less soluble in water resulted in low bioavailability in an aquatic animal application. To increase solubility of the compounds, six different polarity solvents such as hexane, benzene ethyl acetate, acetone, ethanol, and deionized distilled water were employed for extraction. It found that deionized distilled water exhibited the highest extraction percentage yield. However, a high amount of phenolic compounds and flavonoids were obtained in ethyl acetate fraction (EAF). In addition, the results of scavenging radical assay using DPPH IC_{50} showed the highest activity of EAF due to the high content of total phenolic content and flavonoids content. Moreover, EAF, significantly exhibited the highest proliferation activity against MCF-7. Therefore, EAF was selected for developing polymeric micelles. The HPβCD, Pluronic F-68, and Pluronic F-127 were employed with Tween80 to prepare suitable nanoparticles. The proper ratio of EAF-loaded Pluronic F-127 (EAF-F127) product in nanosize range elicited zeta potential value of nearly zero mV and exhibited the transparent aqueous dispersions in DI water. The results promised further *in vivo* studies.

Keywords: *Pueraria mirifica*, antioxidant, MCF-7, polymeric micelles, nanoparticles

INTRODUCTION

Pueraria mirifica (PM) is locally called Kwao Krua Kao in Thai. It is an estrogenic plant that has been used as a traditional herb and a folkloric medicine in Thailand (Intharuksa et al., 2020; Suthon et al., 2016), Myanmar, Laos, Vietnam (Intharuksa et al., 2020; Peerakam et al., 2018) and other southeast Asian countries for over a hundred years (Malaivijitnond, 2012). The roots of PM have been used to improve menopausal stage symptoms or absent periods such as vaginal dryness, hot flashes, irritability, night sweats, depression (Intharuksa et al., 2020). They have been used as a health promoter such as anti-aging, anti-wrinkle agent, increasing hair growth agents (Sirisa-ard, et al., 2018), and bone loss prevention agent. In orchidectomized and ovariectomized rats, the roots are used as an estrogen supplement. (Urasopon et al., 2007; Urasopon et al., 2008). They possess an estrogenic effect on other hormone-sensitive cells (Lin et al., 2017). Unfortunate, most bioactive compounds in PM roots were flavonoids and coumarins (Lee et al., 2017; Jeon, et al., 2005), which were less soluble in water, resulted in low bioavailability in the aquatic animal application. In general, 95% of ethanol was used for the extraction of bioactive compounds from this plant (Peerakam et al., 2018) from the viewpoint of medicinal purposes and the environment. However, to have insoluble compounds with maximum purity, the solvents with different polarities were employed, as indicated by Yodthod et al. (2020).

In this study, we tried to obtain estrogen-like compound fractions using different solvents with different polarities. Moreover, the antioxidant activity, the total phenolic content, the flavonoids content, and MCF-7 proliferative activity were investigated. The selected fraction would be prepared in nano-sized particles by using polymeric micelles formulation technology in order to enhance the solubility. The nanoparticles were developed by using HPβCD ((2-Hydroxypropyl)-β-cyclodextrin), Pluronic F-68, and Pluronic F-127. Particle size and size distribution were determined to characterize the particles. The Zeta potential of surface charge of

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particles was also performed for predicting their stability.

MATERIALS AND METHODS

Plant samples

The mature roots of PM were collected on October 15, 2019, from Tambon Thapa, (18.173540, 99.378623), Muang Lampang District, Lampang, Thailand. The roots were authenticated by herbal experts at Rajamangala University of Technology Lanna (RMUTL). Upon arrival, fresh PM roots were washed with tap water, followed by distilled water consequently. The skin of roots was peeled off by using the paring knife. The thin-sliced samples were dried in a hot air oven at 50 °C until a constant weight was obtained. The dried sample was ground by using an electric blender, and the achieved fine powder was collected by using 80-mesh sieves before wrapping in a vacuum plastic bag and keeping in a deep freezer at -20 °C.

Extraction

Extraction was performed according to the sequential extraction method (Yodthong et al., 2020) using six different solvents, including hexane, benzene, ethyl acetate, acetone, ethanol, and deionized distilled water. Each solvent extraction was performed in triplicate, as described by Yodthong et al. (2020). Ten grams of PM powder were immersed in 300 mL of solvent at ambient temperature and was agitated at 100 rpm assisted by an orbital shaker for 180 min, three times a day, and then filtered using filter paper. The remaining residue was subjected to re-extraction. The filtrates of each cycle were concentrated by using a rotary evaporator, while deionized water filtrate was dried by using a freeze dryer. The fractions were stored at -20 °C in a vacuum bag for further analysis.

DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical scavenging activity was examined using the modified method described by Sassa-deepaenget al. (2019). DPPH solution (oxidized form) was prepared in absolute ethanol to get the final absorbance of 1.0 ± 0.2 at 517 nm. One hundred µL of various concentrations of samples were mixed with 900 µL of DPPH radical solution and then incubated at the ambient temperature for 30 min in the dark. The solution was measured against the blank at 517 nm using V-1200 spectrophotometer with UV-Professional analysis software. The percentage of inhibition of antioxidant was calculated using the equation:

$$\% \text{ Inhibition} = [(OD_{max} - OD_{sample})/OD_{max}] \times 100$$

The linear curves were generated by plotting the percentage of inhibition against the concentration in µg/mL (R^2 = 0.99). After getting the trend line with the regression equation in Microsoft Excel 2019 software, put y=50, and calculated the x value, the IC_{50} of DPPH.

Total phenolic content (TPC)

The content of phenolic compounds was determined by using the Folin-Ciocalteu (FC) colorimetric method using a modified method described by Yodthong et al. (2020). Twenty µL of various concentrations of the extract was mixed with 100 µL of FC reagent in 1,980 µL of DI water; consequently, incubated for 5 min at the ambient temperature. After adding 300 µL of 7% of Na_2CO_3 and incubated for 60 min at the ambient temperature in the dark. The absorbance of the solution was measured at 765 nm using V-1200 spectrophotometer with UV-Professional analysis software. The TPC quantitation was performed in triplicate. The gallic acid at different concentrations was used to generate the standard calibration curve (R^2 = 0.99). The TPC was expressed as microgram gallic acid equivalent (GAE) per milligram dry weight (Sassa-deepaeng et al., 2019).

Flavonoid content

The flavonoid content (FC) was examined using the aluminum trichloride ($AlCl_3$) colorimetric method with some modification by Sassa-deepaeng et al. (2019). Briefly, 100 µL of various concentrations of the extract was added to 300 µL of DI water followed 100µL of 5 % $NaNO_2$ and incubated for 5 min at the ambient temperature. Afterward, 100 µL of 10% $AlCl_3$ was added and then incubated for 6 min at the ambient temperature. Finally, 400 µL of 1M NaOH was mixed and then incubated for 30 min in the dark at the absorbance temperature. The absorbance was measured at the wavelength of 415 nm using V-1200 spectrophotometer with UV- Professional analysis software. The flavonoid content was carried out in triplicate and expressed as microgram quercetin equivalent (QE) per milligram dry weight.

Estrogen sensitive cell lines testing

The MCF-7 human breast cancer cells from the American Type Culture Collection were cultured in a 75 mL sterile culture flask containing DMEM supplemented with 10 % FBS and 10% of antibiotic-antimycotic in a humidified atmosphere of 5 % CO_2

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at 37°C until cells reach 90% of confluent. The proliferative activity assay was conducted by using a modified method described by Sassa-deepaeng et al. (2017). Cells were harvested with trypsin prior to seeding (5×10^5 cells/well) in 96-well plates. Various concentrations (12.5, 25.0, 50.0, and 100.0 μg/mL) of the fractionated PM were added and then incubated at the same temperature for 20 h, while DMSO was also used as the control. Afterward, the medium was removed by using multichannel pipettes for 12-well plates. The 100μL of (5 mg/mL) 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) reagent was added to each well and further incubated at the same temperature for 4 h. Finally, the formazan product in cells was dissolved by adding dimethyl sulfoxide (DMSO), resulting in a purple solution. The absorbance was measured at the wavelength of 570 nm with a microplate reader, and absorbance at 630 nm was used to correct for nonspecific background values. The percentage of proliferative activity was calculated based on comparative growth with control in quadruplicate.

Polymeric micelle preparation

The HPβCD, Pluronic F-68, and Pluronic F-127 were employed for polymeric micelle formation according to the method of Sassa-deepaeng et al. (2016).The selected fraction was prepared by dissolving it in ethanol. The fraction solution was added dropwise with stirring a solution of HPβCD, Pluronic F-68, or Pluronic F-127 solution to obtain the mixture of fraction-polymer at the weight ratios of 1:1,1:2, 1:3, 1:4, and 1:5. Afterward, 100 μL of Tween80 was added prior to adding deionized water to the volume. The mixture was frozen at -20°C prior to lyophilization under vacuum for 24 h. Finally, the obtained dry fraction-loaded HPβCD, Pluronic F-68, and Pluronic F-127 micelles were re-suspended in deionized water to the desired drug concentration for further studies.

Particle size, Size distribution, and Zeta potential

Particle size and size distribution (PDI) of polymeric micelles were determined using Zeta-sizer Nano Zs working on the principle of photon correlation spectroscopy (PCS) as described by Sassa-deepaeng et al. (2019). One milliliter of each suspended product in water was transferred into a quartz cuvette prior to exposure to laser light diffraction. The intensity of the peak with the highest population was recorded. The Zeta potential was also measured using the same instrument in a folded capillary zeta potential cell. The average data were automatically calculated by the instrument software.

Statistical analysis

The data were analyzed using a tool pack of the Microsoft Excel 2016 software. One-way analysis of variance, and the least significant difference (LSD) were used to examine significant differences, and the differences at $P< 0.05$ were considered significant.

RESULTS AND DISCUSSION

Sequential extraction procedures and different polarity solvents are the critical steps responsible for dissolving the endogenous active compounds from PM root. To achieve the highest yield of active compound, the solvents including hexane, benzene, ethyl acetate, acetone, ethanol, and DI water with the polarity of 0.0, 0.1, 0.2, 0.4, 0.6, and 1.0 were used. Based on the solubility in the different polarity of the solvent, active compounds in samples could be extracted in different fraction by using appropriate solvents. The yield obtained from the extraction shows in Table 1.

The maximum yield was obtained in DI water fraction followed by ethanol, hexane, acetone, ethylacetate, and benzene fractions, respectively. It can be suggested that the major phytochemicals in PM root have mostly high polarity, as indicated by Peerakam et al. (2018). Interestingly, the color of hexane extract was creamy white with an oily odor, while the others were yellowish without an oily smell. The possible description for this inspection was the presence of lipid or lipophilic compounds in hexane fractions, such as lipids or sterols, as indicated by Jeon et al. (2005). However, the results cannot be indicated in which fractions possess the active ingredients which exhibited antioxidant property. Therefore, the DPPH assay was firstly conducted, and the result is indicated in Figure 1.

Table 1. Extraction yields of using the different polarity solvent extraction (%)

Solvent	Hexane	Benzene	Ethyl acetate	Acetone	Ethanol	DI water
Yield (%)	2.03±0.46	1.37±0.31	1.44±0.23	1.47±0.21	3.63±0.5	11.87±1.5

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The free radical scavenging activity was determined in terms of IC_{50} value (µg/mL). The smaller IC_{50} value means a higher antioxidant activity. It was found that there was a significant variation in the IC_{50} of the different extracts (2.5±0.91 to 2125.95±18.76). The ethyl acetate fraction showed the highest DPPH radical scavenging activity comparing to other fractions, while the water faction exhibited the lowest IC_{50}. The value of scavenging activity increased from acetone, benzene, and ethanol to hexane fraction, respectively. It was indicated that mainly antioxidant compounds were deposited in moderate polar solvent extracts contributed to reducing power, as documented by Peerakam et al. (2018). To investigate the source of scavenging activity, the TPC and flavonoid contents were measured, and the result is shown in Figure 2.

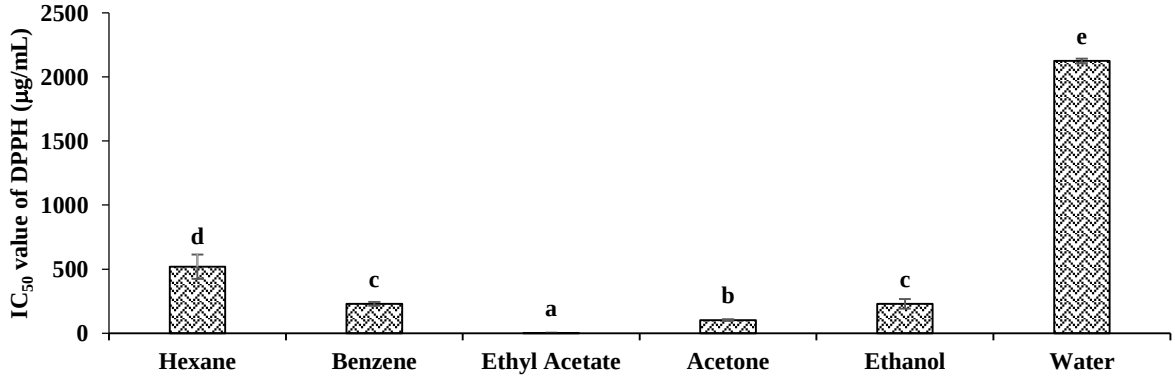

Figure 1. Antioxidant activity of different polarity extracts measured by the DPPH assay.

Different letters indicate statistically significant values (P< 0.05).

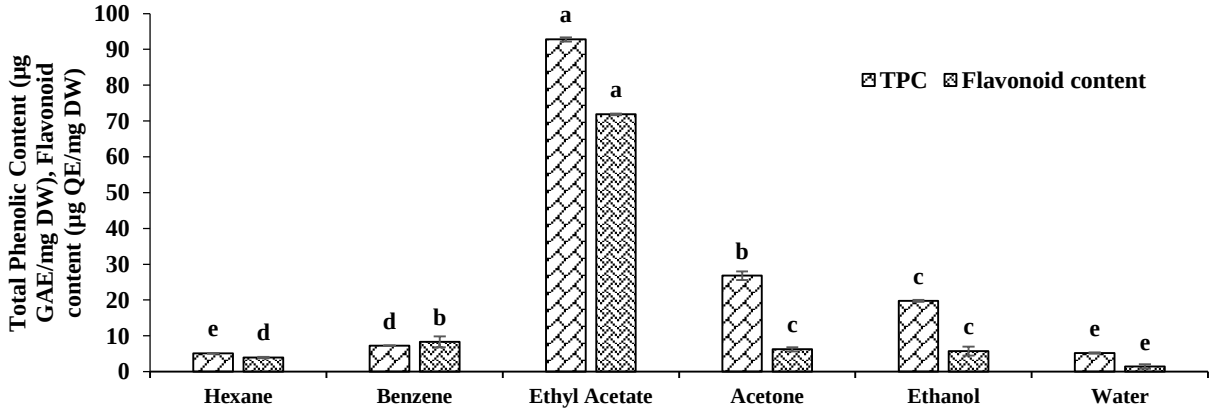

Figure 2. Total phenolic content and flavonoid content of different polarity fractions.

Different lower case letters within the bar indicate significant differences (P< 0.05) analyzed by using the ANOVA calculation.

It was found that TPC values in different polarity fractions ranged from 5.11±0.05 to 92.78±0.58 µg GAE / mg dried weight (DW). The highest TPC value was obtained in ethyl acetate fraction (EAF), and the lowest TPC value was found in DI water fraction. Interestingly, the result was positively related to flavonoid content. The highest amount of flavonoid content was also found in EAF (71.86±0.21µg QE/mg DW), while the water fraction exhibited the lowest flavonoid content. The result confirmed that phenolic compounds, as well as flavonoids, were the most responsible for the antioxidant property, as documented by Kaurinovic and Vastag (2019). However, some researchers recommended that the appropriate ratio of ethanol was also effective and economically used for single-step extraction (Peerakam et al., 2018). To confirm the bioactivity correlations of flavonoids and total phenolic contents with their estrogenic activity, the proliferative activities of fractions on the estrogen-sensitive cell line, MCF-7,were investigated by MTT method. The percentage of cell growth curve was generated and presented in Figure 3.

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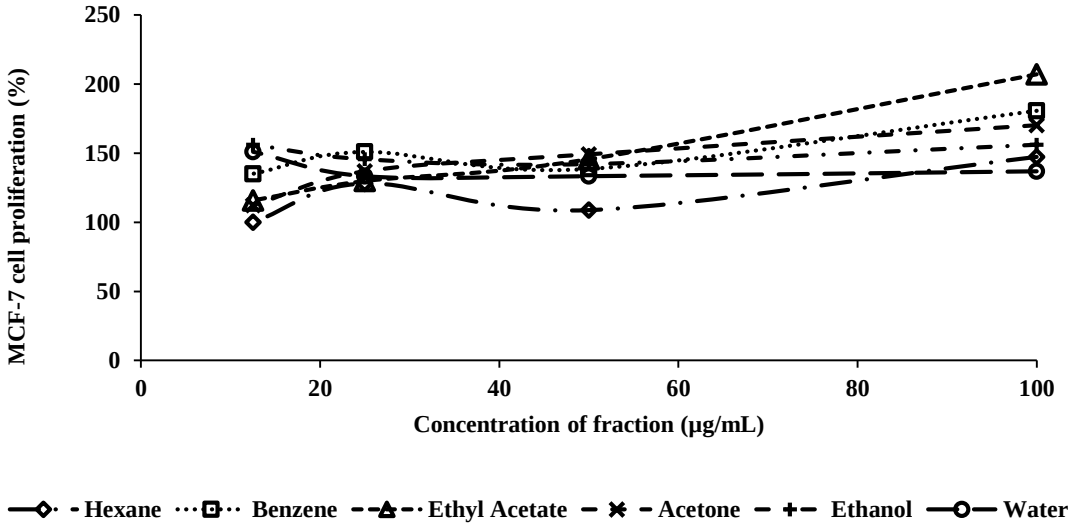


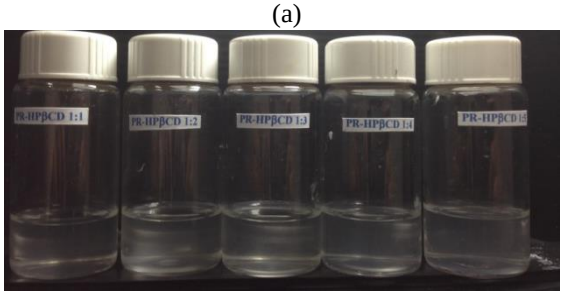
Figure 3. Proliferation curves of MCF-7 cell lines

The MCF-7 proliferation assay is an accepted method for evaluation of estrogenic effect challenged by phytochemicals or extract based on estrogen receptor alpha (ERα) mediates proliferation (Wanda et al., 2006; Innocenti et al., 2007). The mean EAF at 100 µg/mL exhibited a significantly higher proliferation against MCF-7. The result was similar to the report of Cherdshewasart et al. (2008), who summarized that a high dose of *P. mirifica* extract revealed proliferation in MCF-7. Therefore, the EAF was selected for nanoparticle nucleation through polymeric micelles formulation.

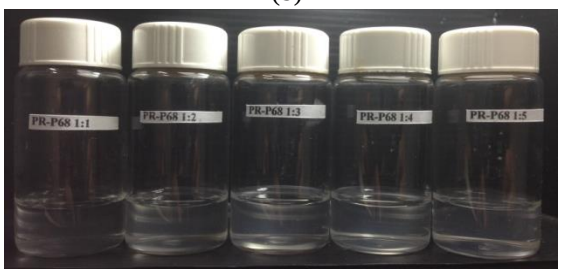
The solubility of complex prepared in water shows in Figure 4.

In the process of polymeric micelle development, Tween80 was added for incorporation into the micelles to solubilized substances. Moreover, it was used in conjunction with nanoparticles to improve specific delivery and was adsorbed on the surface of specific receptors of the blood-brain barrier (Sassa-deepaeng et al., 2016). It was found that the incorporation of Pluronic F127 and Tween80 into EAF resulted in transparent aqueous dispersions were obtained. After the freeze-drying process, the products could be solubilized in water absolutely and still being transparent, as shown in Figure 4(c). The result was in agreement with the previous results that the practically insoluble flavonoid chrysin could be solubilized by polymeric micelles, and transparent aqueous mixtures were obtained (Sassa-deepaeng et al., 2016). To confirm that EAF-F127 was in the nanosize range, size, size distribution (PDI), and zeta potential were determined using Zetasizer Nano Zs. The result is shown in Figure 5.

(a)



(b)



(c)

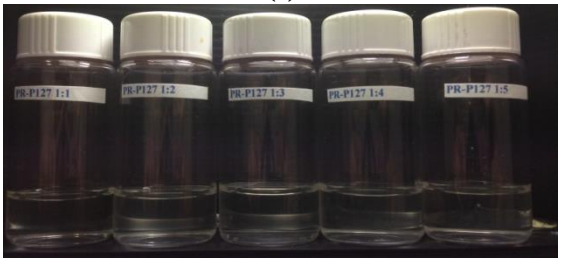


Figure 4. Solubility of (a) EAF-HPβCD, (b) EAF-F68, and (c) EAF-F127 in DI water.

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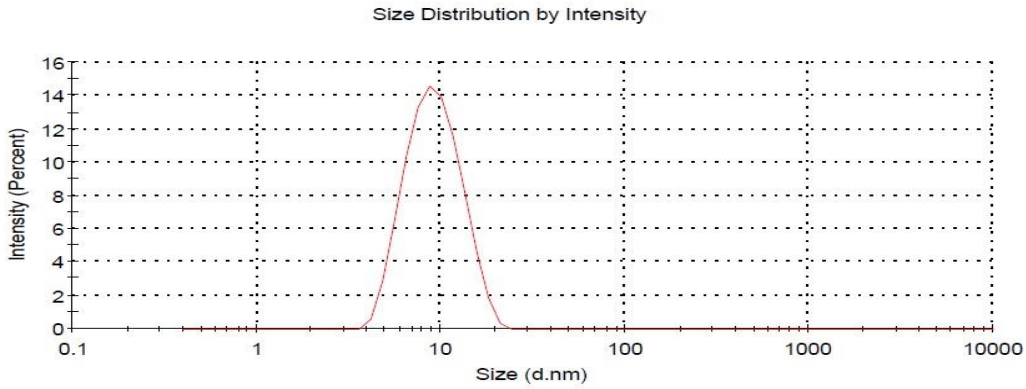

Figure 5. The size distribution by the intensity of EAF-F127.

Dynamic Light Scattering (DLS) analysis was occupied to determine the sized distribution and the polydispersity of colloidal particles of EAF-F127. The major size distribution peak was in the nanosize range of 9.564±3.17 nm with an excellent polydispersity index (PDI) of ~0.2. The dimeter of EAF-F127 was in the size range of polymeric micelles of other phytochemicals (Sassa-deepaeng et al., 2016). To predict the stability of EAF-F127, the zeta potential measurement was performed and revealed in Figure 6.

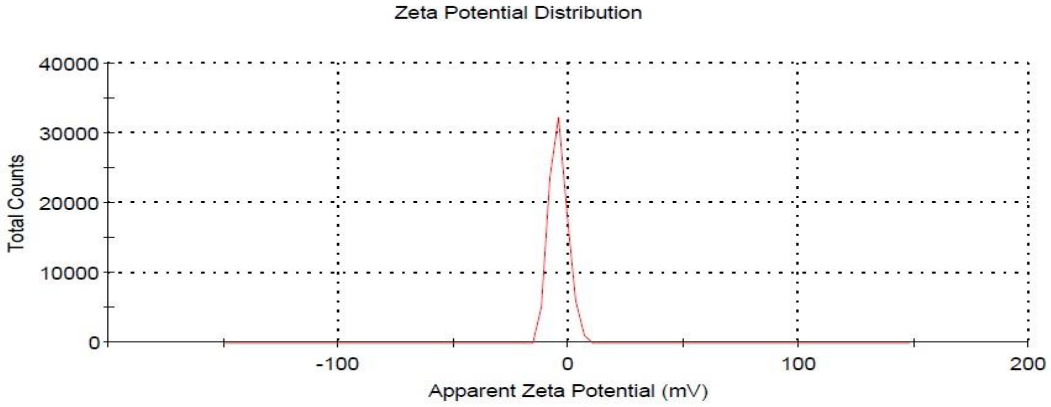

Figure 6. Zeta potential graph of EAF-F127.

The zeta (ζ) potential value is resulted by the net electrical charge contained within the region bounded by the clipping plane of nanoparticles elicited the appearance of a maximum peak at -4.32±3.394 mV. In general, the ζ potential is a parameter commonly used to diagnose cellular interaction with surface charged nanoparticles (Mikolajczyk et al., 2015). Particles that possess a large positive or negative ζ potential are repulsed each other resulted are relatively stable (Sassa-deepaeng et al., 2019). Unfortunately, the ζ potential value of EAF-F127 elicited the appearance of a major peak nearly zero, indicating that the developed EAF-F127 might be less stable and the possibility of aggregation formation. However, the strong interaction between the hydrophilic moiety of Tween80 and the water molecules would support the self-assembly of micelles resulted in the re-solubilization of particles.

CONCLUSIONS

The results demonstrated that the extraction method concerning the solvent selected affects the activity of the obtained PM root. It was observed that ethyl acetate extraction could provide high phenolic content and flavonoid content, which are positively related to proliferative activity against estrogen-sensitive cell line MCF-7. To overcome the less soluble fraction in water, Pluronic F-127 and Tween 80 were employed in the proper ratio. Finally, the obtained EAF-F127 was in the nanosize range with low PDI. Although the ζ potential value was

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nearly zero, the particles were still stable due to the existence of Tween 80 in the system and resulted in the re-assembly of micelles in water to prevent aggregation of nanoparticles. The results indicated enhanced water solubility of EAF- F127 product was promising for further use *in vivo* studies.

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Comparison of fattening Thai native steers on grassland grazing and in feedlot fed corn silage-base with supplemental two protein concentration diets

Kecha Kuha[1]*, Panuwat Chaiyong[1], and Sornthep Tumwasorn[2]

[1]Department of Animal Science and Fisheries, Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Nan 55000, Thailand
[2]Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

***Corresponding author**: kkuha@rmutl.ac.th*
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ABSTRACT

This study determined growth performance and blood biochemical values of Thai native steers during 120 days of fattening. Twelve healthy three-year-old steers were assigned equally into two groups. One group was allowed free grazing on grassland improved mainly with purple guinea grass (*Panicum maximum*), and the other group was reared in feedlot fed with corn silage-base. Both groups supplemented with different crude protein concentrate diets due with 12 and 14 percent of about 1 percent of the bodyweight a day. The results found that steers on grassland were significantly higher average daily gain than those steers reared under feedlot conditions. There was no significant difference in these values between steers supplemented with either of the two protein diets. Blood biochemical values at the end of the trial were not significantly different for the steers fed with different dietary treatments. Glucose, alkaline phosphatase, albumin, mean corpuscular volume and, mean corpuscular hemoglobin after the trial increased, but free serum thyroxine and triiodothyronine decreased and differed significantly from the values at the beginning of the trial. Other blood biochemical values, triglyceride, total cholesterol, total bilirubin, direct bilirubin, aspartate serum transferase, alanine transaminase, blood urea nitrogen, and creatinine were not significantly different between the inception and the conclusion of the trial. Differences in dietary protein supplementation caused no differences in the blood biochemical values of the steers. These results imply that the Thai native steer should fatten on grassland with a 1 percent BW dietary supplemental concentrate of 12 percent of CP.

Keywords: performance, blood biochemical, grassland, feedlot, Thai native steer

INTRODUCTION

Thai native cattle are a kind of Zebu breed (*Bos indicus*). They play an important role in the livestock-cropping production system of smallholder farms in Thailand. Native cattle can be a savings account of farmers that can be changed in cash when necessary (Tumwasorn, 2002). Moreover, they still have many more advantages, such as tolerance to tropical parasites, can use low-quality roughage efficiently, and high fertility (Tumwasorn, 2007). However, they have a small frame size and a low growth rate (Tumwasorn, 2002; Sethakul, 2010). The frame size was more suitable for usage in Hari Raya Aidilfiti festival (Angkulasearanee and Wattanachant, 2010; Pakeechay et al., 2014). Moreover, native cattle meat has a low-fat content (Sethakul, 2010) and can be produced as natural beef production (Duanyai, 2010). So, the interest in fattening native beef is increasing by an opportunity of market increasing (Pakeechay et al., 2014). In Nan province, there are many residuals from the cropping system, especially sweet corn production. After sweet corn was harvested, there is plenty of green stems and leaves of corn that can be cut and carried to feed cattle as a fresh roughage. However, the green corn stem and leaf can become yellow shortly after that; therefore, making corn silage could be a good choice for farmers to reserve corn stem and leaf for feeding cattle. Corn silage can be a good source of quality roughage in fattening beef. During fattening, moreover, farmers needed to pay attention to the health, quality, and welfare of steers. For this reason, blood biological values are needed to assess health status, evaluate body response to nutrition, and indicate adaptability to adverse environmental conditions and the associated stress and welfare (Hall et al. 1995; Jain, 1996; Keneko et al., 1997; Boonprong et al., 2007a; Aengwanich et al., 2009). Given these conditions in northern Thailand, farmers will have two options available to fattening native cattle due to rearing cattle in feedlots or allow grazing in the grassland. A study of these options is best suited for fattening Thai native cattle that have not been performed, and diet recommendations have been based largely on analogies to other breeds.

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This research aimed to determine the growth performance and blood biochemical values of Thai native steers during fattening. One group of the steer was allowed free grazing on grassland, and the other group was reared in feedlot fed corn silage base. The kind of roughage and feeding regime makes a difference in the nutritional intake of the steer, in particular the protein intake. Therefore, the question of protein supplementation was also addressed and compared for both feed modalities by supplying the steer diets with a protein concentrate at two different levels.

MATERIALS AND METHODS

Experimental design and procedure

The 2 x 2 factorial in a completely randomized design was conducted for this trial. Factor A was maintained under steer feeding regiment due to free grazing on the grassland and stay in feedlot fed corn silage-base procedures. Factor B has two levels of crude protein concentrate diet with 12 and 14 percent. Twelve three-year-old healthy Thai native steers, bodyweight about 183.5 kg by average, were randomly assigned to the experimental unit. Six steers were allowed free grazing on the grassland about 10 hrs a day (7:00 am – 5:00 pm). The grassland was mainly purple guinea grass (*Panicum maximum*). The other six steers were reared in a feedlot fed corn-silage base diet for two times a day. Steers in both groups were fed a protein concentrate diet of about 1% body weight per day during the evening with an individual feeder. The ingredient and composition of diets showed in Table 1. Steers were allowed free access to the water basin all the time. Before the experiment starts, all steers were castrated and waiting until fully recovered. Steers, after that, were treated for internal and external parasites (Abentel, Attantic Laboratories Corp., Ltd, Thailand, and Ivomec F®, Merial (Thailand) Ltd.) and were injected with a vitamin complex (Catosal®, OLIC (Thailand) Ltd., under supervision of Bayer Leverkusen, Germany). The body weights of the steers were measured at the inception of the trial and then every 30 days during the experiment until the conclusion at 120 days.

Table 1. Ingredients and composition of the experimental diets

Ingredients	Diet 1 (kg)	Diet 2 (kg)
Corn	70.00	70.00
Rice bran (from local rice mill)	25.00	20.00
Soybean meal	-	5.00
Urea	2.00	2.00
Di-calcium phosphate	1.90	1.90
Sodium chloride	1.00	1.00
Sulfur	0.10	0.10
Total	100.00	100.00
CP, %	12.00	14.00
TDN, %DM	86.16	85.76
ME, MJ/kg	3.34	3.27

Preparation of grassland and corn silage

Preparation of grassland, mainly purple guinea grass, was improved using cattle manure about 100 kg/rai and urea about 10 kg/rai. Then, grassland was supplied water twice a week using a sprinkler for 2 months. For corn silage production, the whole stem and leaf of corn were cut into 2-3 cm lengths with a chopper machine. Then, the chopped corn was tightly packed in a plastic bag and tied with a plastic band or rope after removing air using a blower. After that, the silage bags were stored in a closed room at ambient temperature for three weeks at least and carried for feeding steers.

Blood biochemical analysis

Blood samples were collected at the experiment start and after finishing at the 120-days trial. Blood samples were taken from the jugular vein during fasting (7:00-8:00 am) using 10 ml disposable syringes. The specimens, after that, were put into K3 EDTA and NaF tubes about 2.5 ml each, and the remainder were allowed clotting in a blood clot tube. Serum was obtained following centrifugation at 600 x g for 10 minutes and stored at 4-6 °C before analysis. The blood serum biochemical profile was determined automatically using a COBAS INTEGRA® 800 (Roche, Switzerland). The blood biochemical values were glucose (GLC), total

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bilirubin (TBIL), direct bilirubin (DBIL), aspartate serum transferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CR), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), albumin (ALB), free serum thyroxine (FT4) and free triiodothyronine (FT3). The complete blood cell counts (CBC) were analyzed using an electrical impedance technique by Sysmex K4500 (GMI, Inc., Minnesota, the United States). The hematological values were white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HB), hematocrit (HCT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)

Statistical analysis

The productive traits were analyzed using analysis of covariance (ANCOV) that determined initial body weight as a concomitant variable. The least-squares mean and standard error of the mean were compared using a significant t-test ($P \leq 0.05$). Blood biochemical and hematological values were compared between and within the feeding regiment group and between the periods of the experiment using the paired t-test. The statistical model was following the experimental design using the generalized linear model (GLM) procedure of the Statistical Analysis System (SAS, 1999)

Table 2. Productive performance of the Thai native steers during fattening: Feedlot, fed corn silage-base, and grassland, free grazing

Items *	Feedlot fed corn silage-base		Grassland free grazing	
	Diet 1	Diet 2	Diet 1	Diet 2
	LSM ± SEM	LSM ± SEM	LSM ± SEM	LSM ± SEM
BW_{ini}, kg	180.33 ± 7.44	178.50 ± 9.25	188.00 ± 6.79	187.17 ± 3.68
BW_{fin}, kg	237.57 ± 4.30^{a}	243.17 ± 4.38^{ab}	252.12 ± 4.35^{b}	241.48 ± 4.32^{ab}
BW_{fin},kg$BW^{.75}$	60.49 ± 0.80^{a}	61.56 ± 0.81^{ab}	63.23 ± 0.81^{b}	61.25 ± 0.80^{ab}
BWG, kg	54.07 ± 4.30^{a}	59.67 ± 4.38^{ab}	68.62 ± 4.35^{b}	57.98 ± 4.32^{ab}
ADG, g/d	439.67 ± 3.50^{a}	485.14 ± 3.56^{ab}	558.14 ± 3.54^{b}	471.39 ± 3.51^{ab}
ADG, g/kg$BW^{.75}$/d	7.24 ± 0.45^{a}	7.89 ± 0.46^{ab}	8.78 ± 0.45^{b}	7.70 ± 0.45^{ab}
DMI_{con}, kg/d	1.98 ± 0.15^{b}	1.96 ± 0.15^{b}	1.15 ± 0.15^{a}	1.42 ± 0.15^{a}
DMI_{rou}, kg/d	3.17 ± 0.01	3.13 ± 0.01	-	-
tDMI, kg/d	5.16 ± 0.01	5.10 ± 0.01	-	-
FGR, kg	12.05 ± 0.02	10.71 ± 0.16	-	-

* BW_{ini} = initial body weight, BW_{fin} = final BW, BWG = body weight gain, ADG = average daily gain, DMI_{con} = dry matter intake of concentrate, DMI_{rou} = DMI of roughage, tDMI = total DMI, FGR = feed per gain ratio, LSM = least square mean, SEM = standard error of mean

^{a,b} Different superscript on the same row indicated significant differences ($p \leq 0.05$).

RESULTS AND DISCUSSION

Productive performance

The result found significant differences in an interaction effect ($P < 0.05$), as showed in Table 2. So that, the values of final body weight (BWfin), body weight gain (BWG), and average daily gain (ADG) were not significantly different ($P > 0.05$) between steer in the feedlot and on grassland grazing when fed concentrate at 14% CP. Those values found significant differences ($P < 0.05$) between steers of two feeding regimes when supplemented 12% CP concentrate diet. Dry matter intake of concentrate indicated that steers in feedlot consumed more concentrate than that of steers in the grassland significantly ($P < 0.05$).

The steers allowed free grazing on grassland tended to have higher cumulative BW and ADG than those of steer in feedlot fed corn silage-base during 60, 90, and 120 days of the experiment ($P = 0.08$). Furthermore, during 30 to 60 days of the experiment, the steers on grassland free grazing were the highest ADG, differed from steers in the feedlot significantly ($p<0.05$) as showed in figure 1. This is because compensatory growth was affecting to steers that body condition score of steers was quite low before the experiment starts. The steers were mostly loose in bodyweight, which may cause by the scarcity of roughage supply (Drouillard et al., 1991; Kuha et al., 2009). This can be influenced by BWG and ADG of steers during the early stages of fattening. These agreed to Horton and Holmes (1978), who found that cattle with restricted feed intake gained weight more rapidly during subsequent full feeding in the first 8 weeks. They claimed the compensatory gain paralleled increased intake with no change in ration

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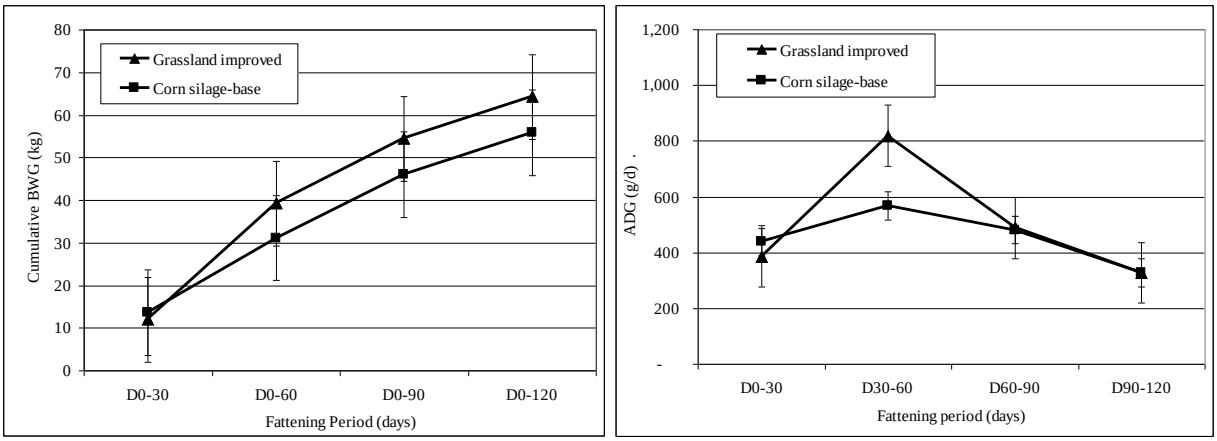
digestibility. Compensatory growth was influenced more by differences in severity of restriction than by the duration of the restriction period (Drouillard et al., 1991).

The ADG of this study was lower than observed in a crossbreed of native cattle with Brahman breed (0.60 kg/d) (Wanapat et al., 1995), and Brahman x Charolais is crossbred (0.89 kg/d) (Tumwasorn, 2007). The ADG and DMI of this study were nearly the same as those reported in native cattle (Bunseelarp et al., 2010; Harnsamer et al., 2010; Kaewpila et al., 2010). This study was accepted as a recommendation of standard nutrient requirements for native cattle in Thailand (WTSR, 2008). Although ADG is related to the dietary proteins reported by Chantiratikul et al., (2009), ADG of steers fed corn-silage base in this research was not significantly different by the level of dietary crude protein. This indicated that concentrate about 12% CP could be effectively and more suitable for fattening native cattle. These agreed to the recommendations by Wanapat et al. (1995).



(a) (b)

Figure 1. Cumulative body weight gain (a) and average daily gain (b) of Thai native steers during 120 days fattening between grassland grazing and in feedlot fed corn silage base

Blood biochemical values

The blood biochemical and hematological values were not significantly different between steers randomly assigned to the different treatments at the beginning of the trial. Those values of the final experiment were also found to be not significantly different between steers given the different dietary treatments in both the grassland grazing and the feedlot, as shown in Table 3. The PLT values of steers in the feedlot with supplementation of diet 1 were found to be lower than that of the other treatments (P=0.068). Steers on grassland free grazing tended to have a higher PLT count than that of steer in the feedlot throughout the 120 days of the experiment. This may indicate that steers in grassland produced more PLT against blood parasites than that of steers in the feedlot, which may embed more in tropical regions. In contrast, low PLT counts could increase bleeding risks. However, the value of this research was in the range of normal (1.0 – 8.0 $x10^5/\mu L$) (Kaneko, et al., 1997).

The values of GLC, ALP, ALB, MCV, MCH at the end of the trial increased significantly different (P<0.05) from those of beginning the trial. The value of TG of the steers by average tended to be higher than that of at the beginning (P=0.07). In contrast, the values of FT4 and T3 decreased significantly at the end of the trial. The blood biochemical values of steers due to TBIL, DBIL, ALT, BUN, and CR were not significantly different between the beginning and the end of the trial. These were the same as hematological values due to WBC, HB, HCT, RBC, MCHC, and PLT that found to be not significantly different between the initiation and conclusion of the trial (Table 4). Plasma GLC concentration increased dramatically during the experiment for steers supplied with the dietary protein treatment. Although GLC concentration at initiation (53.33 ± 3.56 mg/dL) was in the normal range (Kaneko, et al., 1997); however, this was closer to the lower limit of normal values (50.0 mg/dL). This may indicate that before the trial, steers may lack energy supply caused by the restriction of roughage (Kawashima, 2002). At the conclusion of the trial, plasma GLC of steers by average was high (65.08 ± 1.36 mg/dL), indicated an adequate energy supply as a result of the dietary treatment. Serum ALB at the end differed significantly from that at the onset of the trial (p<0.001). Serum ALB is produced in the liver and plays many important functions, including the indicator of plentiful nutritional supply. These may indicate that before starting the experiment, steers were at the risk of malnutrition and

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malabsorption. The fattening process can achieve an abundant nutrition supply to steers. However, for both sets of steers, ALB was in the range given by Kaneko et al. (1997).

At the end of the 120-day trial, steers tended to have higher plasma triglyceride (TG) than that at the beginning ($p = 0.07$). The TG value before starting the trial was nearly the same as for Japanese black crossbred-fed rice straw receiving enough total digestible nutrients (Kita et al., 2003). The value of total cholesterol (TC) was not significantly different between steers fed different dietary treatments and between inception and conclusion of the trial. The TC value of this study was higher than that of Japanese black crossbred cattle (129.33 ± 3.15 mg/dL; Kita et al., 2003) and buffalo calves (129.4 ± 4.96 mg/dL; Kumar and Dass, 2006). The values of TC, TG, and LDL at the end of the trial correlated positively with each other (TG vs. TC = 0.99, TG vs. LDL = 0.97, TC vs. LDL = 0.98). The values of TC, TG, and LDL were negatively correlated to TBIL, which were -0.63, -0.65, and -0.68, respectively. The values of AST, ALT, and ALP levels showed no significant difference between the dietary treatments for the duration of the trial. This study also found lower AST and ALT values than reported in Thai native cattle by Boonprong et al., (20007b) but was in the range reported by Kaneko et al. (1997). The ALP value of steers at the end of the trial was significantly different from that of steer at the beginning of the trial ($P<0.05$). The values of AST and ALT were highly positively correlated with T3 (0.74 and 0.69).

The serum FT4 and T3 were significantly lower at the end of the trial when compared to the beginning (Table 4). The increasing availability of T3 can induce more extensive protein degradation than synthesis (Hersom et al., 2004). The decreasing concentrations of T3 can decrease the requirements to maintain energy (Murphy and Loerch, 1994) and decrease protein degradation (Buttery, 1983; Ellenberger et al., 1989). This result either indicates that proteins mobilized from muscle for maintenance before the trial, or it was evidence of low protein intake. Then, during fattening, steers consumed enough protein, and that could be decreased secretion of T3 and FT4, and this may be responsible for increasing energy in the used for growth. Hersom et al. (2004) claimed that lower concentrations of T3, and T4 in steers, which entered the feedlot, did not inhibit their growth as a response to the previous restriction. The BUN value showed no significant difference between the periods of this research, although BUN relates closely to protein intake in beef cattle (Hammond, 1998).

The values of MCV and MCH were not significantly different between dietary treatments within the study period. However, the values at the end of the trial were significantly higher than those at the beginning. Those values for Thai native steers in this study were lower than Holstein Friesian crossbreed (Angwanich, 2002) and Brahman crossbred cattle (Angwanich et al., 2009). Both values, however, were within the reference range (Kaneko et al., 1997). This may indicate that RBCs of Thai native steers were larger in cell size than those of other breeds. The values of TBIL, DBIL, CRT, WBC, HB, HCT, RBC, and MCHC were not significantly different between dietary treatments and were in the normal range (Jane, 1996; Kaneko et al., 1997). These values for the beginning were not different from the conclusion of the trials.

CONCLUSIONS

There was no significant difference in daily gain between steers supplemented with either of the two protein diets. Steers on the grassland free grazing had a significantly higher daily gain than those steers in the feedlot. Differences in dietary protein supplementation caused no differences in the blood biochemical values of the steers. Blood biochemical values at the end of the trial were not significantly different for the steers fed the different dietary treatments. The results imply that the most effective way to fatten Thai native cattle is to allow it to graze on grassland and be given a 1% BW protein dietary supplemental concentrate of 12% CP.

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Table 3. Blood biochemical values of Thai native steers at the conclusion of the trial (120 days)

Items	Feedlot		Grassland		SEM	P-values
	Diet 1	Diet 2	Diet 1	Diet 2		
GLC, mg/dL	65.67	66.00	67.00	61.67	2.84	0.347
TBIL, mg/dL	0.37	0.17	0.37	0.33	0.07	0.288
DBIL, mg/dL	0.08	0.03	0.03	0.02	0.02	0.294
AST, U/L	50.00	51.00	61.00	68.00	5.85	0.622
ALT, U/L	19.00	22.00	26.67	26.00	1.72	0.317
ALP, U/L	246.33	144.00	171.33	162.00	29.14	0.149
BUN, mg/dL	14.00	15.00	15.67	14.00	0.97	0.207
CR, mg/dL	1.46	1.50	1.27	1.37	0.07	0.663
TC, mg/dL	143.00	222.67	177.67	163.67	50.25	0.379
TG, mg/dL	63.00	146.00	82.67	67.00	50.67	0.359
HDL, mg/dL	40.33	36.67	51.33	48.00	8.12	0.984
LDL, mg/dL	90.07	156.80	109.80	102.27	40.64	0.388
ALB, g/dL	3.20	3.30	3.00	3.17	0.11	0.760
FT4, ng/dL	0.97	0.83	0.90	0.87	0.06	0.412
T3, ng/dL	94.33	92.00	145.00	114.33	12.72	0.298
WBC, $x10^3/_{\mu}L$	13.03	11.66	10.59	12.15	1.51	0.362
RBC, $x10^6/_{\mu}L$	8.52	8.18	7.11	8.91	0.85	0.241
HB, g/dL	12.70	12.23	10.80	13.73	1.33	0.237
HCT, %	39.00	38.33	34.00	43.00	4.46	0.310
MCV, fL	45.87	46.90	47.67	47.97	1.59	0.823
MCH, pg	14.97	15.00	15.17	15.33	0.48	0.893
MCHC, g/dL	32.63	32.03	31.80	32.00	0.56	0.494
PLT, $x10^5/_{\mu}L$	1.47	2.55	2.64	2.30	0.34	0.068

Table 4. Comparison of blood biochemical values of Thai native steers at the beginning and the ending of the trial

Items	Beginning of the trial		End of the trial		pr> |t|	Reference
	Mean ± SEM	range	mean ± SEM	range		range *
GLC, mg/dL	53.33 ± 3.56[a]	29-72	65.08 ± 1.36[b]	55-71	0.002	50.0-75.0
TBIL, mg/dL	0.28 ± 0.02	0.22-0.39	0.31 ± 0.04	0.01-0.5	0.453	-
DBIL, mg/dL	0.04 ± 0.01	0.01-0.1	0.04 ± 0.01	0.01-0.12	0.885	-
AST, U/L	55.58 ± 3.81	39-81	57.50 ± 3.35	43-81	0.622	75.0-135.0
ALT, U/L	23.42 ± 1.57	17-33	23.42 ± 1.19	17-29	1.000	11.0-40.0
ALP, U/L	148.75 ± 11.97	87-228	180.92 ± 17.11	100-327	0.034	0-488
BUN, mg/dL	13.88 ± 0.77	9.6-18.9	14.67 ± 0.47	12-17	0.421	10.0-20.0
CR, mg/dL	1.43 ± 0.05	1.27-1.92	1.40 ± 0.04	1.2-1.67	0.556	0.7-1.5
TC, mg/dL	146.00 ± 4.68	112-167	176.75 ± 23.17	115-419	0.299	-
TG, mg/dL	40.25 ± 1.78[a]	28-53	89.67 ± 23.83[b]	38-347	0.073	-
ALB, g/dL	2.82 ± 0.05[a]	2.35-3.01	3.17 ± 0.06[b]	2.8-3.5	<.0001	2.8-3.5
FT4, ng/dL	1.64 ± 0.14[b]	1-2.4	0.89 ± 0.03[a]	0.7-1.0	<.0001	-
T3, ng/dL	213.08 ± 11.07[b]	151-287	111.42 ± 8.39[a]	79-182	<.0001	-
WBC, $x10^3/_{\mu}L$	12.08 ± 0.73	8.8-18.7	11.86 ± 0.70	7.6-16.7	0.653	4.0-20.0
RBC, $x10^6/_{\mu}L$	8.76 ± 0.20	7.71-9.81	8.18 ± 0.41	6.2-11.3	0.176	5.0-10.0
HB, g/dL	12.53 ± 0.30	10.9-14.3	12.37 ± 0.65	9.3-17.8	0.780	8.0-15.0
HCT, %	39.08 ± 1.09	33-45	38.58 ± 2.13	28-56	0.789	24.0-46.0
MCV, fL	44.58 ± 0.64[a]	40.4-48.5	47.10 ± 0.72[b]	41.4-50.1	<.0001	40.0-60.0
MCH, pg	14.31 ± 0.20[a]	12.8-15.3	15.12 ± 0.21[b]	13.7-16.1	<.0001	11.0-17.0
MCHC, g/dL	32.13 ± 0.31	30-34.1	32.12 ± 0.26	30.3-33.3	0.925	30.0-36.0
PLT, $x10^5/_{\mu}L$	2.11 ± 0.35	1.24-5.61	2.24 ± .20	1.4-3.7	0.547	1.0-8.0

[a, b] Different superscript on the same row indicated significantly different ($p \leq 0.05$).

* adapted from Jain (1996)and Kaneko et al.(1997)

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Small RNA sequencing studies of populations of plant virus families in tropical rainforest of Xishuangbanna

Yue Liu[1,4], Xu Shan Shan[1,2], Wang Kan[1,2], Baohua Kong[1,2*], Hongxiang Li[3], Chongde Wang[1,2], Taiyuan Yang[3], Chengyun Li[1,2], Yuanhua Tan[3], Hao Qu[4], Dexi Wu[1,2], Lianchun Wang[5], and Hairu Chen[1,2]

[1]State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, No.452, Fengyuan Road, Panlong District, Kunming, Yunnan 650500, China
[2]Key Laboratory of Ministry of Education Agriculture Biodiversity for Plant Disease Management, Yunnan Agricultural University, Kunming, Yunnan 650500, China
[3]Xishuangbanna Plant Protection Plant Quarantine Station, Jinghong, Yunnan 666100, China
[4]Tea Research Institute, Yunnan Academy of Agricultural Sciences, Menghai, Yunnan 666201, China
[5]Yuxi Normal University, Yuxi, Yunnan, 653100 China

***Corresponding author**: *baohuakong@126.com*
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ABSTRACT

The tropical rainforest of Xishuangbanna has the most abundant and cryptic plant species biodiversity in China. To explore the populations of plant virus families in Xishuangbanna tropical rainforests, fifteen pools of plant samples from different forest locations were collected and used for small RNA analyses by high throughput sequencing. All contigs were classified and annotated with the NCBI Nt database to determine the species distributions, and comparisons were conducted using the Blast algorithm. The viral sequences in the Clean Reads were compared with the relevant sequences, using the Kraken software system, to infer their possible classification and to analyze the abundance of each species statistically. The number of Clean reads of every pool sample was from 1573897 to 26878598, and the average number of Clean reads was 20322116. The average number of clean reads is taken as 0.0036% from the average number of Raw reads. The results from a total of 3703 viral sequences were annotated, and these represented a total of 16 plant virus families. Among these, 1952 Geminiviridae sequences comprised the dominant annotated virus proportion. 732 sequences belonged to the Potyviridae, and 192 sequences were characteristic of members of the Caulimoviridae. The viral sequences primarily originated from dicots and monocotyledonous plants, including herbaceous and woody plants. Generally, the total viral sequences from herbaceous plants represented 66.7 %, and 33.3 % were from woody plants, and amongst these, 57.1% dicots represented the plant hosts, whereas 42.9 % were derived from monocotyledonous families. The presumptive Geminiviridae and Potyviridae viruses have broad host ranges, and different families were annotated from each sample site in Xishuangbanna. This study lays a foundation for future research on the evolution and utilization of viruses within tropical rainforests and those of cultivated agricultural areas.

Keywords: virus, family, population, tropic forest, Xishuangbanna

INTRODUCTION

Plant viruses affecting agriculture production have been investigated extensively, but aside from cultivated plants, little is known about virus distribution in other plant ecosystems. According to the 9th report of the International Committee on Taxonomy of Viruses (ICTV) (Roossinck 2011; Owens et al., 2012) about 900 species of plant viruses were listed, and almost all of these are plant pathogens (Roossinck, 2011), and these accounted for 77% of the viruses that were recognized in 2012 by the ICTV. However, due to host ambiguities and the difficulties of virus isolation using traditional virology research methods, it has been difficult to carry out research on plant virus distribution in alternative habitats. Hence, most plant virus research has focused on agriculturally important viruses, but it is slowly being recognized that some viruses contribute to the survival of the host (Marquez et al., 2007). These studies suggest that some viruses are actually beneficial to the host in various ways, often by helping the host take advantage of the fierce competition in the natural environment. Studies such as those of Marquez et al. (2007) have found that symbiosis with fungi can play an important role in helping plants survive drought and other adverse environmental conditions (Marquez et al., 2007; Roossinck, 2010; Roossinck et al., 2010; Roossinck, 2011). These studies provide strong evidence that viruses and their symbiosis play an important role in the evolution of life on earth (Roossinck, 2011; Roossinck, 2005). Nevertheless, little is currently known about the role and

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significance of plant viruses in non-agricultural ecosystems. Next-generation sequencing technology provides new and rapid analyses to identify known or unknown viruses that are difficult to isolate and characterize in different ecosystems, such as lakes, oceans, plants, plant virus, and even infected animals (Wei, 2012; Yang et al., 2013; Ge et al., 2013; Grover et al., 2010; Roossinck, 2012; Roossinck, 2010; Ma et al., 2016). Muthukumar et al. (2009) studied the presence of plant viruses in the Tallgrass Prairie Preserve in Oklahoma, USA, and identified genome sequences from different organisms, including plants, bacteria, fungi, and viruses. The results indicated that the viruses in the Highland Grassland Reserve were different from those in adjacent agricultural systems. Scheets et al. (2011) detected the members of Tombusviridae, and Min et al. (2012) studied the molecular characteristics, ecology, and epidemiology of Tymovirus infections of *Asclepius viridis*. Vaskar et al. (2015) also studied the taxonomic composition of viruses in Oklahoma Highland Grassland Reserve to explore the role and significance of plant viruses in this ecological niche. However, until the present time, there have been no reports about plant virus distributions in tropical rainforests.

Xishuangbanna is located in southwest central China at the northernmost end of the Southeast Asia tropical rainforests which represent one of three major tropical rainforest areas in the world. The Xishuangbanna Tropical Rainforest is a member of the biodiversity conservation circle of the United Nations, and a Chinese ecological demonstration zone, and contains a wealth of plant, animal, and microbial resources (Yang, 2011; Zhu, 2015; Zhu, 1994). The rich ecological diversity of these regions consists of a wide variety of plant species, but essentially nothing is known about plant virus populations or their composition within this region or any other tropical rainforest area in the world. Therefore, we have initiated a study of plant virus populations in the Xishuangbanna region to provide preliminary information about this important ecosystem. Our study provides a preamble for more extensive systematic investigations of plant and other viruses using small RNA analyses to clarify the distribution of plant virus families and their diversity within the region and their role in the ecological stability of the region. The study also provides a foundation to compare the distribution and evolution of crop viruses in rejoins adjacent to the tropical rain forest.

MATERIALS AND METHODS

Plant samples

From 2015 to 2016, 15 pools of mixed plants were collected from 15 random sites in Xihsuangbanna, as shown in Figure 1 and Table 1. The leaves from each sampling site were frozen in liquid nitrogen wrapped in aluminum foil and stored at -80 °C.

Main Reagent

The EASYspin plant microRNA extraction kit (Aidlab Biotechnologies Co.) was used for mRNA isolation and protein identification.

Small RNA extraction and library construction for sequencing

A total 15 pools of mixed plants from Xishuangbanna were used as samples, and their small RNAs were extracted using the EASY spin methodology according to the kit instructions, which were immediately used to construct cDNA libraries. For cDNA library constructions, total RNAs from the plant were used as templates for polymerize chain reaction (PCR) analyses. A joint sequence was linked at the small RNA 3' end before reverse transcription to avoid self-interactions between the 3'and 5' termini. Subsequently, PCR amplification using random primers were conducted, and the products were separated in PAGE gels for fragment purification. In preliminary tests, the sequences were quantified with Qubit 2.0, and the sizes of synthesized cDNAs were determined with Agilent 2100. Then, the effective cDNA concentrations (>2nM) were determined with qPCR, and sequenced with the Illumina HiSeq 2500 sequencing platform (Biomarker Technologies Co. LTD).

Data Processing and Virus Annotation

The original image data files sequenced by Illumina HiSeq2500 platform were transformed into Raw Data or Raw Reads by base calling, and then snRNAs, Sc RNA repeat sequences, rRNA, and tRNAs were filtered and annotated for RNA classification. The small RNA contigs were further spliced using the Velvet de novo Assembler for short-read sequencing technologies with velvet software (Zerbino, 2010). The resulting contigs were classified and annotated with the NCBI Nt (NCBI non-redundant nucleotide sequences; ftp://ftp.ncbi.nlm.nih.gov/blast/db/) database to determine the species distributions, and comparisons were conducted using the Blast algorithm (Korf, 2003), in which the parameter limitation is 1e-5 for BLASTN. The viral sequences in the Clean Reads were compared with the relevant sequences, respectively, using the Kraken software system

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(Wood and Kraken, 2014), to infer their possible classification and to analyze the abundance of each species statistically. The viral sequences were annotated and classified in the Xishuangbanna Tropical Forest.

RESULTS

Sample distribution

A total of 15 pool Leaf samples was collected from 13 sites (A to O) throughout the Xishuangbanna Tropical Rainforest in Yunnan Province of China (Figure 1 and Table 1). The pool samples distributed at Mengyang Town Nature Reserve in Jinghong City, the nature reserve of Menglun Town, Mengla County, Menglun Town Nature Reserve, Mengla County, and other sites where were abundant plant resources in Xishuangbanna tropical Rainforest in Yunnan, from elevation was from 795 m to 1381 m, the plants resourced from herb to wood, and from Dicots and Monocotyledons, representative the plant distribution in tropic rainforest Xishuangbanna.

Data output of small RNA sequencing

In these experiments, the quality of the sequences from each plant sample was managed, and after the construction of the cDNA library, we used Q-PCR to quantify the effective library sequence concentration to ensure the quality of the library. High-quality sequences (Clean Reads) were obtained by removing low-quality sequences. Clean Reads were filtered NCRNAs and repetitive sequences, such as ribosomal RNA (rRNA), transfer RNA (tRNA), intranuclear small RNA (snRNA), and nucleolar small RNA (snoRNA) to ensure the quality of the experimental samples and the accuracy of the analysis results in each step of the study. The statistics data of small RNA sequencing show in Table 2. The results that the number of original Raw reads sequence was from 18618044 to 30056704, with an average of 2485974. The number of small RNA sequences whose length was less than 18 bp after removing joints ranged from 480406 to 3026389, with an average of 1368999. The number of sequences whose length was greater than 30 bp after removing joints ranged from 654641 to 6254772, with an average of 254326. Because the content of N exceeds 10%, the number of reads filtered out ranged from 89010 to 18005, with an average of 95025. Finally, the number of Clean reads was gotten from 1573897 to 26878598, with an average of 20322116. These manipulations resulted in 304.83 M Clean Reads, no less than 15.74 M Clean Reads, was used to classify and annotate 320.5 contigs obtained by stitching and assembling to ensure the accuracy of the information of 3703 virus-related sequences and 16 plant virus families (Figure 2). Throughout our experiments, the annotated sequences were submitted to NCBI (SRA accession: SRP158336, Temporary Submission-ID: SUB43936 22).

Plant virus families annotated in the tropical rainforest of Xishuangbanna

All contigs were classified and annotated with the NCBI Nt database to determine the species distributions, and comparisons were conducted using the Blast algorithm. The viral sequences in the Clean Reads were compared with the relevant sequences, respectively, using the Kraken software system, to infer their possible classification and to analyze the abundance of each species statistically. Among the viruses annotated in the A to O sampling sites are shown in Figure 1. The families Caulimoviridae, Geminiviridae, and Potyviridae were the most widely distributed. The families Alphaflexiviridae, Bunyaviridae, Luteoviridae, and Tymoviridae had lower distributions in the sites and were annotated at three or fewer locations, whereas the Pospiviroidae and Rhabdoviridae samples were each detected at only one sampling site. The primary sequences annotated in this study consisted of more than 90% homology for each family listed in Figure 2. A total of 3703 viral sequences was annotated to 16 plant virus families, and among these, nine primary families were annotated and detected > 10 times. These included the families Alphaflexiviridae, Bunyaviridae, Caulimoviridae, Geminiviridae, Luteoviridae, Pospiviroidae, Potyviridae, Rhabdoviridae, and Tymoviridae (Table 3). Among these, 1952 Geminiviridae sequences were annotated and represented the dominant family. The 732 Potyviridae sequences represented the second most abundant family, and 192 Caulimoviridae sequences were annotated, followed by decreasing amounts of 14 other families. The remaining families shown in Figure 2 were detected < 10 times and hence are not included in Table 3. These included the families Bromoviridae, Nanoviridae, Nyamiviridae, Partitiviridae, Secoviridae, and Tombusviridae. Many viruses in these families cause major losses to crop production and rely on insects (aphids, whiteflys, thrips etc.) for local and long-distance transmission.

Description of plant families from which the virus sequences were derived

The pool samples from which the annotated families originated came primarily from herbaceous and woody dicots with a smaller proportion from monocotyledonous plants as well as more primitive families (Table 3). The Dicot representatives

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included the widely distributed Compositae and Euphorbiaceae, Malvaceae, Rosaceae, and Solanaceae families, each of which included a large number of herbs, shrubs, and succulents of agricultural importance. The Caricaceae family includes papayas, and the Thymelaeceae are a cosmopolitan family of flowering Dicots that are mostly of tropical origin that include Daphne, and other ornamental species were also represented. Monocots were not prevalent but did include the Zingiberaceae, which contains a number of aromatic perennial shrubs that are prevalent in tropical forests. Potyvirus sequences were also detected in the Pepper family (Piperaceae), a primitive family with a large number of flowering shrubs belonging to basal Angeosperms that were prevalent prior to the evolution of the Dicots and Monocots. The broad geminivirus host range also included the Taxaceae (Yew Family), a primitive coniferous family of small trees and shrubs. This diversity of hosts and the limited relationship of several of the families to crop plants grown in southern China suggests that many of the virus species in the Xishuangbanna Tropical Rainforest are endogenous, rather than having arisen from contiguous agricultural areas. Hence, it is entirely likely that the Xishuangbanna Tropical Rainforest could serve as a virus reservoir for crop species that are introduced near the rainforest in the future.

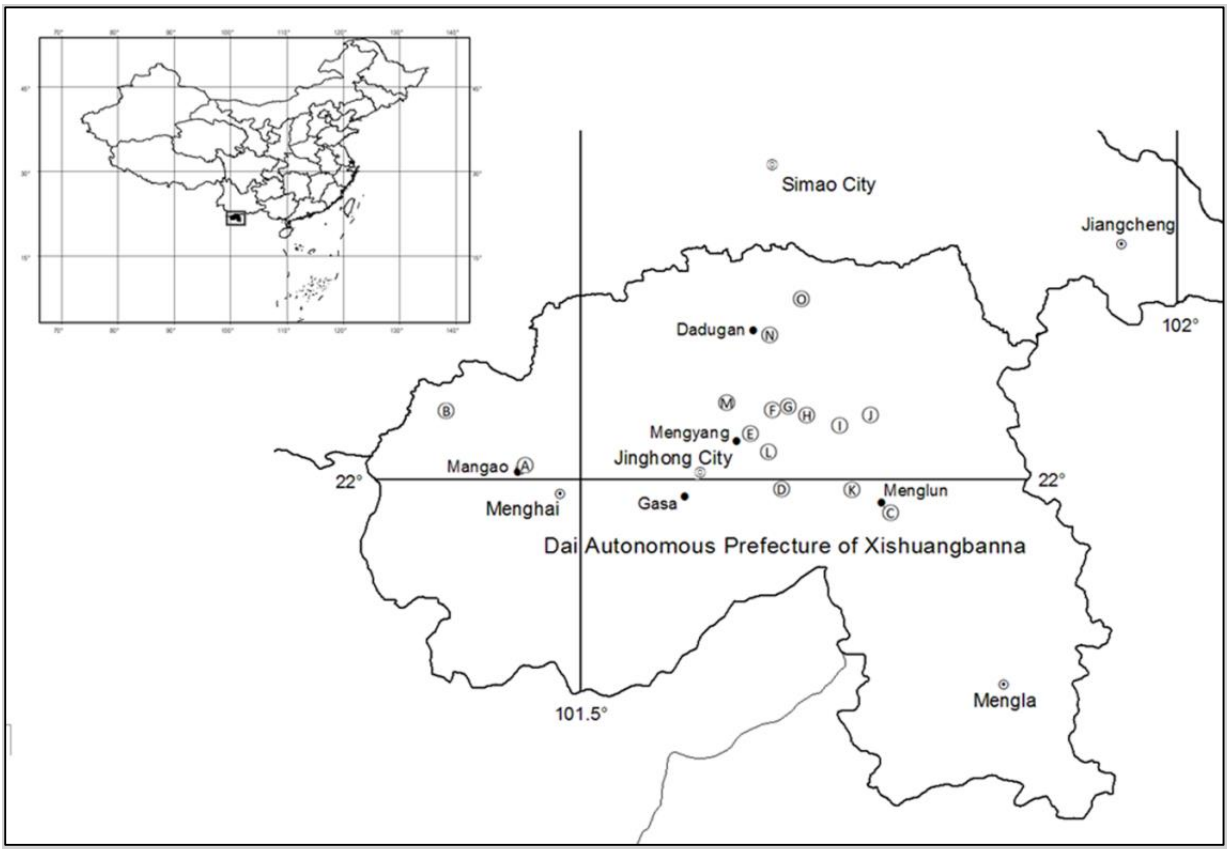

Figure 1. Distribution of virus sampling sites at the tropic forest in Xishuangbanna, Yunnan Province of China
Notes：city; town； county；Ⓐ to Ⓞ：15 sample collection sites from A to O

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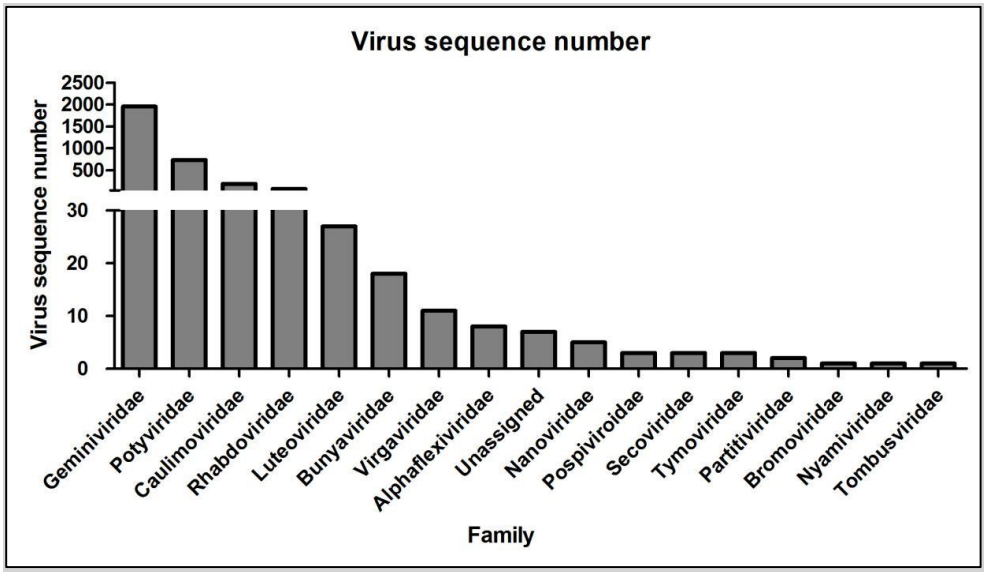

Figure 2. Numbers of virus sequences of virus families

DISCUSSION

In total, the viral sequences of nine plant virus families consisting of the Alphaflexiviridae, Bunyaviridae, Caulimoviridae, Geminiviridae, Luteoviridae, Pospiviroidae, Potyviridae, Rhabdoviridae, and Tymoviridae were annotated according to the viral sequencing identity (>90%) and detection number (>10). Among those families, the Geminiviridae sequences dominated and had broad distribution. Potyviridae and Caulimoviridae were common at most sample sites; however, fewer viral sequences were annotated to other families. The Tomato leaf curl Joydebpur virus and Tomato leaf curl Karnataka virus species in the Begomovirus genus in the Geminiviridae, had the highest detection rates of all sequences. However, Alphaflexiviridae, Bunyaviridae, Luteoviridae, and Tymoviridae sequences were detected at only 2 and 3 sampling points, respectively. Specific viruses annotated were tomato spotted wilt Tospovirus in the Bunyaviridae, Pepper vein yellows Polerovirus in the Luteoviridae, Tobacco vein distorting Potyvirus in the Potyviridae. Wild tomato mosaic virus, a possible of Potyvirus species in the Potyviridae, also had very high detection rates, and these are worthy of further identification and verification. Pospiviroidae and Rhabdoviridae sequences were detected at only one sample site each. The of nine main virus family sequences were annotated both from herbaceous and woody plants, and more sequences were detected in dicots than monocotyledons. Generally, the virus sequences were more prevalent in herbaceous than woody plants and in more dicots than monocotyledons, and these hosts collectively account for hosting the vast majority of the annotated viral sequences. Therefore, we infer that herbaceous dicots derived are more likely to be virus-infected in the tropical rain forest than monocotyledons and woody plants. Plant virus diseases of Solanaceae, Gramineae, Leguminosae, Cruciferae, and Cucurbitaceae plants are predominant in Yunnan, and the most harmful are tobacco mosaic diseases, potato virus diseases, lily virus disease, maize dwarf mosaic disease, etc. (Zhang and Li, 2001 ; Hong et al., 2001), so there are more the host plants of farmland plant viruses are herbs and dicots. Therefore, the plant types of herbs and dicots are more likely to be the source of farmland plant viruses. It is of great significance to study their evolution and the interaction between viruses and hosts comparing the tropic forests and farmlands.

The viruses of Geminiviridae are the important pathogen affecting crops in Yunnan and southern China (Yan et al., 2002 ; Yang et al., 2011 ; Zhang et al., 2002). The viruses of Potyviridae have a wide host range, causing serious economic losses to food crops, cash crops, and horticultural crops（Hong et al., 2001; Zhang, 2010）while the host range of Caulioviridae is narrow (Owens, 2012). They were indeed the main pathogens of crop diseases in agricultural cultivation areas. The interesting thing is that our study shows that Geminiviridae, Potyviridae, and Caulioviridae are dominant populations in the tropical rain forests of Xishuangbanna. Are these viruses of Geminiviridae, Potyviridae, and Caulioviridae from tropical rainforests related to the viruses of ones from agricultural areas? The pathogenic evolution of plant viruses in the tropical rainforest deserves further study in the future.

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Table 1. Pool sample geography and plant resources

Pool sample number	Place	Longitude and latitude	Elevation /m	Herb or wood	Plant type (M:Monocotyledons D: Dicotyledons)
S01	Nature reserve of Mangao at Menghai County	N22°01'12.59" E100°19'937.61"	1212	H	D
S02	Nature reserve of Xishuangbanna River Basin, Gasa Town, Jinghong City	N22°10'03.45" E100°9'36.92"	712	H	D
S03	Huludao, Menglun Town, Mengla County	N21°55'12.47" E101°16'06.65"	545	W	D
S04	Basha Laozi Village, Jino Mountain Jino Nationality Township, Jinghong City	N21°59'28.47" E101°00'01.19"	1034	H	D
S05	Basha Laozi Village, Jino Mountain Jino Nationality Township, Jinghong City	N21°59'28.47" E101°00'01.19"	1034	H	D
S06	Jinghong City nearby	N22°06'29.32" E100°55'11.33"	806	H/W	D
S07	Tiaoba River Village, Mengyang Town, Jinghong City	N22°10'15.84" E100°59'16.89"	849	H/W	D
S08	Mengyang Town Nature Reserve in Jinghong City	N22°09'22.83" E101°03'30.67"	749	H	D/M
S09	Mengyang Town Nature Reserve in Jinghong City	N22°07'55.32" E101°08'45.09"	1381	H/W	D
S10	Nature reserve of Menglun Town, Mengla County	N21°58'38.77" E101°10'33.33"	940	H	D
S11	Menglun Town Nature Reserve, Mengla County	N22°03'49.54" E100°58'01.06"	1032	H	D
S12	Wild Elephant Valley, Mengyang Town, Jinghong City	N22°11'11.97" E100°51'48.41"	795	H/W	D
S13	Dadugang Township, Jinghong City	N22°21'23.16" E100°58'16.14"	1328	H	D/M
S14	Dadugang Township, Jinghong City	N22°27'01.55" E101°02'57.87"	886	W	D
S15	Dadugang Township, Jinghong City	N22°09'22.78" E101°13'23.97"	1129	H	D/M

Note: H: Herb; W: Wood; D: Dicotyledons; M: Monocotyledons

Table 2. The statistics data of small RNA sequencing

Number of Pool Samples	Raw reads	Length<18	Length>30	N%>10%	Clean reads
S01	26262871	813765	4611684	18005	20819417
S02	23478580	1023143	4337951	16109	18101377
S03	23258049	956255	6254772	15793	16031229
S04	23457569	852400	4847823	16171	17741175
S05	22099253	1719675	3650579	14847	16714152
S06	25558415	1051545	2397810	116019	20733120
S07	26032741	2013554	1415993	143493	21683556
S08	23893777	3026389	1104308	102656	18914701
S09	23835377	1422754	1888847	133992	19648756
S10	24621159	616569	1410155	137951	21677193
S11	26934996	2622017	1381004	146751	21847629
S12	18618044	2224506	654641	89010	15738897
S13	27455472	1000918	1458737	154457	23963604
S14	27336604	711092	1184827	149788	24338340
S15	30056704	480406	1559762	170322	26878598
Average	24859974	1368999	2543926	95025	20322116

Note: Raw reads: Sequencing raw data; <18nt reads: reads less than 18 nucleotides after removing the junction; >30nt reads: reads with a mass value greater than 30 nucleotides; N>10%: reads with at least unknown base N>10%; Clean reads: Reads with a mass value greater than or equal to 30 nucleotides.

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Table 3. The main 9 plant virus families annotated in tropic rainforest of Xishuangbanna

No	Plant Virus Family in Alphabetical Order	Nucleic Acid Type	Average % Identity	Average E-value	Average Sequence Length	Numbers of times Detected	Source plants			
							Plant type			
							H: Herbaceous or W :Woody	M: Monocotyledonous or D: dicotyledonous	Plant Family	Sampling Site
1	*Alphaflexiviridae*	Plus Strand RNA	98.15	2.11×10^{-7}	54	11	H/W	D	*Compositae*	C, D
2	*Bunyaviridae*	Minus Strand RNA	94.36	3.02×10^{-7}	57.85	30	H	M/D	*Compositae*、*Malvaceae*	A, B, G
3	*Caulimoviridae*	Retrovirus DNA	91.35	2.16×10^{-7}	23	20	H/W	M/D	*Taxaceae*、*Compositae*	E, H, I, M, O
4	*Geminiviridae*	Single Strand DNA	93.27	1.71×10^{-7}	76.11	257	H/W	M/D	*Taxaceae*、*Compositae*、*Malvaceae*、*Solanaceae*	A, B, D, E, K, O
5	*Luteoviridae*	Plus Strand RNA	93.77	2.70×10^{-7}	58.85	250	H/W	M/D	*Caricaceae*、*Thymelaeceae*	F, G, J
6	*Pospiviroidae*	Circular Viroid RNA	94.67	2.19×10^{-7}	72	20	H/W	D	*Solanaceae*	E
7	*Potyviridae*	Plus Strand RNA	93.4	2.41×10^{-7}	86.31	350	H/W	M/D	*Zingiberaceae*、*Piperaceae* *Euphorbiaceae*、*Rosaceae*、*Solanaceae*	B, C, D, E, F, G, J, K, M,
8	*Rhabdoviridae*	Minus strand RNA	100	2.42×10^{-7}	67	11	H	D	*Compositae*	A
9	*Tymoviridae*	Plus Strand RNA	90	1.66×10^{-6}	20	11	H/W	M/D	*Solanaceae*、*Euphorbiaceae*	H, I, M

Note: H: herb; W:wood; M: monocotyledons ; D: dicotyledons

CONCLUSIONS

This study clarifies the plant virus family population in the tropical rainforest of Xishuangbanna. The viral sequences from herbaceous plants were obviously greater than from woody plants, and virus sequences isolated from dicots were obviously more extensive than from monocotyledons. This work lays a foundation for further research on the pathogenesis and evolution of plant viruses in the tropical rainforest virus and those in agricultural areas.

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Compatibility of entomopathogenic fungi with Derris extracts

Malee Thungrabeab[1*] and Patcharin Krutmuang[2]

[1]Agricultural Technology Research Institute, Rajamangala University of Technology Lanna Lampang 52000, Thailand
[2]Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

***Corresponding author**: sriwanmal@yahoo.com*
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ABSTRACT

Integrated pest management (IPM) strategies have been suggested for the control of various insect pests. Entomopathogenic fungi and botanical insecticides such as derris extract have considerable potential to become significant components in sustainable IPM. However, the compatibility of entomopathogenic fungi with derris extracts is still unknown. Therefore, the present study was evaluated the compatibility of *Beauveria* sp., *Metarhizium* spp., and *Paecilomyces* spp. with derris extracts under *in vitro* conditions. The derris extract was used at 1% and 0.1% incorporated to a culture medium (malt extract peptone agar) and distributed into petri dishes for their effects on vegetative growth and conidia viability. The vegetative growth of all fungi tested was inhibited by 1% and 0.1% of derris extracts, but they did not affect the viability of conidia.

Keywords: entomopathogenic fungi, biological control, Derris extracts, IPM

INTRODUCTION

Integrate Pest Management (IPM) to be the basis for pest control. There is no single criterion that guarantees the successful uptake of biological control agents, and the difficulties that need to be overcome include scientific, economic, social, and political aspects. However, entomopathogenic fungi have considerable potential to become major components in sustainable IPM. Entomopathogenic fungi are important as natural control agents of many insect pests (St Leger and Wang, 2009). Previous research reported that many isolates of entomopathogenic fungi have effectively controlled various insect pests and were relatively safe on non-target beneficial insects (Malee and Sirinun, 2002, Sengonca et al., 2006, Thungrabeab et al., 2006, Thungrabeab, 2007). Integrated pest management with entomopathogens should be considered as an important reduction factor in insect pest population density. Therefore, we must understand the compatibility of entomopathogenic fungi with other strategies for sustainable pest control, such as botanical insecticides, which may inhibit to a smaller or larger extent the development and reproduction of entomopathogenic fungi.

Derris extract has insecticide activity against a wide variety of insect pests (Aroon et al. 1997, 2001, 2006). *Derris malaccensis* (Benth.) Prain is a climbing leguminous plant found in the southeastern parts of Asia. The root of *D. malaccensi*, which contains rotenone used as a source of natural insecticide. It can be lethal to insects and even fish. Rotenone affects not only the stomach of an insect that may digest some of it but also can transmit poison on contact. Derris has been used for years against fleas and lice, as well as it is also effective against aphids, beetles, caterpillars, maggots, bagworms, cabbage worms, thrips, leafhoppers, Japanese beetles, vegetable weevils, codling moths, sawflies, and slug sawflies (Rey, 1991). Additionally, it is also safe to use on grain, fruits, and vegetables. Rotenone may take a few days to terminate insects, but the insects will die, and feeding on the plants will end. Rotenone is biodegradable, so with the presence of the sun and heat, the chemicals will break down in about a week, as well as, it also rapidly breaks down in soil and water. Rotenone, although widely used, happens to be a very safe natural insecticide. The only warning that is more of a safety principle is not to enter a treated area for about 12 hours after using rotenone. Compatibility of entomopathogenic fungi with derris extract was never observed. The compatibility data are essential before such associations are employed in the field. Therefore, the objective of this study was to evaluate the compatibility between entomopathogenic fungi with derris extract *in vitro*, using concentrations that showed potential for control insect pests under field conditions.

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MATERIALS AND METHODS

Fungi

The following fungi isolates were used; *Beauveria bassiana* 5 isolates (Bb. 2637, Bb. 4591, Bb. 5335, Bb. 5736, Bb. 6241), *Metarhizium anisopliae* 5 isolates (Ma. 6071, Ma. 6079, Ma. 6171, Ma. 7965, Ma. 6491), Metarhizium flavoviride isolate Mfl. 1164, as well as *Paecilomyces fumosoroseus* isolate Pfu. 2507 and *Paecilomyces tenuipes* 3 isolates (Pt.7968, Pt. 7996, Pt. 8003).

Preparation of derris extracts and incorporation to the culture medium

To prepare derris extract, the roots of *Derris malaccensis* were harvested from 2 years of age. A hundred grams of fresh root were crushed and was then added to 1,000 ml of ethanol 95%. The extract remained at rest for approximately 24 h at room temperature, and it was then filtered through filter paper No.1 to separate the solid components. The concentration of rotenone obtained from the derris extracts was analyzed by HPLC (High-Performance Liquid Chromatography) method according to Aroon, 2008

After that, a concentration of derris extract recommended for control insect in the field, including 1 ml of derris extract was added to 100 ml of malt extract peptone agar (MEA, 3% malt extract; 0.5% Soybean peptone; 1.5% Agar), for the concentration to 1%. To obtain the 0.1 % concentration, 100 µl of derris extract was added to 100 ml MEA. The culture media treated after homogenization was dispensed in 90 mm diameter petri dish. There were five plates per treatment; each plate served as a replication. There were two controls, the first was untreated MEA, and a second was added 1% and 0.1% ethanol in MEA (diluted from ethanol 95%). The experiment consisted of 75 treatments (15 isolates × 2 concentrations of derris extract × 2 concentrations of ethanol 95% × control).

Vegetative fungal growth

For recording vegetative fungal growth, the mycelial mats were obtained by using water agar (WA, 15 g agar in 1000 ml H_2O). WA was dispensed in 90 mm diameter petri dish; after solidification, 100 µl of conidial suspension containing 1×10^6 conidia/ml of each isolate was spread on water agar. Plates were then incubated at 25±2°C, 12 h photophase for 3 days. Mycelial mats were cut from culture plates into round agar plugs using a 7 mm. diameter cork borer. Each agar plug was then transferred singly onto the center of media Petri dishes, prepared as the procedure described in capital 2.2. Plates were sealed with Parafilm membrane and incubated at 25±1°C under continuous light conditions. Colony growth was recorded daily for 14 days using two cardinal diameters through two orthogonal axes previously drawn on the bottom of each petri dish to serve as a reference. The diameter of the colonies was estimated by calculating the mean of two perpendicular measurements.

Conidia viability assessments

In order to determine conidia viability, conidial suspension (100 µl of 1×10^6 conidia/ml) was spread on each media plate, prepared as the procedure described in capital 2.2. Plates were then incubated individually at 25±1°C, 12 h photophase. After 24 h inoculation, germination was halted by transferring 1 ml of 0.5% formaldehyde onto each plate. A drop of lactophenol cotton blue stain was dropped into the plate. Germination and non-germination conidia in three separated (1×1 cm^2) squares of agar (100 conidia per square of agar) cut from WA were counted under the microscope by using microscope slides through moving the field of view of the microscope in order to cover a large area of agar square. Three counts were made for each replication for every treatment. Each plate was served as a replication; six replications were used for each treatment.

Statistical analysis

The experimental design for all trials was completely randomized. The data were submitted to ANOVA, and the mean values were compared by using Duncan's multiple range test at a probability level of < 0.05. Statistical analyses were performed using the Statistical Analysis System software (SAS).

RESULTS

Productive performance

Percent of rotenone content derived from the root extracts of *Derris malaccensis* was 7.41%, which analyzed by Aroon (2008)

Vegetative fungal growth

Derris extracts affected the colony growth of all *B. bassiana* isolates tested. Significant inhibition was observed in colony growth as compared with control. No significant difference was observed between the concentration of derris extract at 1% and 0.1%. No significant inhibition of colony growth with derris extract was observed between the third and the seventh days of inoculation (Table 1).

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Table 1. Colony growth (diameter± SE) of *Beauveria bassiana* cultured on MEA medium amended with different concentration of derris extract, at 25 ± 2 ºC and 12 h photophase

Isolates	Concentration	Colony growth			
		3 days (cm ± SE)	Inhibition (%)	7 days (cm ± SE)	Inhibition (%)
Bb. 2637	Control	2.782 ± 0.086 a	00.00 A	5.026 ± 0.120 a	00.00 A
	0.1%	1.844 ± 0.026 b	33.81 A	3.392 ± 0.143 b	32.95 A
	1%	1.706 ± 0.065 b	38.66 A	2.702 ± 0.187 b	46.60 A
Bb. 4591	Control	2.610 ± 0.087 a	00.00 A	4.472 ± 0.235 a	00.00 A
	0.1%	1.962 ± 0.037 b	24.90 A	3.418 ± 0.099 b	23.77 A
	1%	1.762 ± 0.031 b	32.57 A	2.832 ± 0.152 b	36.83 A
Bb. 5335	Control	2.738 ± 0.067 a	00.00 A	4.912 ± 0.447 a	00.00 A
	0.1%	1.652 ± 0.055 b	39.67 A	2.848 ± 0.118 b	39.71 A
	1%	1.536 ± 0.044 b	43.87 A	2.582 ± 0.078 b	47.45 A
Bb. 5736	Control	2.522 ± 0.098 a	00.00 A	4.186 ± 0.183 a	00.00 A
	0.1%	1.966 ± 0.056 b	19.84 A	3.402 ± 0.173 a	18.89 A
	1%	1.978 ± 0.022 b	21.62 A	3.372 ± 0.032 a	25.93 A
Bb. 6241	Control	2.520 ± 0.034 a	00.00 A	4.062 ± 0.033 a	00.00 A
	0.1%	1.656 ± 0.073 b	34.32 A	2.980 ± 0.118 b	26.60 A
	1%	1.466 ± 0.036 b	41.86 A	2.698 ± 0.193 b	33.57 A

Means in a column with different small letters indicated significant differences among different concentrations of derris extract within the same isolate (one way ANOVA, $P \leq 0.05$; Duncan's multiple range test). Means in the same row followed by the same capital letters indicate no significant difference between two days after inoculation within the same isolate at $P \leq 0.05$ (T-test)

Effect of derris extract on colony growth of *Metarhizium* spp. showed the same tendency as *Beauveria bassiana*, derris extract inhibited the colony growth of all fungi test, but percentage inhibition was isolates – dependent. (Table 2)

Table 2. Colony growth (diameter ± SE) of *Metarhizium* spp. cultured on MEA medium amended with different concentration of derris extracts, at 25 ± 2 ºC and 12 h photophase

Isolates	Concentration	Colony growth			
		3 days (cm ± SE)	Inhibition (%)	7 days (cm ± SE)	Inhibition (%)
Ma. 6071	Control	2.386 ± 0.063 a	00.00 A	4.758 ± 0.100 a	00.00 A
	0.1%	1.382 ± 0.033 b	42.13 A	2.728 ± 0.043 b	42.69 A
	1%	1.112 ± 0.026 b	53.45 A	2.112 ± 0.064 b	55.62 A
Ma. 6079	Control	1.766 ± 0.041 a	00.00 A	3.002 ± 0.079 a	00.00 A
	0.1%	1.606 ± 0.034 ab	9.06 B	2.270 ± 0.046 ab	21.66 A
	1%	1.386 ± 0.024 b	21.82 A	2.214 ± 0.032 b	26.33 A
Ma. 6171	Control	2.616 ± 0.042 a	00.00 A	4.836 ± 0.062 a	00.00 A
	0.1%	1.792 ± 0.054 b	31.55 A	3.488 ± 0.025 b	27.92 A
	1%	1.506 ±0.006 b	42.44 A	2.588 ± 0.078 b	46.54 A
Ma. 6491	Control	3.022 ± 0.107 a	00.00 A	5.580 ± 0.137 a	00.00 A
	0.1%	2.236 ± 0.043 b	25.99 A	3.908 ± 0.085 b	30.02 A
	1%	1.956 ± 0.028 b	35.26 A	3.478 ± 0.029 b	37.72 A
Ma. 7965	Control	2.618 ± 0.045 a	00.00 A	4.728 ± 0.060 a	00.00 A
	0.1%	1.762 ± 0.025 b	32.67 A	3.152 ± 0.086 b	33.33 A
	1%	1.416 ±0.032 b	45.89 A	2.422 ± 0.033 b	48.78 A
Mfl. 1164	Control	1.512 ± 0.032 a	00.00 A	2.762 ± 0.080 a	00.00 A
	0.1%	1.360 ± 0.046 a	9.93 A	2.512 ± 0.060 a	9.05 A
	1%	1.202 ± 0.022 a	20.52 A	2.268 ± 0.052 a	17.93 A

Means in a column with different small letters indicated significant differences among different concentrations of derris extract within the same isolate (one way ANOVA, $P \leq 0.05$; Duncan's multiple range test). Means in the same row followed by the same capital letters indicate no significant difference between two days after inoculation within the same isolate at $P \leq 0.05$ (T-test)

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The effect of derris extract on colony growth of *Paecilomyces* spp is illustrated in table 3. The colony in culture media containing derris extract had their vegetative growth reduced compared to control, even though the difference was not always significant.

Table 3. Colony growth (diameter ± SE) of *Paecilomyces* spp. cultured on MEA medium amended with different concentration of derris extracts, at 25 ± 2 ºC and 12 h photophase

Isolates	Concentration	Colony growth			
		3 days (cm ± SE)	Inhibition (%)	7 days (cm ± SE)	Inhibition (%)
Pfu. 2507	Control	1.436 ± 0.021 a	00.00 A	3.142 ± 0.069 a	00.00 A
	0.1%	1.224 ± 0.034 a	14.98 A	2.866 ± 0.037 a	8.75 B
	1%	1.178 ± 0.026 a	18.11 A	2.620 ± 0.040 a	16.71 A
Pt. 7968	Control	1.9080 ± .037 a	00.00 A	3.642 ± 0.121 a	00.00 A
	0.1%	1.302 ± 0.041 a	32.41 A	2.930 ± 0.025 a	19.29 B
	1%	1.082 ± 0.021 a	43.30 A	2.236 ± 0.066 a	38.59 A
Pt. 7996	Control	2.498 ± 0.024 a	00.00 A	4.742 ± 0.040 a	00.00 A
	0.1%	1.808 ± 0.035 ab	27.65 A	3.614 ± 0.147 ab	21.20 A
	1%	1.566 ± 0.025 b	37.27 A	3.444 ± 0.324 a	34.28 A
Pt. 8003	Control	2.378 ± 0.070 a	00.00 A	4.546 ± 0.187 a	00.00 A
	0.1%	1.906 ± 0.089 b	19.79 B	3.068 ± 0.097 b	32.56 A
	1%	1.730 ± 0.035 b	27.37 A	3.058 ± 0.102 b	32.78 A

Means in a column with different small letters indicated significant differences among different concentrations of derris extract within the same isolate (one way ANOVA, $P \leq 0.05$; Duncan's multiple range test). Means in the same row followed by the same capital letters indicate no significant difference between two days after inoculation within the same isolate at $P \leq 0.05$ (T-test)

Conidia viability

The results showed that the conidia germination was isolates–dependent (Table 4). The germination varied among fungi species and extract concentrations. Mostly, no significant differences were observed between control and treatment.

Table 4. Percentage germination of *Beauveria bassiana* conidia at 24 h on MEA medium amended with different concentration of derris extracts, at 25 ± 2 ºC and 12:12 h (L:D) photoperiod

Isolate	Percentage germination (% ± SE)		
	control	0.1% Derris extract	1% Derris extract
Beauveria bassiana			
Bb. 5335	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Bb. 4591	100 ± 0.00 a	100 ± 0.00 a	87.38 ± 1.35b
Bb. 6241	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Bb. 2637	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Bb. 5736	100 ± 0.00 a	100 ± 0.00 a	74.09 ± 1.67b
Metarhizium anisopliae			
Ma. 6071	100 ± 0.00 a	94.7 ± 5.30 a	55.08± 0.00b
Ma.6079	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Ma.6171	100 ± 0.00 a	100 ± 0.00 a	96.78 ± 3.22a
Ma.6491	100 ± 0.00 a	80.53 ± 4.91b	67.84 ± 5.65c
Ma.7965	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Metarhizium flavoviride			
Mfl.1164	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Paecilomyces fumosoroseus			
Pfu. 2507	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
***Paecilomyces tenuipes*:**			
Pt.7968	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Pt. 7996	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Pt. 8003	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a

Means in a column with different small letters indicated significant differences among different concentrations of derris extracts within the same isolate (one way ANOVA, $P \leq 0.05$; Duncan's multiple range test).

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DISCUSSION

The roots of derris contain rotenone, in which rotenone is a botanical pesticide. It has pesticide activity against a wide variety of insects and arachnids encountered in both domestic and commercial horticulture and field crop. It has been shown a smaller negative effect on mycelial growth of plant disease fungi (Sookchaoy, 2007). However, the mechanism of action of derris on vegetative growth and conidia viability of entomopathogenic fungi is still unknown. The results of these experiments demonstrated that the derris extract did not affect the germination of conidia of entomopathogenic fungi but inhibited the colony growth of all fungi test. In fact, spores of the entomopathogenic fungi have been used successfully to control insects. High toxicity *in vitro* did not always mean that the same will occur in the field, but it shows a possibility for this to occur. These corroborate with Depieri et al. (2005), who stated that extracts had high toxicity *in vitro* does not mean that the extracts will always be toxic for the fungi in the field. This might be explained by the fact that fungi vegetative growth will occur or will be inhibited only inside the host. Concentration inside the insect is probably smaller than that used in in vitro tests. Thus, in vitro results represent what happens with the vegetative growth of fungi inside the insect body in a more drastic way. Moreover, under field conditions, environmental factors decrease the impact of toxic components on the fungus. Furthermore, Wood et al. (2005) reported that rotenone rapidly decomposes upon exposure to light and air. Its persistence is therefore limited to 2-3 days. In this situation, inhibition of vegetative growth might be a less representative indication of fungitoxicity than the viability of conidia or the effect of germination; due to under field conditions, compatibility between derris extracts and germination was necessary because insects become infected by means of conidia germination, by contact. The compatibility in the field should consider the effect of the extract on conidia germination as one of the most critical factors. This was due to the fact that fungi infect insects through the conidium germination by contact. In addition, the inoculum survival of the entomopathogenic fungus in the field was made by conidia. At the beginning of an epizootic, the conidia were responsible for the occurrence of the first disease focuses. Thus, if germination inhibition occurs, the pathogen control efficiency will be committed if the fungi have been applied in inundative form, together or separately with derris extracts, or the fungi was natural present in the insects and contacts the derris extracts.

CONCLUSIONS

This study had identified that derris extract had no negative effect on germination of promising entomopathogenic fungi; *Beauveria* sp., *Metarhizium* spp., and *Paecilomyces* spp. as biological control agents, even though effects on vegetative growth. Therefore, in the IPM program, they can be recommended for pest control where these entomopathogenic fungi are important natural enemies, and derris extracts are important botanical insecticides.

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2.3) Abstract and keywords

Abstract - A concise abstract with maximum of 200-250 words identifying the new and significant results of the study must be provided for all manuscripts.

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Author should prepare the introduction with suitable length and keep it as short as possible.

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This section contains details about materials, techniques, apparatuses, experimental design, and environment. Sufficient detail should be provided to permit the reader to repeat the experiments. For the manuscript reports the utilization of chemical, please provide the purity of all chemicals used in this part.

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In this part, the results and discussion can be combined or separated depends on author design. Please use tables, graphs, diagrams, and photographs to provide a clear understanding of the results. Quantitative measures of significance (P-values) should be presented. Authors may use either absolute P-values or a defined significance level as long as usage is consistent.

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Clear and significant conclusions should be provided and related to the results and objectives of your work.

2.8) Acknowledgments

In this section, financial and material support should also be mentioned. Authors should list all funding sources in this acknowledgments section. The names of funding organizations should be written in full. List those individuals who provided support during the research

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2.9.1) In text citations, please use name-year system such as:

a. Before texts: Smith (2018)…., Tsuji and Hayate (2012)…., Smith et al.(2019)…., Smith (2018); Tsuji (2012)…., Smith et al.(2018a); Smith et al.(2008b)…..

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2.9.2) References list

Published literatures or unpublished works must be listed alphabetically by surname of author at the end of the manuscript. Each reference to a periodical publication must include, in order, the name(s) of the author(s), the year of publication, the full title of the article, the publication in which it appears, the volume and inclusive page numbers, and the digital object identifier (DOI), if available. Examples of references are listed as follow:

• Journal article

Halmilton, M.B., Pincus, E.L., Fion, A.D., and Fleischer, R. C. 1999. Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques. 27: 500-507. https://doi.org/xx.xxxxxxxxxx

Gupta, A.P., and Kumar, V. 2007. New emerging trends in synthetic biodegradable polymers-polylactide: A critique. European Polymer Journal. 43: 4053-4074.

• Book

Carr, R.L. 1976. Powder and granule properties and mechanics. Marcel Dekker Publisher, New York.

• Chapter in book

Jackson, M.B. 1982. Ethylene as a growth promoting hormone under flooded conditions. In: Wareing, P.F. (ed) Plant growth substance. Academic Press, London. p.291-301.

• Proceeding, symposia etc.

Pratt, A., Gilkes, R.J., Ward, S.C., and Jasper, D.A. 2000. Variations in the properties of regolith materials affect the performance of tree growth in rehabilitated bauxite mine-pits in the Darling Range, SW-Australia. In: Brion, A., and Bell, R.W. (eds) Proceeding of Remade Land 2000, the International Conference on Remediation and Management of Degraded Lands. Fremantle, 30 Nov-2 Dec 2000. Promaco Conventions, Canning Bridge. p.87-88.

• Dissertation

Senthong, C. 1979. Growth analysis in several peanut cultivars and the effect of peanut root-knot nematode (Meloidogyne arenaria) on peanut yields. Ph.D. Dissertation. University of Florida, Gainesville, Florida, USA.

3. Equation, figure, table and unit of measurement

Equation

All equations presented in the text can be prepared by Equation editor from Microsoft word or Math Type.

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Data presented in figure must be written in English. Color or gray scale figure should be prepared as TIFF format (.tif) with at least 600 dpi. Photograph should be prepared as TIFF (.tif) or JPEG (.jpg) format with at least 600 dpi. Figure with caption should be placed next to the relevant text, rather than at the bottom of the file.

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Unit

Author should use the International System of Units (S.I.)

4. Structure of manuscript

- Title
- Author names and affiliations
- ABSTRACT
- Keywords
- INTRODUCTION
- MATERIALS AND METHODS

- RESULTS AND DISCUSSION (The results and discussion can be combined or separated depends on author design)
- CONCLUSIONS
- ACKNOWLEDGMENTS (if any)
- REFERENCES

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