

# MASS PRODUCTION AND APPLICATION OF *SPODOPTERA PECTINICORNIS* AS BIOLOGICAL CONTROL AGENT OF WATER LETTUCE (*Pistia stratiotes*)

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## ABSTRACT

Water lettuce (*Pistia stratiotes*) is one of the problematic aquatic weeds because it can cause many problems for humans and environment. In addition, the declining quality and quantity of water is also due to the invasion of water lettuce weeds covering the surface of waters, which can lead to the increasing transpiration and destruction of plankton making the balance of the ecosystem disrupted. This study was conducted in an attempt to control water lettuce (*Pistia stratiotes*) by utilizing the biological control agent *Spodoptera pectinicornis* with mass production and its releasing applications in South Kalimantan's waters. The study was started by collecting *S. pectinicornis* from several places/ fields. The moths were then placed in trays of water and put in a gauze cage of 75 cm x 75 cm x 75 cm in order to keep the air circulation. They were nourished in a laboratory to produce groups of eggs. The groups of eggs were then transferred to rearing ponds. When a fair number of agents were obtained, the treatment of liquid fertilizer AB Mix was carried out. The results showed that the treatment with fertilization to water lettuce weeds as the feed for the biological control agent *S. pectinicornis* did not make any difference when compared to the treatment without fertilization either in *S. pectinicornis*' feeding ability, weight of larvae and pupae or in fitness of imago. However, its destructive ability is so high that it has a big potential as a biological control agent of water lettuce.

Keywords: Mass production, *Spodoptera pectinicornis*, *Pistia stratiotes*

## INTRODUCTION

Water lettuce (*Pistia stratiotes*) is one of aquatic weeds that pose serious problems to humans and the environment. The presence of weeds can inhibit a variety of human activities, such as agricultural activities in the waters of wetlands, shipping, and fisheries, and disrupt hydroelectric energy sources. Its environmental impact is a decrease in water quality due to reduced oxygen levels in the water which result in decreased water levels of biodiversity in both plants and animals that live in the water area (BioNET-Eafrinet, 2011 and Queensland Government 2016).

Various attempts have been made to overcome the issues such as the lifting of water lettuce onto the land but it is less

successful because in the short time the water surface will be filled with water lettuce due to its very rapid growth. Moreover, the method requires high cost. Actually herbicide shows a good hope but its use in waters is very worrying because there are many people using the waters for life.

South Kalimantan is a region that has a large area of waters, and water lettuce weeds still become a serious problem (EPPO, 2014 and CABI, 2015). However, a control solution which is good and environmentally friendly has not been found and it becomes an unresolved problem. It is suspected that the existence of *S. pectinicornis* which is environmentally friendly biocontrol agents in Indonesian (Mangoendihardjo *et al.*, 1979) is still limited, leading to the abundant population of water lettuce, because it is unable to keep pace with the rate of growth and development of water lettuce.

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Based on the results of the research conducted by Wheeler *et al.* (1998), it showed that the quality of the feed in the form of fertilization treatment used in mass propagation of *S. pectinicornis* will affect the distribution of eggs, pupae biomass. Furthermore, the larvae treated with high concentrations of fertilizer will produce imago with greater fitness and increase the total production of eggs compared with the fertilization treatment with lower concentration. However, the imago given with fertilization treatment with low concentrations will apparently last longer than the imago with high concentrations of fertilizer. It is critical to the success of the biological agents in colonizing the water lettuce weeds.

Based on the fact above, the manipulation action should be taken so that the biological agents are in sufficient quantities to be able to control the target weeds. With the potential of such natural enemies, it is important to do some efforts to make the natural enemy *S. pectinicornis* work well to control water lettuce.

The initial effort was the mass production of biological agents in the laboratory. The next step was the preparation for discharging them into the field. However, there is always a limiting factor of natural enemies in the field. The existence of predators and parasitoids is one of the limiting factors that can influence and reduce their performance; hence it is important to observe both of them.

For those reasons, the study with a specific purpose was conducted by conducting mass propagation of biological control agent *Spodoptera pectinicornis* to control water lettuce (*Pistia stratiotes*).

The urgency of this study was as an effort to save the waters from the dominance of water lettuce which could cause damage and imbalance of ecosystem. In this study, we would get the potential natural enemies and the efforts to manipulate the mass production so that the application of augmentation techniques where the natural enemies flood simultaneously into the invaded region could control the population of target weeds.

## MATERIALS AND METHODS

The methods used for collecting and maintaining the biological control agent *S. pectinicornis* adopted from Wheeler *et al.* (1998a)

For rearing the biological control agent *S. pectinicornis*, we created two artificial ponds with the size of 3x3x1 m<sup>3</sup> for each, which were protected by paranet. For the experiment in the greenhouse, we made 20 pieces of gauze containments. The liquid fertilizer used in the experiment was AB Mix with a dose of 5 ppm. For rearing the agents in the laboratory, plastic tanks were prepared. The other needed tools were digital scales for weighing the larvae and pupae, loupe, insect dissection devices, and hand counter.

### Taking and Collecting *S. pectinicornis* in Field

Water lettuce weeds which were visibly damaged were taken and collected and then observed carefully and thoroughly to find out whether the damage was caused by the larvae of *S. pectinicornis*. Water lettuces with the symptoms were then put in containers of big plastic bags and well-arranged so that it was not getting worse. They were then placed in trays of water and put in a gauze cage measuring 75 cm x 75 cm x 75 cm in order to keep air circulation.

### Rearing Agent *S. pectinicornis* in Laboratory

Water lettuces that were taken from the field and put on a tray filled with water, which were also damaged by feeding activity of larvae, were placed at the top of the fresh water lettuces so that the larvae could move and got a new food until it became the pupae. The pupae were collected and placed in a plastic jar with a diameter of 14 cm. On its base was placed tissue paper and wet cotton to keep it moist. Imago male and female which appeared were moved into the enclosures and provided with honey of 10% put on hanged cottons. The imagoes were allowed to mate and lay eggs on the water lettuces.

## Transferring Agent *S. pectinicornis* to Rearing Ponds

Groups of eggs contained in the water lettuces were transferred to a pond that already contained fresh water lettuces. The eggs were left to hatch into larvae and eat the leaves of water lettuce. The Fresh water lettuces had to always be available for the feeding needs of the larvae to reach the pupae stage and then became imagoes. The rearing continued until the insects could reproduce themselves.

## Treatment of Applying Fertilizer to Water Lettuce

Water lettuces taken from the field in medium size were put into an artificial pond of 1 x 1 x 1 m until the entire surface was covered. Then in the other pond were also put water lettuces until covering the surface of the pond but it was given extra liquid fertilizer (AB Mix 5 ppm) and it was left for 1 week. The description of the treatments that would be given was as follow:

A = Water lettuce without fertilizer

B = Water lettuce with fertilizer.

Each treatment would be replicated 5 times so that there were 10 experimental units. The water lettuces in the ponds were used as the feed for *S. pectinicornis*. The implementation of the experiments was carried out by taking the individual water lettuce on each treatment and it was placed in a tray of water; 100 larvae aged 1 day old were then invested in the tray. The larvae were allowed to eat and when the water lettuce was run out, it should be immediately replaced by fresh water lettuce for each treatment. Larvae were left until reaching the pupae. The pupae were then taken and placed in a plastic container padded with tissue paper and closed with the lid with gauze. Pupae were reared until reaching the imago phase. The imagoes were allowed to mate and lay eggs.

The parameters that were observed:

1. Feeding ability: determined by counting the number of individual water lettuce damaged by larvae eating it.

2. Size of the larvae: found out by weighing, using an analytical balance, 25 instar III larvae which were then averaged.
3. Pupae size: determined by weighing, using an analytical balance, 25 pupae which were then averaged.
4. Fitness of imago: determined by counting the number of eggs produced by imago.

Based on the results and the treatments showing positive results, the trial of mass production of biological control agent *S. pectinicornis* was carried out.

## Mass Production of Biological Control Agent *S. pectinicornis*

The mass production was performed by setting up a pond of 3 x 3 x 1 m as many as 2 ponds which were covered with gauze cover. The fresh water lettuces which were taken from the field were put in the ponds if the treatment with liquid fertilizer to the water lettuce gave a positive result. The early instar larvae (aged 1 day) were invested as many as possible and kept alive until they became pupae and imago. The imagoes were allowed to mate and lay eggs. The treatment was repeated until the sufficient population was obtained enough for a mass release.

## RESULT AND DISCUSSION

The result of the taking and collecting the biological agent *S. pectinicornis* in the field showed that the presence of natural enemies was easily found in waters containing water lettuce weeds (*Pistia stratiotes*). Water lettuce which was damaged by larval activity was reared in rearing containers in the laboratory until the larvae reached pupae and imago phases. Imagoes which were copulated would produce groups of eggs that would be used as the materials for further study.

From some of the results of the study conducted by Aphrodyanti *et al.* (2009) it can be noted that *S. pectinicornis* was not able to control the presence of water lettuce weeds in the field. It was evaluated from the water lettuce weeds which were still in abundance whereas there was always the biological agent

*S. pectinicornis* that was associated with weeds. It could be concluded that the populations of existing biological agent was not able to follow the growth and development of water lettuce. Based on this conclusion, the control recommendations that were considered good and right were a form of augmentation techniques. In such conditions, the stage of mass production should be done first. Mass production would be done in an artificial pond and well-conditioned so that it would be obtained optimal results.

Before conducting mass production of *S. pectinicornis*, the experiments with the treatment without fertilization and the treatment with fertilization in water lettuce weeds, which were used as the feed for the biological agents, were carried out. The fertilization treatment used a liquid fertilizer AB mix that was generally used for the nutritional needs of hydroponic plants, thus considered suitable for the treatment in this study.

The results of the study would be found out through the parameters observed. The observation of the feeding ability of *S. pectinicornis* was proportional to the damage they caused because the more biological agents ate the water lettuce weeds, the bigger the damage was. During the feeding activities, the measurements of the larval and pupal weight were also carried out. After the pupae entered the imago phase the eggs produced by female imagoes would be calculated.

### **Feeding Ability**

The feeding ability of biological agent *S. pectinicornis* was one of the indicators of effectiveness or potency of natural enemies to control the target weeds. The observation was carried out by counting the number of individual damaged water lettuce due to the feeding activity of the *S. pectinicornis* larvae.

The placement of the 100 instar larvae was conducted on the water lettuce weeds that had been treated with fertilization and without fertilization. The time given after the treatment was  $\pm 1$  week in the hope that the fertilizer, which was the nutrient, could be absorbed by the water lettuce weeds. The emergence of a new individual of stolon vegetative organ of water lettuce provided a positive result and indicated that the fertilization affected the growth and development of water lettuce weeds. In the process of mass propagation of biological agent *S. pectinicornis*, the feeding needs had a very important role in terms of both quantity and nutritional content.

One important factor that often limits the growth and development of herbivorous insects is the nutritional quality of plants, especially the percentage of nitrogen (Bansode & Purohit, 2013) and leaf toughness (Wheeler *et al.*, (1998). Nitrogen is needed to meet the needs of living creatures like human beings, plants, animals, or other small microorganisms.

The percentage of the damage suffered by the water lettuce weeds in the treatment without fertilization was faster than the one in the treatment with fertilization. That was because the water lettuce weeds that had the fertilization treatment formed new saplings that it took a long time to be eaten (Figure 1).

The results of the observation on the feeding ability of larvae showed highly significant results both in non-fertilization treatment and in fertilization treatment. The Instar II larvae were applied to the water lettuce weeds which initially were only able to be damaged by 20 % but increased on the following day, reaching up to 100 % damage along with the increase of instar larvae. The percentage of damage caused by feeding activity of *S. pectinicornis* larvae is presented in the graph below (Figure 2).



Figure 1. Water lettuce Weeds. A. Treatment without fertilization B. Treatment with fertilization

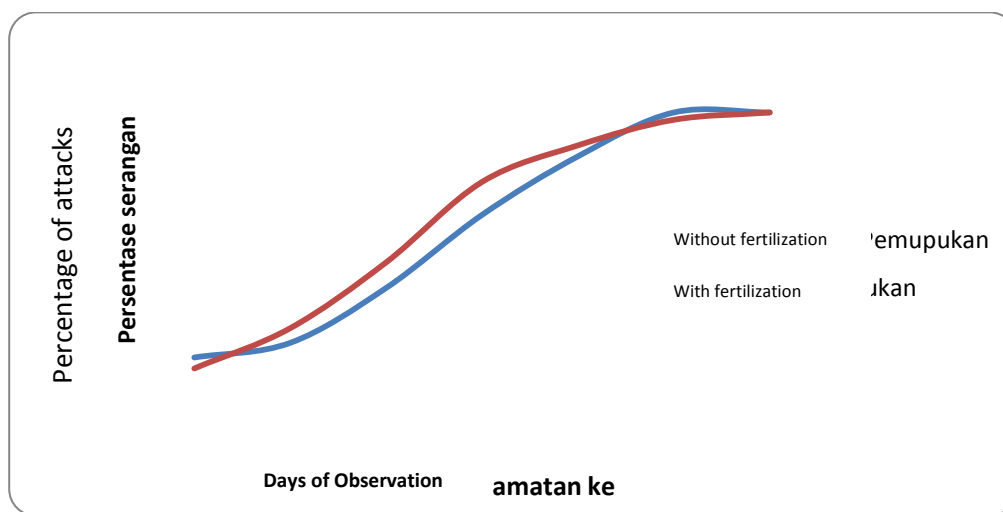


Figure 2. Graph of the percentage of damage caused by feeding activity of *S. pectinicornis* larvae

According to Aphrodyanti *et al.* (2009) on the third instar it was found out that the body size of larvae began to increase rapidly and was accompanied by the increase of the amount of larval food consumption. For the nutritional requirements, the larvae began to eat all the leaves of the water lettuce and caused a real damage. The intensity of the damage to water lettuce was increasing, which entered the fourth instar larvae. This illustrates that the third and fourth instar larvae were very active to eat water lettuce, showing the high potential damage. In addition, the number of larvae would also affect the time for the larvae to damage the water lettuce.

The damage suffered by water lettuce weeds as a result of the feeding activity of *S.*

*pectinicornis* suggested that the potential of biological agents was very high but human intervention was required to manipulate its presence in the field. The damage can be seen in Figure 3.

Insects that would be used as the natural enemies should have the ability to keep pace with the growth of weeds so that the biological agents must have the quick ability to damage and the effort was started from mass propagation in the laboratory, it was therefore expected that after they were being released into the field they could enhance the reproductive system and produce enough eggs that could work as the potential biological control of weeds (Kasno, 2003).



Figure 3. Damage on water lettuce due to the feeding activity of *S. pectinicornis*

The results showed that *S. pectinicornis* was capable of causing severe damage to the water lettuce with or without fertilization treatment, so the addition of feed and preparation of pupae stage were anticipated by the provision of fresh water lettuce. It reinforced the previous study, as stated by Ningrum (2015) that the damage suffered by the water lettuce in NPK fertilizer treatment with a concentration of 0.5 ml/l, 1.5 ml/l, and 2.5 ml/l had the respective percentage by 96.88%, 70.31%, and 62.50% while without fertilizer by 78.13%. It could be stated that the magnitude of the damage inflicted by *S. pectinicornis* was not affected by fertilization treatment on water lettuce.

### Weight of Larvae and Pupae

Larval weight calculation was performed to determine whether fertilization treatment affected the body size of the biological agent *S. pectinicornis*. Based on the calculation using the analytical balance, it was found that the average weight of larvae without fertilization treatment was 0.10 g, while with fertilization treatment 0.07 g. It was found out from the pupal weight calculation that the weight of pupa was 0.049 g in the treatment without fertilization and 0.045 g in fertilization treatment. The significant difference cannot be seen visually in the weight of larvae and pupae of *S. pectinicornis*. It had also been studied by Ningrum (2015), which showed that

fertilization had no effect on the weight of larvae and pupae of *S. pectinicornis* but the damage it had done was very great, even reaching 90%. This was not in line with the results of the study conducted by Wheeler *et al.* (1998a) that the larvae feeding on water lettuce with high level of NPK fertilization would produce biomass of pupae larger than that on the water lettuce with low level of NPK fertilization.

Some possibilities that made the results of this study were different were the relatively short time required by water lettuce weeds to absorb nutrients resulting its limited development in saplings only. The different conditions of climate and weather could also cause differences affecting the growth and development of natural enemies. There was no information on the exact concentration of fertilization to water lettuce as the feed for *S. pectinicornis* in the mass production of biological agents. However, the magnitude of the damage caused by the feeding activity of *S. pectinicornis* gave a great hope for the success of these natural enemies to overcome the water lettuce problems in Kalimantan waters particularly and Indonesia in general.

### Imago Fitness

Imago fitness could be seen from the number of eggs produced by female imago. The biological control agents feeding on water lettuce in the treatment without fertilization produced eggs in an average of 135 eggs per

female parent while in fertilization treatment it produced 146 eggs per female parent. It showed that eggs produced by female imago were not much different in either the treatment with fertilization or the treatment without fertilization. In the study conducted by Center *et al.* (2000), it was noted that the eggs were laid in groups of approximately 150 eggs, with an average of 94 eggs in each group. Similarly, the results of Suasa-ard' and Napompeth study (1976) showed that the number of eggs per group were about 94.3.

According to Wheeler *et al.* (1998), the larvae treated with high concentration of fertilizer would produce imago with greater fitness and increase the total production of eggs than those treated with lower fertilization treatment. However, apparently the imago in the treatment with low concentration of fertilizer would last longer than the imago in the treatment with high concentration of fertilizer. Plant-herbivore interactions are influenced by host plant quality which in turn is affected by plant growth conditions, but at some levels of fertilization can decrease the number of herbivore (Mahdavi *et al.*, 2014)



Figure 4. Pond of propagation/ mass production of biological control agents

## CONCLUSIONS

The treatment of giving fertilization to water lettuce weeds as the feed for biological control agent *S. pectinicornis* showed no different results from the treatment without fertilization in feeding ability, weight of larvae and pupae, and imago fitness of the

## Mass Production of Biological Control Agent *S. pectinicornis*

Based on the results that had been obtained, the provision of fertilizer to water lettuce weeds as feed for *S. pectinicornis* did not give any difference in feeding ability, weight of larvae and pupae, and imago fitness of *S. pectinicornis* in producing eggs, but the damage it caused to water lettuce was very high, so the mass production of biological control agent *S. pectinicornis* was done in the treatment without fertilization. The mass propagation was carried out in a pond of 3 x 3 x 1 m (Figure 4), and the investment of larvae was made and then constantly reared, which was able to produce a large quantity of biological agents. The availability of fresh feed was the critical factor of the success of the mass propagation so that the larvae could eat and successfully entered the pupal stage. The pupae were periodically harvested and placed in a plastic container and kept in it until they reached the imago phase and were allowed to copulate and reproduce eggs ready to be applied to the field.

agent. The damaging ability of the biological control agent *S. pectinicornis* was very high and potential as the biological control agent of water lettuce weeds, and the mass production of biological control agent *S. pectinicornis* could be carried out well with the availability of fresh feed so that they could live and reproduce optimally.

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