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Effect of Pyriproxyfen, a Malaria Vector Larvicide, on the Growth and Mortality of Vannamei Shrimp

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Abstract

Anopheles mosquito shared a breeding site with the vannamei shrimp (*Litopenaeus vannamei*). Anopheles mosquito larvae commonly live in both stagnant fresh and brackish water. The potential breeding habitat of Anopheles mosquito larvae may be rice fields, pools or ponds, swamps, ditches, and slow-current streams. One of the effective strategies to control malaria is to apply chemical insecticides. Pyriproxifen is a larvicide that contains juvenile hormone-like compounds. Juvenile hormone-like compounds can affect mosquito morphogenesis, characterized by the failure of the larvae to develop into pupae. With a completely randomized design, the shrimp fry was divided into 4 groups, 6 repetitions, 20 individuals each. Shrimp fry in each experimental unit was placed and maintained in plastic jars containing 2.5 L of brackish water. The results showed that the insect growth regulator (IGR) with the active ingredient pyriproxyfen at various concentrations had no significant effect on the growth, mortality, and survival of vannamei shrimp fry. It can be concluded therefore that pyriproxifen larvicide was safe to be used for controlling malaria mosquito vectors in hatcheries and breeding ponds of vannamei shrimp.

Keywords**:** *Anopheles* sp., insect growth regulator, larvacide, pyriproxyfen, vannamei shrimp

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INTRODUCTION

In Indonesia, about 35% of the population lives in areas at risk of malaria. Here the annual incidence of malaria reaches 15 million, with a mortality rate of up to 30,000 [1]. In 2021 there are 304,607 cases of malaria in Indonesia [2].

Indonesia is a malaria endemic country where at least 167 regency areas or cities that show high indications of malaria. Among the Indonesian provinces Lampung is one of the malaria endemic area that has the potential for transmission this mosquito borne disease.

There are 223 villages or 10% of the total number of villages in Lampung Province that contribute to malaria morbidity rate up to 0.17 per 1,000 population. Pesawaran, one among 15 regencies in Lampung Province, is the highest contributor of positive malaria cases. Such an assumption is based on the survey conducted by Ministry of Health in 2017 using Man Biting Rate (MBR) method that results in a rate of 40 bites per person per hour [3].

The breeding habitat of Anopheles mosquito larvae is similar to the breeding habitat of vannamei shrimp (*Litopenaeus vannamei*). Young vannamei shrimp breeding habitat is brackish water [4]. In the breeding habitat of Anopheles mosquito larvae, there are many aquatic animals such as tinhead fish, cere fish, tilapia, shrimp, orchids and dragonfly nymphs [5]. Anopheles mosquito larvae can live in small ponds, large ponds, temporarily stagnant water, or permanent swamps. Some places where Anopheles larvae are found include mangrove forests, estuaries, ponds, beaches, swamps, brackish waters, and mangroves [6].

Vannamei shrimp (*L. vannamei*) is one type of shrimp that is widely cultivated today because vannamei shrimp has very promising prospects and benefits [7]. Shrimp is one of the best seafood products in Indonesia. The more shrimp farming places, the more places for Anopheles larvae to breed.

Malaria control can be initiated by eradicating vector mosquito larvae and taking preventive measures to reduce the spread of infection. Efforts to control malaria-carrying organisms aim to reduce the number of human contacts with Anopheles mosquitoes. This can be done in several ways, including the use of mosquito nets, spraying in homes, environmental management, and controlling mosquito larvae in certain areas [8].

Another strategy to eradicate malaria mosquitoes is applying chemical control using compounds that has insecticidal properties. In this regards the World Health Organization (WHO) strongly recommended the uses of safe insecticides. One of the insectides in question is pyriproxyfen. This insecticide was catageroized as an Insect Growth Regulator (IGR) due to its content of juvenile hormone-like compounds. IGRs consist of two classes, namely juvenile hormone analogs that result in the failure of larvae to pupate and chitin synthesis inhibitors that inhibit the formation of chitin during the molting process. Juvenoid compounds can affect mosquito morphogenesis characterized by the inability of larvae to develop into pupae [9].

Given the role of pyriproxyfen as IGRs in mosquito larvae morphogenesis, it is important to know whether the use of pyriproxyfen insecticides in Anopheles mosquito larvae breeding sites is safe for non-target animals, in this case vannamei shrimp fry. (*L. vannamei*).

METHOD

Time and Place of Reserach

This research was conducted from January to February 2022 at the Zoology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung.

Materials

The tools used in this research are scope net, aerator, aeration hose, measuring cup, and plastic jar. The materials used in this study were PL 12 vannamei shrimp fry, Insect Growth Regulator (IGR) larvicide with sumilary trademark, shrimp feed, and brackish water.

Procedure

The were 5 levels of concentration of pyriproxyfen prepared for this experiment namely 0 ppm, 25 ppm, 50 ppm, and 100 ppm respectively with six repetitions each. The vannamei shrimp fry (*L. vannamei*) were acclimatized first in temporary rearing tanks with adequate feed and aeration.

For maintenance containers, plastic jars were prepared and filled with brackish water of 2.5 liters. Then, 20 vannamei shrimp (L. vannamei) fry that had been acclimatized were put into each plastic jar. Furthermore, the IGR larvicides of 0 ppm (control), 25 ppm, 50 ppm, and 100 ppm were added into the jars containing the shrimp fry. During rearing, shrimp were fed with dried artemia three times a day, at 07:00 am, 12:00 pm, and 17:00 pm.

Observations were made every 24 hours and the parameters measured are water quality including pH, temperature and salinity; and the number of mortality of shrimp fry. Growth parameters measured in this study are body weight and length of the shrimp. Measurement of body weight and length of shrimp was done at the beginning (before) and end (after) of treatment

The data of body weight and length were calculated separately using different formulas according to Effendie [10], as follows:

For the weight gain, the formula used is:

$$W = Wt - Wo$$

Where:
W = Shrimp weight gain(g)
Wt= Shrimp fry weight at the end of rearing (g)
Wo= Initial shrimp fry weight (g)

For the body lenght gain, the formul used is:

$$L = Lt - Lo$$

Where:
L = Shrimp length gain (cm)
Lt = Length of shrimp fry at the end of rearing (cm)
Lo = Length of shrimp fry at the beginning of rearing (cm)

Data Analysis

To determine the influence of independent variables on the dependent variable the one way ANOVA at the 0.05 level was used followed by the least significant difference (LSD) for the posthoc test. Whereas mortality data obtained were analyzed descriptively.

RESULT AND DISCUSSION

Mortality

The results of research that has been conducted on the mortality test of vannamei shrimp fry (L. vannamei) by giving IGR made from active pyriproxifen with 4 treatments at different concentration levels, namely 0%. This means that no vannamei shrimp fry died after being treated with IGR during the study.Details are presented in Table 1.

Table1. Vannamei shrimp fry mortality by concentration of IGR

IGR Treatment(ppm)	t_0 (n)	t_1 (n)	Mortality (%)
0	20	20	0
25	20	20	0
50	20	20	0
100	20	20	0

Note: t_0= Number of shrimp fry at the beginning of rearing
t_1 = Number of shrimp fry at the end of rearing
n = Number of individu

The results of the study of giving IGR made from active pyriproxyfen with various concentrations to vannamei shrimp fry did not affect the mortality of vannamei shrimp fry. Presumably because in IGR made from active pyriproxifen there are compounds that resemble juvenile hormones. Juvenile hormone is a hormone that acts as a regulator of developmental processes to physiology in insects. In vannamei shrimp (*L. vannamei*) there is a hormone similar to juvenile hormone, namely methyl farnesoate (MF). Zhang et al., [11], stated that methyl farnesoat (MF) has a function similar to juvenile hormone. Nagaruju [12] stated that MF is an unoxidized form of insect juvenile hormone secreted by the mandibular organ (MO) in crustaceans. MF plays a role in regulating molting, reproduction, osmoregulation, morphogenesis, metabolism, and behavior in crustaceans.

IGRs made from pyriproxyfen are safe to use in vannamei shrimp breeding habitats. The juvenile hormone analog contained in piriproxifen is low in toxicity to invertebrates. The results of research by Khalil et al., [13] stated that pyriproxyfen is harmless or safe for soil organisms, birds, fish, microcrustaceans and other aquatic organisms. The harmlessness of IGR to vannamei shrimp fry is because IGR can be degraded in water within 18-21 days. IGR made from active pyriproxyfen is environmentally friendly. This is in accordance with the results of research by Akbar et al. [14] which states that IGR larvicides made from active pyriproxyfen do not affect water hygiene, are safe for the environment, and do not increase resistance. So that it causes no deaths in vannamei shrimp.

Factors that affect the mortality of vannamei shrimp fry are shrimp stress due to decreased maintenance water quality, such as temperature, pH, and salinity. In the study there were no vannamei shrimp fry that died because the water quality was well controlled. Details are presented in table 2.

Table 2. Effect of pyriproxyfen treatment for 21 days on the quality of rearing water of vannamei shrimps

Parameter	Range	Quality Standard*
pH	7.16-7.25	7.5-8.5
Suhu (◦ C)	28-29	28-30
Salinitas (ppt)	30-32	26-32

*Source: Regulation of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia Number 75 Year, 2016

Haliman and Adijaya [15] stated that in addition to nutritional factors, good rearing water supports optimal growth and development of vannamei shrimp. Therefore, the quality of rearing water must be managed properly. Zulfikar [16] states that another factor affecting mortality of vannamei shrimp fry is food. Excessive supplementary feeding will damage water quality as a cause of disease, but underfeeding can cause cannibalism, shrimp will eat others shrimp.

Weight gain of vannamei shrimp

The weight gain of vannamei shrimp fry (*L. vannamei*) by IGR treatment are presented in Figure 1. The results of statistical analysis on mean values of weight gain of vannamei shrimps treated with pyriproxyfen of different concentration are shown in Table 3. The highest weight gain was obtained by shrimp fry given IGR of 25 ppm with an increase of weight of 0.152g. While the lowest obtained by shrimp treated with IGR of 100 ppm and control with the weight increase of 0.147g. However, the data in Table 3 shows no statistical difference between groups.

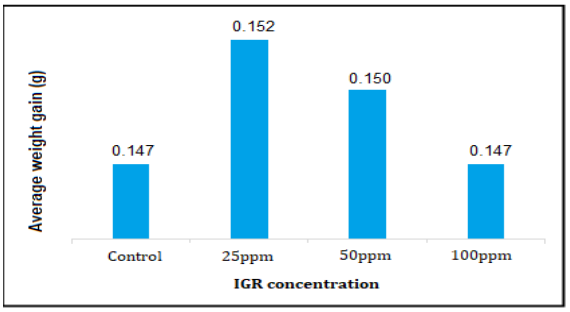

Figure1. Mean values of weight gain of vannamei shrimp fry treated with IGR of pyriproxyfen for 21 days

Table 3. Average weight gain of shrimp fry treated with IGR of pyriproxyfen

IGR treatment (ppm)	Weight gain (g) (Mean±SE)
0	0.147±0.0014
25	0.152±0,0015
50	0.150±0.0018
100	0.147±0.0003

Notes: Values in the same columns followed by the same superscript are not significantly different

Length gain of vannamei shrimp

The data of length gain of shrimp fry (*L. vannamei*) is depicted in Figure 2. The highest average value of length gain of the shrimp was shown by the treatment of 100 ppm IGR and control (0 ppm) by 3.25 cm, while the lowest was in the treatment of 25 ppm IGR by 3.23 cm.

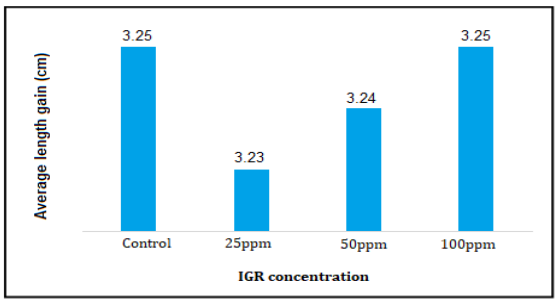

Figure 2. Mean values of length gain of vannamei shrimp fry treated with IGR of pyriproxyfen for 21 days

Table 4. Average length gain of shrimp fry treated with IGR of pyriproxyfen

IGR treatment (ppm)	Length gain (cm) (Mean±SE)
0	3.5±0.0083[a]
25	3.23±0.0095[b]
50	3.24±0.0087[b]
100	3.25±0.0095[a]

Notes: Values in the same columns followed by the same superscript are not significantly different.

Figure 3. Body legth measurement method of vannamei shrimp fry

Based on the results of the One-Way Anova test at a significance level of 0.05, the average length gain of the shrimp treated with different IGR concentrations is not significantly different (Table 4). The technique for obtaining shrimp body length after treated with IRG for 21 days is illustrated in the photograph in Figure 4.

Briefly, the results of this study indicated that both body length and weight gain of the vannamei shrimp fingerlings given IGR were not statistically different ($p>0.05$). This is thought to be due to the sensitivity of juvenoid hormones contained in pyriproxyfen is low to the growth of crustaceans. A previous study by Miyakawa et al., [17] revealed that the juvenoid hormone is very low sensitivity to crustaceans stimulation. Therefore, IGRs made from active pyriproxyfen are safe for the growth of vannamei shrimp (*L. vannamei*). Sullivan [18], in his research, stated that IGRs made from active piriproxifen did not have a significant effect on crustacean organisms.

The absence of a significant effect of piriproxifen on the weight and length gain of vannamei shrimp fry at various concentrations during maintenance, can be seen from the length and weight gain of vannamei shrimp fry during 21 days of maintenance tends to increase. The increase in length and weight of vannamei shrimp fry tends to be normal, in accordance with Nuntung et al., [19] in his research, stating that after 20-25 days post vannamei shrimp larvae will increase in body weight and length and then become juveniles (yuwana). Based on Nababan's research, et al for 30 days of rearing vannamei shrimp fry produced absolute weight growth data of 0.93 g and absolute length of 4.04 cm. Factors that affect shrimp growth include genetics, age, density, water quality, disease, and food. Ariyadi [20], states that water quality is one of the most important factors in the cultivation of vannamei shrimp (L. vannamei). Good water quality stabilizes the breeding environment of vannamei shrimp (L. vannamei). Shrimp growth can be affected by several water quality indicators, such as dissolved oxygen (DO), temperature, pH, salinity, ammonia, and alkalinity [21]. The following are the results of pH, temperature and salinity measurements during the rearing of vannamei shrimp fry.

Table 5. Quality of rearing water of shrimp fry for 21 days

Parameter	Range
pH	7.16-7.25
Temperature (◦ C)	28-29
Salinity (ppt)	30-32

The range of temperature, pH and salinity in this study is a suitable range for the growth of vannamei

shrimp. The temperature of the shrimp rearing water treated with IGR made from pyriproxifen ranged from 28-29 °C. This temperature range is suitable for the growth of the shrimp. As indicated by Kordi and Tanjung [22], the optimum temperature for the growth of vannamei shrimp ranged from 28-31°C and shrimp grow well at a temperature of 24-34 °C. It was also suggested that if the temperature is below 18 °C shrimp appetite will decrease, if the temperature is below 12 °C or above 40 °C can cause death for shrimp [23].

The salinity values in vannamei shrimp rearing water treated with the IGR were range from 30-32 ppt. Salinity is one aspect of water quality that plays an important role because it affects the growth of vannamei shrimp [24]. Maica [25], said that salinity ranging from 4-32 ppt increases alkalinity and total suspended solids concentration, positively affecting the growth performance and survival of vannamei shrimp fry reared in a super intensive system without water exchange.

The pH values of vannamei shrimp culture water treated with IGR containing active ingredient of pyriproxyfen were ranged 7.16 to 7.25. In this range shrimp can grow optimally, in accordance with the results of research by Carbazal and Sanchez [26], that the optimum pH range for the maintenance of vannamei shrimp ranges from 7.0 to 8.5. The pH concentration in water affects the appetite and chemical reactions of shrimp in water. In addition, less pH can cause the skin to be smoother and less active, making it difficult to moult (molting) [27].

CONCLUSION

The results showed that the administration of Insect Growth Regulator (IGR) made from active pyriproxifen, a larvicide for malaria vectors, at a different concentrations did not affect the mortality and growth of vannamei shrimp fry. In conclusion the pyriproxifen is safe to be use as control agent of malaria vectors in hatcheries and ponds of vannamei shrimp (*Litopenaeus vannamei*).

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