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Utilization of Mangosteen (*Garcinia mangostana* Linn.) Pericarp Extract to Treat *Zoothamnium* sp. Infection in White Leg Shrimp (*Litopenaeus vannamei*).

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Zoothamnium sp. is an aquatic protozoan that causes lifeless, defective appendage and immune reduction in the shrimp (*Litopenaeus vannamei*). *Zoothamnium* sp. infections causes secondary infection by bacterial, viruses and fungi. This study investigated optimum concentration levels of mangosteen pericarp (peel, rind or hull) extract, effective against *Zoothamnium* sp. from the *L. vannamei*. The against test, toxicity test and water quality were conducted at the RBRU labarotory experimental. The most effcetive concentration of mangosteen pericarp extract to treat *Zoothamnium* sp. was 0.5 g/L, follow by 1.0, 1.5 and 2.0 g/L respectively, which can eliminated all *Zoothamnium* sp. at 21, 18, 15 and 12 hour, respectively. By consequence, however, the concentration of mangosteen pericarp extract also caused mortality of *L. vannamei*. From the total 10 individual of *L. vannamei* from the begin of the experiment. The concentration at 0.5 g/L caused the *L. vannamei* mortality of 2.33 ± 0.58 (average ± SD) individuals, follow by the concentrations of 1.0, 1.5 and 2.0 g/L, which caused the mortality values of *L. vannamei* 3.00 ± 0.00, 4.00 ± 1.00 and 4.67 ± 0.58 individuals, respectively. For the concentration of 0.5 g/L, during the experimental period, the average DO, pH and water temperature were beginning at 3.87 mg/L, 9.08 and 27.41°C respectively. At the end of the experiment, the average DO, pH and water temperature were 0.39 mg/L, 7.79 and 28.89 °C, respectively.

Keywords: *Garcinia mangostana* Linn., *Zoothamnium* sp., *Litopenaeus vannamei*

Introduction

The White Leg Shrimp (*Litopenaeus vannamei*) is an important agricultural product in Thailand. The value from *L. vannamei* products exports in 2016 was about 99 million tons, values of 29,320 million Thai Baht (Office of Agricultural Economics, 2016). Nevertheless, in the Thai, the white shrimp farmers often face problems of disease infection on *L. vannamei* throughout the culture period, because of the intensive system in which the high density of

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shrimp are released. As a result, this system produces high waste levels in the pond and caused the water quality degradation for *L. vannamei* leading to physiological stress. Resulting decreased immunity can them lead to secondary infections. The most common protozoan shrimp ectoparasite in Chanthaburi province is *Zoothamnium* sp. which are commonly found in fresh, brackish, and sea water The *Zoothamnium* sp. are offen found attached on the outer surface of the gill, appendage, outer body surface and shell of shrimp, crabs, molluscs and fish. Sometimes, they are found floating in water column in zooplankton rearing ponds.

Thailand is located in a tropical zone. The climate and the intensive farming systems for high productivity was an ideal source of growth of pathogens. Currently, many drugs and chemicals for prevention and treatment for aquatic animal parasites and diseases are commonly available and farmers, however, often have limited information concerning drugs and chemicals may use them erroneously. As a consequence, parasites may show increasing resistance for those available drugs and chemicals and can not be prevented and treated for aquatic animals (Chitmanat, 2013). In addition, drugs and chemicals can remain in the animals and environment. It also results in consumers being harmed. For the consequen results, the contamination from drugs and chemicals may find their way to the consumer and also lead to expert problems. With this concern, herbs and natural products may play an increasingly important role in aquaculture, as they are readily available, inexpensive prevention and treatment for disease, can be used as supplements. Presently, plant extracts are very becoming popular because it used in appointment concentrations is not harmful for aquatic animals and no residues are left that may consumers impact. Thailand has many natural plants. And local lower show, as well as, modern technology can be used to extract substances from Thai herbs to inhibit protozoan bacteria fungi and viruses (Chitmanat, 2013). Among numberous herbs, mangosteen (*Garcinia mangostana* Linn.) is a fruit tree from Asia, has been very popular and knowed as the name "queen of fruit". This fruit has a delicious sweet taste and useful to fight free radicals and strengthen the immune system. The extracts of *G. mangostana* have very diverse pharmacological activities including anti-inflammatory, cytotoxic, antioxidant, antitumoral, immunomodulatory, neuroprotective, anti-allergic, antibacterial and antiviral properties (Al-Massarani S.M. *et al.*, 2013). The pericarp of mangosteen contains astringent substances, namely, tannins and xanthones. Xanthone knowed as a potent inhibit growth of protozoan such as *Plasmodium falciparum*, intracellular amastigotes of *Leishmania infantum* and *Trypanosoma cruzi* and free trypomastigotes of *T. brucei*, antibacteria such as *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillius subtilis*,

Staphylococcus aureus, *Mycobacterium smegmatis*, *M. cheleneoi*, *M. xenopi* and *M. intracellulare* (Al-Massarani *et al*., 2013), The α-mangostin is antifungal such as *Candida albicans*, the most important microorganism implicated in oral candidiasis, some derivatives of mangostin were reported that can against three phytopathogenic fungi, such as *Fusarium oxysporum vasinfectum*, *Alternaria tenuis* and *Dreschlera oryzae* (Kaomongkolgit *et al*., 2009). In addition, mangosteen pericarp also can be used to relieve abdominal pain, diarrhea, dysentery, prevent wound infections, reduce and heal inflammation (Chaverri *et al*., 2008). In addition, mangosteen pericarps contain antifungal agents suitable for composting. (Kaomongkolgit *et al*., 2009)

Objectives: This research focus on agricultural and technological development, use of mangosteen pericarp extract as a therapeutic against *Zoothamnium* sp. The aim of this study was to find possibility and promote natural plant extracts as an alternative to combat aquatic parasites and diseases. Results from this study can be use for sustaining the Thailand aquaculture sector and promoting Thai's shrimp products for export.

Materials and methods

Mangosteen extraction

Prepare the mangosteen pericarp by slicing, subsequent and drying at 60 °C for 1 day or until constant weight is obtained. Mangosteen pericarp slices are crushed using a mortar, seived through a fine grinder. Finally, maceration extraction was performed by alocohol extraction of mangosteen pericarp powder with 95% ethanol with a ratio 1: 4 in a 1,000 ml brown color bottle for 1 week. The sludge was removed by filtertion with filter fabric and filter paper No. 1. Finally, the solution was transfered to a rotary vacuum evaporator and processed at a pressure level 175-200 psi at 60 °C and product solution (sticky charecteristic) was stored with brown bottle and keep in refrigerator at 4 °C.

Before start the experimental, product solution were weighted (g) and melted with a drop of 95% ethanol, then, the melted solution were diluted, into different concentrations *i.e.* 0.5, 1.0, 1.5, 2.0 g/L, with 20 ppt sea water.

Zoothamnium sp. Preparation

Zoothamnium sp. was reared on the 1x1 cm. plastic sheets as a substrate. Plastic sheets were placed in the aquatic tanks with sea water (20 ppt). Then, *L. vannamei* with known *Zoothamnium* sp. infection, were reared together in the tanks. *L. vannamei* were leaved in the tanks for 3-4 days or until *Zoothamnium* sp. were found full the 1x1 cm. plastic sheet.

Test against Zoothamnium sp.

For investigated the numbers of *Zoothamnium* sp. which were die from the 1x1 cm. plastic sheet in every 3 hours, within 24 hours. The concentrations (i.e. treatments) of mangosteen pericarp diluted solution extracts were treated at 0, 0.5, 1.0, 1.5 and 2.0 g/L respectivelly. Each concentration (treatment) was repeated three times with a completely randomized design.

Toxicity test on L. vannamei (P15)

Toxicity test of *L. vannamei* (postlarva 15; P15) on mangosteen pericarp extract was performed at concentrations (treatments) 0, 0.5, 1.0, 1.5 and 2.0 g/L respectively. Each concentration was repeated three times with a completely randomized design. The total of 15 beakers (1,000 ml volume) were used for the test. Each beaker contain 10 individual *L. vannamei* reared in 20 ppt sea water. The test ran for a period of 24 hours. The Linear regression analysis of mangosteen extracts (factor X) and percent mortality of *L. vannamei* (factor Y) were used to explaned the toxicity test of *L. vannamei* on mangosteen pericarp extract.

Water quality

From experiment 2, the water quality parameters were measured. Dissolved oxygen (DO), pH, and temperature in each treatment were measured, both, before and at termination of the experiment.

Results

Test for against Zoothamnium sp.

within a 24-hour period, an average of 60.82 ± 12.86 cells of the *Zoothamnium* sp. died at the control concentration of 0 g/L. Total mortality (100%) of the *Zoothamnium* sp. was recorded concentration of 0.5, 1.0, 1.5 and 2.0 g/L in 21, 18, 15 and 12-hour period, respectively. There was statistically significant difference between treatments (Table 1).

Toxicity test on L. vannamei (P15)

Within 24-hour period, no mortality of *L. vannamei* was found at the concentration of 0 g/L mangosteen pericarp extract. Mean while, average 2.33 ± 0.58 individuals of *L. vannamei* died at concentration 0.5 g/L and the average mortality of the *L. vannamei* was 3.00 ± 0.00 at the concentration 1.0 g/L. The

mean mortality (individuals *L. vannamei*) was found at 4.00 ± 1.00 individual at the concentration 1.5 g/L and the mean mortality of *L. vannamei* were found 4.67 ± 0.58 individuals at the concentration 2.0 g/L. The mortality rate of treatment concentration of 0 g/L was significantly different from other treatments (i.e. at 0.5, 1.0, 1.5 and 2.0 g/L) (Table 2).

Based on the linear regression analysis, mangosteen pericarp extracts (factor X) and the mortality rate of *L. vannamei* (factor Y) showed a high correlation, R-square = 0.99 (Fig. 1). The concentration that caused the *L. vannamei* to die in half (LC_{50}) was calculated as 2.04 g/L.

Table 2 Mortality of *L. vannamei* (P15) at the end of experiment

Concentration	Mortality rate
0	0.00 ± 0.00^{a}
0.5	2.33 ± 0.58^{b}
1.0	3.00 ± 0.00^{bc}
1.5	4.00 ± 1.00^{cd}
2.0	4.67 ± 0.58^{d}

The different alphabat "a, b, c and d" in column indicate statistically significant difference ($P<0.05$)

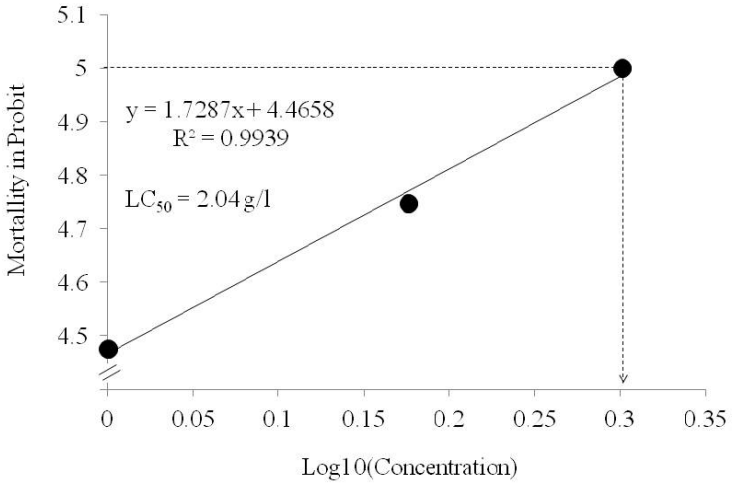

Fig. 1 The linear regression analysis of LC50 calculation for *L. vannamei* reared in differnt concentration of mangosteen pericarb extract.

Table 1 Mortality of *Zoothamnium* sp. in 5 concentrations (g/L) at 3, 6, 9, 12, 15, 18, 21 and 24 hours

Conc.	3-hours	6-hours	9-hours	12-hours	15-hours	18-hours	21-hours	24-hours
0	19.75±0.69^{a}	18.71±6.21^{a}	9.41± 9.70^{a}	58.19±6.18^{a}	56.64±3.47^{a}	54.55±5.68^{a}	51.28±13.79^{a}	60.82±12.86^{a}
0.5	81.96±1.40^{b}	89.92±1.41^{b}	95.38±0.34^{b}	97.90±0.22^{b}	99.54±0.10^{b}	99.92±0.08^{b}	100.00±0.00^{b}	100.00±0.00^{b}
1.0	82.68±1.65^{b}	90.83±1.31^{bc}	96.09±0.36^{b}	98.84±0.03^{b}	99.55±0.07^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
1.5	84.47±4.81^{b}	96.36±1.30^{c}	99.02±0.44^{b}	99.79±0.09^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
2.0	90.77±0.84^{c}	99.26±0.22^{d}	99.86±0.05^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}

The different alphabat “a and b” in column indicate statistically significant difference (P<0.05)

Table 3 Water quality in the begin and the end experimental

Conc.	DO		pH		Temperature	
	Begin	End	Begin	End	Begin	End
0	4.42 ± 0.02^{a}	1.53 ± 0.47^{c}	9.80 ± 0.23^{a}	7.64 ± 0.28^{d}	27.65 ± 0.09^{a}	28.95 ± 0.11^{df}
0.5	4.23 ± 0.68^{a}	0.20 ± 0.13^{d}	9.38 ± 0.14^{b}	8.04 ± 0.19^{e}	27.49 ± 0.15^{ac}	28.91 ± 0.20^{d}
1.0	3.60 ± 0.34^{b}	0.12 ± 0.09^{d}	8.87 ± 0.08^{c}	7.84 ± 0.10^{de}	27.49 ± 0.07^{ac}	29.08 ± 0.11^{de}
1.5	3.54 ± 0.21^{b}	0.06 ± 0.05^{d}	8.72 ± 0.09^{c}	7.70 ± 0.07^{d}	27.13 ± 0.10^{b}	28.65 ± 0.12^{g}
2.0	3.56 ± 0.15^{b}	0.04 ± 0.01^{d}	8.62 ± 0.13^{c}	7.73 ± 0.04^{d}	27.30 ± 0.06^{bc}	28.85 ± 0.05^{f}

* The different alphabat “a, b, c, d, e, f and g” in column indicate statistically significant difference (P<0.05)

Water quality

Within 24 hours, the water quality results of average dissolved oxygen (DO), acidity (pH) and water temperature, at begin and end of experiment, as shown in Table 3.

Discussion

Based on the results, the mangosteen pericarp extract can possibly be utilized for treatment against *Zoothamnium sp*. in *L. vannamei*. The optimum concentration was 0.5 g/L as it eliminated 100% *Zoothamnium* sp. whitin 21 hours. In addition, low mortality (2.33 ± 0.58 individuals) of *L. vannamei* was found at this concentration of mangosteen pericarp extract.

The respond of *L. vannamei* (P15) during the experiment showed that the *L. vannamei* anxiously moved around the beaker and sometimes bounced up from the water surface. That respond may be cuased by mangosteen pericarp extract irritated or also caused by environment changing such as DO and pH. In addition, mangosteen pericarp extract have a sticky appearance and transform to sediment when dissolved with solvents, i.e., saltwater. The sediment can enter and block up the *L. vannamei* gills and adhrerd the swimming legs. In addition, the experiment culture of *L. vannamei* was different from the actual culture in ponds, due to the environment such as the density of *L. vannamei* and water paramiters. From this study, the water quality parameters showed unsutable for *L. vannamei* after treated *Zoothamnium sp*. with mangosteen percrab extract. So that, the next step, could perform study on possibility of adding mangosteen percrab extract in shrimp diet. Talpur (2014) found that using *Mentha piperita* (Papermint) 1-5 g/kg mixed in fish diet can enhanced growth performance, immune respond and disease resistance of Asian seabass (*Lates calcarifer*, Bloch) and can agianst *Vibrio harveyi* infection. In addition, Talpur (2014) also reported the number of red blood cell, white blood cell and serum bectericidal activity was non-significantly between treatment groups, except fish fed a low *M. piperita* diet 1g/kg. Militz *et al*. (2013) used garlic (*Allium sativum*) 50 ml/kg and 150 ml/kg as dietary supplementation to prevent monogene (*Neobenedenia* sp.) infection in *L. calcarifer* for 30 days and reported that can decressed 70% of *Neobenedenia* sp. The results of this study can be used as a basis for reference and also used for further development of the product of mangosteen pericarps for against the aquatic parasites.

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