

# Preferences Spodoptera pectinicornis as biocontrol of water lettuce (*Pistia stratiotes* L.) wetland weeds to various forms of feedstock

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## Preferences *Spodoptera pectinicornis* as biocontrol of water lettuce (*Pistia stratiotes* L.) wetland weeds to various forms of feedstock

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**Abstract.** The dominance of water lettuce as one of the wetland weeds can cause a decrease in biodiversity in an ecosystem. *Spodoptera pectinicornis* can be a biological control agent for these weeds but requires a mass multiplication stage, so it is necessary to research feedstock in their augmentation. The research aimed to study the preferences and survival abilities of larvae *S. pectinicornis* to three forms of feed preparation made from water lettuce leaves. The research method had carried out in 2 stages (1) feedstocks form; an extracted water lettuce, mashed and cut into pieces (2) the addition of nutrients and preservatives to feedstocks. Observations had been made on the preferences and ability of the larvae to survive. The results showed that the larvae of *S. pectinicornis* had a preference and could survive on cut water lettuce feedstock, while in extracted and mashed died. The addition of nutrients and preservatives to the cut-up feed turned out to cause the larvae to stay away from the feed preparation and eventually die. Based on these, larvae *S. pectinicornis* only prefer to eat the feedstock in cutting form than enrich it with nutrients and preservatives.

**Keywords :** feedstock, mass-rearing, *Spodoptera pectinicornis*, water lettuce (*Pistia stratiotes*), wetland weed.

### 1. Introduction

Water lettuce (*Pistia stratiotes*) is one of the wetland weeds that causes various problems such as inhibiting the flow of water, hosting many kinds of organisms that can cause disease in humans and animals, reducing the quality and quantity of agricultural products, and so on. In addition, its ability to dominate an aquatic area due to its rapid proliferation can reduce biodiversity, so that it can disrupt the balance of the ecosystem [1–4].

Biological weed control is an effort to overcome these problems by considering various aspects that do not cause environmental damage. One of them is by using the insect *Spodoptera pectinicornis* (Lepidoptera: Noctuidae) to control water lettuce weeds. *S. pectinicornis* is a herbivorous insect associated with water lettuce weeds. Based on field observations, the population of *S. pectinicornis* is mealy, so it is necessary to make augmentation efforts.



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The augmentation method or the abundance of natural enemies requires a mass propagation stage in the laboratory. Mass propagation of *S. pectinicornis* is quite complicated because it needs a spacious space to grow *S. pectinicornis* that contains water lettuce plants as food. Water lettuce growing media in the form of water is another problem because it can be a nesting place for various types of mosquitoes and snails. In addition, monitoring the growth and development of *S. pectinicornis*, such as observing early instar larvae or the presence of pupae in submerged, is constrained [2,5].

The success of mass-rearing other insects such as *Spodoptera litura* using artificial feedstock also gives hope to *S. pectinicornis*. Mass rearing of insects in the laboratory requires in large numbers with uniform stadia and age. It is needed for research purposes, both biological studies and control efforts. Rearing the insect using artificial feed is considered more economical and easier to prepare [6–8]. It requires many scientific disciplines because it covers much knowledge such as nutrition, physiology, behaviour, pathology, ecology, genetics, food science, and more [9–11]. Under the rearing conditions causes the insects are finite to find their food so, the provision of food is prominent. The feed used in the form of fresh or artificial, processed based on the test results, can support the growth and development of these insects [6,9].

Due to those reasons, *S. pectinicornis* can be growing using more efficient feedstock. The provision of water lettuce plants as a source of feed is still needed. It is because water lettuce is the specific host of *S. pectinicornis*. So, the artificial substitute feed is quite troublesome. Therefore, the making of it had conducted with water lettuce base material in several forms. The research had carried out on feedstock with added nutrients and preservatives, also.

The research aimed to study the preferences and survival ability of larvae *S. pectinicornis* to three feedstocks forms. The results that gave a positive response had continued with efforts to improve nutrition and the addition of preservatives. The results of this study are valuable as an alternative to providing feed for the mass rearing of insects *S. pectinicornis* that are more efficient for controlling the weed of water lettuce.

10

## 2. Materials and Methods

### 2.1. Place and time

The research had conducted at the Laboratory of Biological Control, Department of Plant Protection, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru. It had conducted from June – October 2020.

### 2.2. Materials and tools

Materials used in this research were water lettuce (*Pistia stratiotes*), larvae *S. pectinicornis*, agar powder, aquadest, yeast powder, casein, L-ascorbic acid, cholesterol, multivitamin multi-mineral capsule, sorbic acid, and formaldehyde solution.

The tools used include plastic containers, fine brushes, microscopes, porcelain dishes, blenders, hot plate magnetic stirrer, analytical balances, thermometers, measuring cups, Petri dishes, beakers, and refrigerators.

### 2.3. Research methods

#### 2.3.1. Feedstock forms

- Extracted. Water lettuce leaves that had washed and dried were weighed as much as 50 g and then mashed and added 50 ml of distilled water and filtered. Then 0.5 g of agar powder was added and heated until the powder dissolved. Further poured into a Petri dish, then cooled and stored in the refrigerator.
- Mashed. Water lettuce leaves that had washed and dried were weighed as much as 5 g and then mashed using a blender and placed into a 650 ml mold container. Afterwards, 2 g of agar powder dissolved in 200 ml of distilled water and boiled in a beaker with a stirrer, cooled at

13

50°C, and then put into a container containing mashed water lettuce leaves and stirred evenly. Then poured into Petri dishes and stored in the refrigerator.

- Cut into pieces. The washed and dried water lettuce leaves were weighed 5 much as 50 g, then cut into ±2 cm pieces and placed in a 500 ml mold container. Later, 2 g of agar powder dissolved in 300 ml of distilled water and boiled in a beaker with a stirrer, cooled at 50°C and, then poured into a container containing water lettuce leaves that had been cut into pieces and then flattened using a spatula and stored in the refrigerator.

#### 2.3.2. Improved nutrition and addition of preservatives.

Increased nutrition by adding yeast powder (7.9 g), casein (3.8 g), cholesterol (0.125 g), multivitamin multi-mineral capsule (1 capsule), ascorbic acid (0.8 g). The preservatives used sorbic acid (0.325 g), formaldehyde solution 5% (1 ml). These ingredients had based on the feed formulation for the mass rearing of insects *Spodoptera litura* [6]. Additions made to the results of previous studies showing the feeding preferences of larvae *S. pectinicornis*.

#### 2.4. Treatments and observations

The third instar larvae had placed in each rearing container containing feedstock according to each treatment and repeated ten times. Observations had made on the processed feedstock included changes in shape, colour, and smell. Observations on larval behaviour had carried out every day to determine preferences for feedstock, while survival was calculated based on the following formula:

$$Viability = \frac{a}{b} \times 100\%$$

Where:

a = number of live larvae

b = number of observed larvae.

### 3. Results and Discussion

#### 3.1. Feedstock forms

The processed feedstock for each treatment that extracted, mashed, and cut into pieces showed differences in size and texture. Based on the composition and formulation that has been processed, the extracted, mashed, and cut were 50, 250, and 350 ml, respectively. Extracted and mashed feedstock placed in a Petri disk was 15 ml each, while cut was in a 500 ml mold container. The extracted feedstock had a smoother texture compared to those mashed and cut. The feedstock processing showed a slight colour change but, in general, still showed a green leaf colour. In all treatments of feedstock, there was no pungent aroma. The feedstock forms of water lettuce for each treatment had provided in Figure 1.



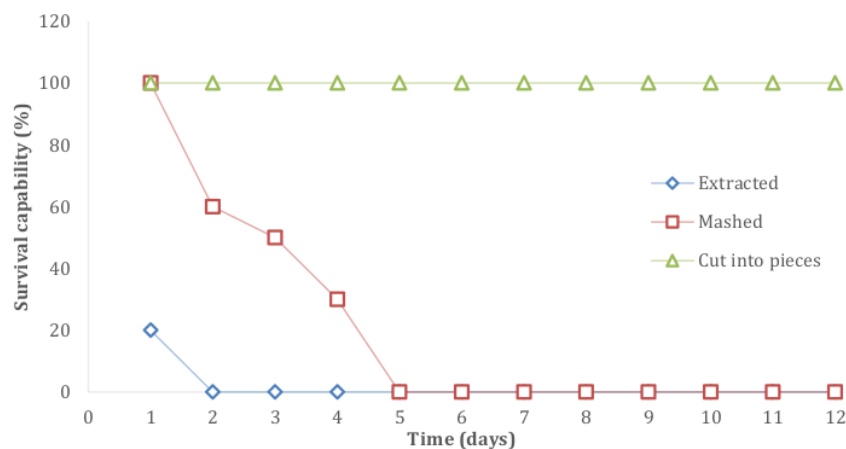
**Figure 1.** Water lettuce feedstock forms (a) extracted (b) mashed (c) cut into pieces.

During the feedstock processing, compounds or nutrients contained in water lettuce seemed no changes it might have caused by the agar liquid poured into the feedstock were in the temperature range of 50°C. Temperatures in the range of 60-70°C in a sample material do not cause decomposition of the compounds contained in it, while the high temperatures around 70-100°C cause certain compounds such as carbohydrates to decompose [12].

### 3.2. Behavior and survival ability of larvae *S. pectinicornis*

The observations on the behaviour of larvae *S. pectinicornis* that gave extracted, mashed, and cut of feedstock showed the differences. In the extracted feedstock, the larvae approached the feed to eat a small amount then died after 1-2 days. In the treatment of mashed feedstock, the larvae also came closer and even entered the feed to eat a few amounts, then died following 2 to 5 days later. Different things showed in the treatment of water lettuce preparations which had cut into pieces the larvae approached the feedstock and carried out feeding activities.

Of the three feedstock forms tested, the larvae could only survive on the cutting form of water lettuce. The suitable quality and quantity of an available feed affected their growth and development. The food consumed by insects must meet the needs of insects to grow and develop optimally. The suitable indication had been observed through the ability of insects to complete their life cycle followed by biological parameters such as their morphological and physiological [13]. The capability of larvae of *S. pectinicornis* to survive until they reached the pupal stage in each feedstock treatment had presented in the graph below (figure 2).



**Figure 2.** Survival capability of larvae *S. pectinicornis* in various feedstock forms.

The survival ability of *S. pectinicornis* larvae treated with three types of feedstocks showed the differences. On the first day of observation, the survival ability of the extracted feedstock was only 20%, while the mashed and cut into pieces were 100% each. On the following day, it was found that all the larvae given the extracted feedstock died, while the mashed feedstock continued to decrease until all the larvae finally died on fifth day. The larvae fed with cut into pieces feedstock were able to survive until the end of the observation.

The behavior of larvae *S. pectinicornis* approaching all the tested feedstock forms indicated an introduction process to the host as a food source. Herbivorous insects can recognize the host plant through its physical form (morphology) and chemical content. The physically, such as the leaf thickness, leaf hairs (trichomes), colour, and others, while chemically based on the compounds contained [14–19].

The physical appearance of plants can be an attraction or repel for herbivorous insects. One of them is the presence of trichomes or hairs on leaves that grow from plant epidermal tissue. The density of trichomes could be an inhibitor (antixenosis) for the herbivorous insect *Spodoptera cosmiodes* (Lepidoptera: Noctuidae) [20]. However, the presence of trichomes may be a preference for other insects. Water lettuce is known to have many trichomes, and the insect *S. pectinicornis* has a desire for this plant. Herbivorous insects also have visual stimuli (photoreceptors), a capability to recognize the host plant based on its colour [21]. However, the stimuli visual of larvae *S. pectinicornis* take a minor

part because they have categorized to holometabola larvae group that has stemmata eyes. Stemmata in insect larvae are known to have only a small number of light receptors (photoreceptors) [15].

For herbivorous insects, a specific chemical compound in a plant plays an essential role in determining the host plant. Herbivorous insects have a close relationship with their hosts, especially related to the compounds or nutrients contained in these plants [22]. The chemical composition, especially plants nutrients, can affect the survival and fitness of herbivorous insects so that the compound content of plants can determine the suitability of their hosts [23]. Larvae tended to move from low-quality to higher-quality feed and can affect their development [24,25].

Water lettuce plants contain various nutrients such as protein, carbohydrates, fat, fiber, vitamins, and minerals [26–28]. These nutrients have needed by insects *S. pectinicornis* for their growth and development. In addition, water lettuce also contains various chemical compounds such as alkaloids, glycosides, flavonoids, and steroids. These compounds are secondary metabolites from plants that can have used as plant defences against herbivorous insect attacks [29–31]. However, the content of these secondary metabolites can act as food stimulants (phagostimulants). Especially for host-specific (monophagous) herbivorous insects [32]. The *S. pectinicornis* is an insect that has the specific character of the host plant lettuce timber so that it can allegedly contain certain compounds that function as phagostimulant.

Herbivorous insects also can recognize their hosts through their olfactory receptors [21,33]. Furthermore, herbivorous insects have an intense attraction to host plants by recognizing them through smell and can even be derived from their heritage [34].

The results showed that larvae *S. pectinicornis* ate a little in the extracted and mashed feedstock, thus proving that the color, smell, or compounds possessed by the water lettuce were not enough to larvae *S. pectinicornis* attract to eat. It caused the larvae to starve and cannot survive. Besides the compounds contained in the water lettuce, both as a source of nutrition and other roles, it predicted that the larvae of *S. pectinicornis* attract to the morphology of the water lettuce. It had proven in the cut feedstock, which has the shape and texture of the leaves of water lettuce so that the larvae are interested in eating and can complete the stages of development into pupa and adult (Figure 3).



**Figure 3.** Larvae (a), pupa (b), and adult (c) of *S. pectinicornis* in the treatment of cut feedstock.

The interest of insects in specific plant morphology is affected by plant morphology as if the presence of leaf hairs and/or wax layer [16]. Moreover, they can carry on adaptation to the physical and chemical circumstances of their host plants [35]. Based on this, the herbivorous insects can determine whether or not to eat the plant. The feed supply on insect growing activities is a necessary thing to do. Ease of providing feed and efficiency in both time and cost must be considered but still meet the needs of insects to grow and develop optimally [10].

### 3.3. Addition of nutrients and preservatives

Based on the results of previous research, the addition of nutrients and preservatives in the cut form of water lettuce feedstock was not preferred. The feedstock that added nutrients and preservatives changed their colour to yellowish then released a plenty pungent odour (figure 4).



**Figure 4.** Feedstock that added nutrients and preservatives.

The larvae fed with those enriched feedstocks seemed to move away, and after 1-2 days were died. It indicated that the enriched feedstock was not suitable for larvae *S. pectinicornis*. The incompatibility of insects to their feed had marked by the minimum food consumption, low endurance, and decreased fertility that implies lowering population density. It caused by the presence of certain compounds so that insects not to be interested in consuming them [23].

The addition of yeast and casein as a protein source in the artificial feed composition is common. Yeast added to insect feed can serve as a source of essential nutrients that can affect larval development and oviposition of imago [36]. Casein is also a source of protein as an enriched nutrient in the formulating of insect feed. However, these two types of protein have a plenty sharp odour and cause discoloration. Therefore, it caused larvae *S. pectinicornis* to move away, starve and eventually die.

Some studies suggested that certain compounds in artificial feed can cause larvae to refuse to eat. The content of compounds such as aromatic essential oils produced by certain plants can cause effects antifeedant, growth inhibitors, and sterilant on *S. littoralis* (Lepidoptera: Noctuidae) insects [37]. Furthermore, the content of specific compounds can cause host incompatibility so that it affects the period and number of egg-laying, life cycle, and insect fecundity [38].

#### 4. Conclusion and Suggestion

##### 4.1. Conclusions

Preference and survival ability of larvae *S. pectinicornis* performed only in the cut form of water lettuce leaf feedstock without nutrients or preservatives enriched. Therefore, it is more efficient during the rearing process.

##### 4.2. Suggestions

Further research on enriched nutrition of water lettuce through fertilization is necessary that needed for the mass rearing of *S. pectinicornis* for biocontrol purposes.

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PAGE 1

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PAGE 2

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PAGE 3

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PAGE 4

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PAGE 5

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PAGE 6

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PAGE 7

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