

THE GROWTH AND DEVELOPMENT OF *Spodoptera pectinicornis* (Lepidoptera: Noctuidae) PROVIDED WITH ENHANCED NUTRITION FROM *Pistia stratiotes* FEEDING VIA FERTILIZATION

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ABSTRACT

A monophage herbivorous insect called Spodoptera pectinicornis can be utilized to biologically control the weed Pistia stratiotes. Insect mass reproduction followed by release into the target weed area is one technique that can be used. The quality of the feed must be taken into consideration during maintenance in order to increase the fitness of S. pectinicornis insects and enable them to function at their best. The fertilization procedure can improve the nutritional value of P. stratiotes as a feed source. The experiment involved applying NPK fertilizer to P. stratiotes at 6 different levels: 0 g (control); 5 g; 10 g; 15 g; 20 g; and 25 g. The findings demonstrated that when NPK fertilizer was applied to Pistia stratiotes, the growth and development of S. pectinicornis larvae, pupae, and adult were improved compared to controls. S. pectinicornis showed improved insect growth and development since its lifespan was shorter than controls at every developmental stage. The ideal NPK fertilizer dose for maintaining P. stratiotes as feed during S. pectinicornis propagation was 5g/20 L of water.

Keywords: *fertilization, nutrition, Pistia stratiotes, Spodoptera pectinicornis*

INTRODUCTION

The intensive use of synthetic pesticides to control various types of plant organisms needs to be a serious concern. The same holds true for efforts to address issues related to nuisance plants or weeds in aquatic areas. One problematic aquatic weed is water lettuce (*Pistia stratiotes*), often controlled using herbicides. Chemical compounds that can act as herbicide such as glyphosate, diquat, bispyribac sodium, flumioxazin and imazamox can reduce water lettuce biomass by more than 99 percent (Glomski & Mudge, 2013; Mudge & Netherland, 2014). The convenience factor is one reason for resorting to hazardous chemicals due to their quick and lethal results. This approach frequently becomes the primary choice, potentially leading to unwise use. Synthetic herbicides are known to cause environmental pollution and disrupt both biotic and abiotic balances within the ecosystem. Herbicide residues can disrupt ecosystem functions, one of which is through changes in microbial populations which ultimately also affect the main cycle of nutrients and trophic interactions (Ruuskanen et al., 2023).

To mitigate the various impacts caused by synthetic herbicides, environmentally friendly control measures are necessary. The utilization of herbivorous insects can be recommended to address the issue of weed colonization in aquatic environments. *S. pectinicornis* (Lepidoptera: Noctuidae) is one such insect that is closely associated with the aquatic weed *Pistia stratiotes*. This insect is known to be monophagous, making it a suitable candidate for use as a biological control agent (Aphrodyanti, 2007).

The results of the research on its existence indicate that *S. pectinicornis* is consistently present in water habitats colonized by the weed *P. stratiotes*. Calculations of the number of individual *S. pectinicornis* larvae show fluctuations but generally range from 3 to 5 per individual *P. stratiotes* plant (Aphrodyanti et al., 2022). This quantity is considered very low to counterbalance the population of *P. stratiotes* in the field (Aphrodyanti et al., 2022). Therefore, human intervention is needed to facilitate initial reproduction and subsequent release into water bodies infested with the target weed.

Several factors can contribute to the low population of insects, and one of these factors is related to the quality of their food source. The quality of food, especially its nutritional content, significantly affects the survival of insects. Nutritional requirements for insects, in the form of compounds from chemical substances, are quite similar to other organisms. For herbivorous insects like those in the orders Orthoptera, Lepidoptera, and Coleoptera, the protein, amino acid, and carbohydrate needs are generally balanced (Susrama, 2017). Nutrition serves to maintain body tissues, support reproductive processes, and serve as the primary source of energy. When these requirements are adequately met, insects can grow and develop optimally (Bansode & Purohit, 2013a; Gupta et al., 2005; Susrama, 2017).

The monophagous nature or specific host preference of *S. pectinicornis* is highly advantageous when considering it as a biological control agent. Concerns about it becoming a pest, particularly for cultivated plants, are less

likely to occur. However, for insect reproduction, providing *P. stratiotes* as the sole food source is necessary. To enhance the nutritional quality of this food, fertilization processes can be employed.

Fertilization involves adding essential nutrients to the growing medium or directly to the plant to enhance production outcomes. Common types of fertilizers typically contain nitrogen (N), phosphorus (P), and potassium (K), often supplemented with other elements to support plant growth and development. Nitrogen, phosphorus, and potassium are key nutrients because they play roles in cell division, leaf development, and root growth in plants (Uchida, 2000).

Several research findings indicate that NPK fertilization significantly affects the proximate composition of plants, including protein, fat, carbohydrates, fiber, minerals, and vitamins (Keraf et al., 2015; Neumann et al., 2017; Simo et al., 2017; Peace & Monisola, 2020). Based on the aforementioned studies, it is essential to conduct research aimed at enhancing the nutritional content of *P. stratiotes* through NPK fertilization. This is expected to improve the growth and development of *S. pectinicornis* insects that feed on enriched food sources.

MATERIALS AND METHODS

Research Methods

The experiment consisted of 6 levels of NPK fertilizer dosages: 0 (control), 5, 10, 15, 20, and 25 g. Each NPK fertilizer dosage was dissolved in 20 L of water, with each treatment replicated 3 times. Fertilization was applied to 10 individual *P. stratiotes* plants and they were

maintained for 26 days in plastic containers (u 50 cm). Afterward, the *P. stratiotes* plants were ready to be used as feed for *S. pectinicornis*. The 26-day period of maintaining the *P. stratiotes* plants was considered sufficient for nutrient absorption from the NPK fertilization, as indicated by the emergence of flowering. This suggests that the plants had undergone the appropriate growth and development stages. *P. stratiotes* is known for its rapid growth and development capabilities. This is supported by the research findings of Samal et al., (2021), which stated that the biological reproduction, growth, and development, including nutrient uptake, of *P. stratiotes* are rapid. The testing was conducted on young *P. stratiotes* plants with a diameter of 13-15 cm and 5-6 leaves. The results of the testing on the duration of development indicated that 25 days were the maximum period for *P. stratiotes* plants, as shown by an increase in plant density and the number of leaves per plant.

Preparation of *S. pectinicornis* and *P. stratiotes*

***S. pectinicornis* insects.** The *S. pectinicornis* were collected from the field based on the signs of damage to *P. stratiotes* caused by their larval feeding. *P. stratiotes* containing larvae was placed in plastic containers with a diameter of 50 cm (Figure 1).

The larvae were reared by providing fresh feed until they reached the pupal stage. The formed pupae were collected and placed in Petri dishes with a moistened tissue paper on the bottom. A sufficient amount of water was added to keep the environment humid. Subsequently, groups of pupae were placed in cages along with fresh

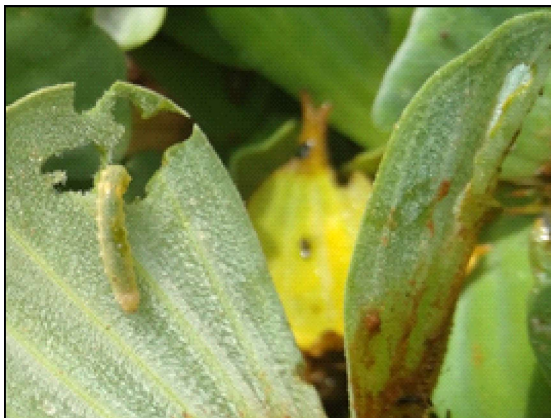


Figure 1 Larvae *S. pectinicornis*

P. stratiotes to prepare for the emergence of female adults to lay eggs. Additionally, a 10 percent honey solution was provided on cotton pads placed in small bottles, creating a feeding source that resembled a wick. The top of the cage was covered with a mesh cloth to allow for air circulation and protect the insects from predators. The clusters of eggs found on *P. stratiotes* were then ready to be used as the research material.

***P. stratiotes* plants.** The *P. stratiotes* plants used for the research were collected from the field. Specifically chosen were plants with characteristics of young leaves that were bright green in color and had a thin texture. The selected plants had a diameter ranging from 10 to 15 cm.

Preparation of Food for *S. pectinicornis* Testing

Food from *P. stratiotes* plants: The water lettuce from each NPK fertilizer treatment were placed in plastic containers with a diameter of approximately 20 cm. These containers were filled with water to maintain the freshness of the water lettuce. Each experimental unit containing water lettuce was covered with a lid. The water lettuce that had been subjected

to different NPK fertilizer treatments were prepared as larval food.

Measurement of growth and development of *S. pectinicornis*. Water lettuce plants was subjected to treatment by infestation of 20 larvae. Observations on the larvae were conducted by recording their lifespan (days). The larvae were reared until they reached the pupal stage, and then calculations of pupal age were performed. Next, the emerging adults were counted for the number of males and females, allowing the sex ratio to be determined. The adults were allowed to mate and lay eggs to determine female fecundity.

Data Analysis

The average data obtained from calculations of each stage in the life of *S. pectinicornis* were presented in the form of graphs, and polynomial analysis was conducted to derive equations for predicting the optimal values for each variable. If the results show a significant effect, these equations can be used for predictions. Polynomial regression analysis was carried out using Minitab 17th.

RESULTS AND DISCUSSIONS

Like herbivorous insects in general, *S. pectinicornis* also requires nutrition derived from plants to survive and maintain the continuity of its offspring. Nutrition refers to chemical compounds that are absorbed to facilitate growth and development, tissue maintenance, reproduction, and as a source of energy for insects. The suitability of nutrition can be depicted through various conditions at each developmental stage of the *S. pectinicornis* insect.

Larvae of *S. pectinicornis*

The calculation of the average survival ability of larvae fed with water lettuce, treated with varying doses of NPK fertilizer ranging from 43.3 percent to 75.0 percent, revealed that larvae can survive under all given treatments. However, at doses of 15 and 20 g/20 L of water, the larvae exhibit better survival capabilities compared to the other treatments. The survival ability of larvae is illustrated in Figure 2.

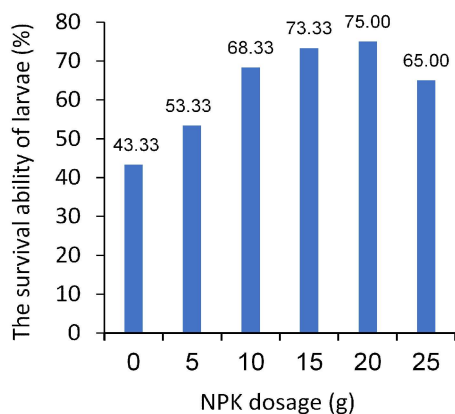


Figure 2 The survival ability of *S. pectinicornis* larva

Based on the results of the percentage calculation of larval survival ability, there were components that can serve as a reference, indicating that a feed composition for insects was considered good if the survival percentage of adult insect stage or larval viability is ≥ 70 percent (Ribeiro et al., 2018). The larval survival ability that reaches this percentage was observed in the NPK fertilization treatments with doses of 15 and 20 g.

Provision of feed is a crucial stage as it is closely related to efficiency in insect rearing for the purpose of mass propagation in augmentation

processes. Efforts to enhance the nutritional value of feed through fertilization processes are expected to optimize the survival ability of larvae. This can improve insect propagation efforts, which, with conventional methods, are often deemed challenging and typically only yield insects in limited quantities (Gupta et al., 2005; Han et al., 2016; Rahayu et al., 2018).

The success of larvae progressing into the pupal stage is known to be greatly influenced by their strong adaptation abilities and feeding activities. Nutritional requirements for insects should ideally encompass both the quality and quantity of optimal food sources. Reducing these aspects mentioned above can also lead to a decline in the performance of insect larvae (Couture et al., 2016). Observations of the feeding activity of *S. pectinicornis* larvae indicate that the larvae possessed a relatively high adaptability and were capable of carrying out feeding activities effectively.

Based on the observations, it was also evident that there were larvae that were unable to survive and experience death. Among the causes of larval death was when larvae fall into water and were unable to climb back to the host plant. This phenomenon is also noted by (Mangoendihardjo, 1982), stating that the mortality of early instar larvae is mostly due to falling into water, which serves as the growing medium for water lettuce. Larvae that fall into water generally become weak and eventually die. Additionally, larval mortality can also result from insect pathogen attacks (entomopathogens). Based on the symptoms observed, these larvae were suspected to be infected with the nuclear polyhedrosis virus (NPV) (Figure 3). Larvae

infected with this virus exhibit initial symptoms such as slow movement, loss of appetite, swollen body, a pale or reddish body color and if touched will emit a thick liquid that contains virus particles. At a latter stage, the larvae will gradually turn from dark brown to black before ultimately dying, it will die due to the cessation of metabolism and damage to body tissue. If the decaying larval body ruptures, a foul-smelling black liquid was expelled. At death, the body wall ruptures and releases the Occlusion Bodies (OBs) (Aphrodyanti, 2007; Kaur et al., 2021; Zufahmi et al., 2015).



Figure 3 Larvae infected by disease

The NPV virus is a common type of insect pathogen that affects the larvae of the Lepidoptera group and can lead to death. Some insects that are frequently infected include *S. frugiperda*, *S. litura*, and *Helicoverpa armigera*. Furthermore, this virus can also cause mortality in herbivorous insects belonging to the orders Diptera and Hymenoptera (De Fouchier et al., 2017; Raghunandan et al., 2019).

The results of the polynomial regression analysis indicated that there was a significant effect of NPK fertilizer dosage on the survival ability of larvae, following a linear regression pattern (Figure 4). The regression resulted show that as the dosage given increases, the survival ability of the larvae also increases.

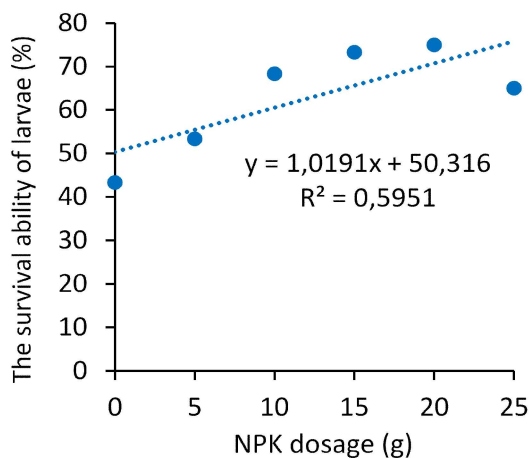


Figure 4 Polynomial regression analysis of larval survival ability

The result of calculations on the age of larvae (days) showed that the NPK fertilizer dosage treatments 0; 5; 10; 15; 20; and 25 g have mean values of 15.75; 15.28; 14.74; 14.87; 14.67; and 14.38, respectively. This data provided information that without NPK fertilizer application, larvae had a longer lifespan compared to other treatments. This aligned with research findings on *Plutella xylostella* larvae (Lepidoptera: Plutellidae), which were known to have faster developmental stages when their host plants were given increased fertilizer doses, especially nitrogen (N) fertilizer. The relationship between N fertilization, larval growth enhancement, and potential modification of larval feeding preferences was not exactly known, but there was a possibility that N variable shifts the plant’s protein balance towards carbohydrate ratios or reduces the level of plant protective compounds, or a combination of both (Bansode & Purohit, 2013a). Similar findings were reported by (Bansode & Purohit, 2013b), indicating that increased NPK fertilizer doses on host plants can shorten the developmental period of *H. armigera* larvae (Lepidoptera: Noctuidae).

The results of the polynomial regression analysis indicated a significant effect of fertilizer dosage on the age of larvae, following a linear regression pattern. The regression demonstrated that as the fertilizer dosage increases, the age of the larvae decreases (Figure 5). This suggested that higher fertilizer dosages, regardless of type, lead to a shortened duration in the larval phase.

Herbivorous insects are known to recognize host plants through their physical forms (morphology) and chemical compounds, including their nutritional content. The physical characteristics of plants can include leaf thickness, leaf hairs (trichomes), color, and others. On the other hand, chemical recognition involves the compounds present within them (Bernays, 1998; Karmakar et al., 2018; Lhomme et al., 2018; Gallon & Gobbo-Neto, 2021)

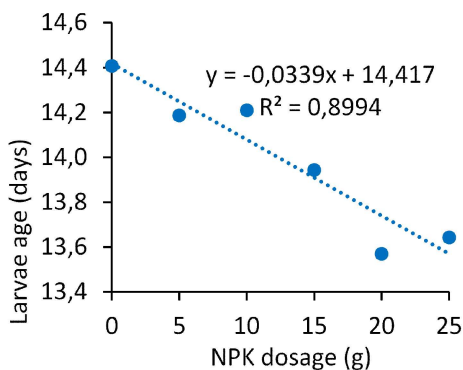


Figure 5 Polynomial regression analysis of the age of *S. pectinicornis* larvae

The larval stage is a phase during which insects engage in feeding activities, making it a crucial determinant for their progression into subsequent phases. Larvae that do not move away from their food source can indicate their suitability with the nutritional requirements. This indication is observed in the larvae of the *H. armigera*, which will remain close to the food source and not move away if provided with nutritionally complete feed (Wang et al., 2019).

Pupae of *S. pectinicornis*

The calculation of the percentage of pupal survival ability in *S. pectinicornis* across all tested treatments ranged from 43.3 percent to 75.0 percent. The percentages of pupae that were able to survive were presented in Figure 6.

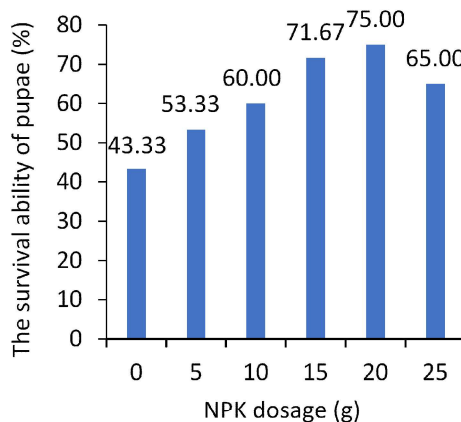


Figure 6 The survival ability of *S. pectinicornis* pupae

The results of the polynomial regression analysis indicated a significant effect of fertilizer dosage on the pupal survival ability, following a linear regression pattern (Figure 7). The regression results suggested that as the fertilizer dosage increases, the pupal survival ability also increases. The pupal survival ability appeared to be in line with the larval stage, strengthening the indication that *S. pectinicornis* provided with enriched nutritional feed exhibits better survival ability.

The calculations for pupal age show that without NPK fertilization, the pupal development takes longer compared to other treatments. For the fertilizer dosage treatments 0; 5; 10; 15; 20; and 25 g, the average pupal ages (days) were 7.43; 7.14; 6.92; 7.05; 6.94; and 7.19, respectively. From this data, it can be observed that pupal survival ability was also correlated with the duration of the pupal stage itself.

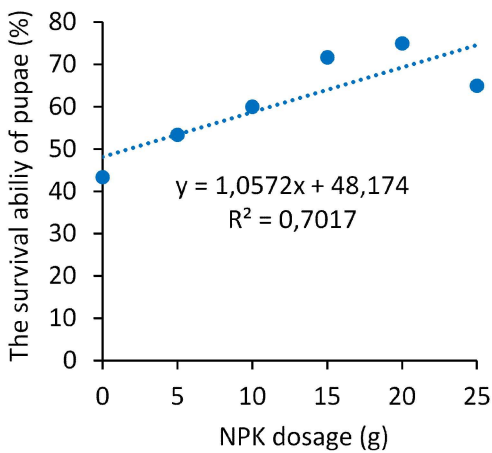


Figure 7 Polynomial regression analysis of the pupal survival ability of *S. pectinicornis*

The results of the polynomial regression analysis indicated a significant effect of fertilizer dosage on the pupal age, following a quadratic regression pattern (Figure 8).

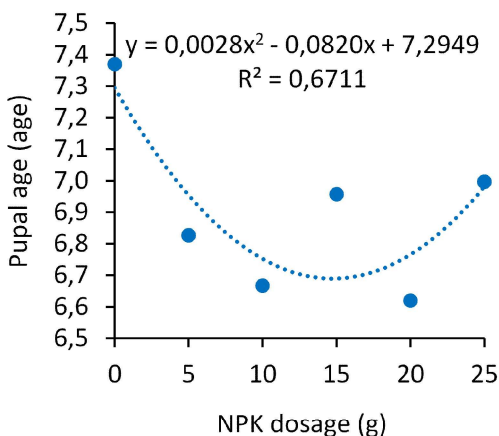


Figure 8 Polynomial regression analysis of the pupal age of *S. pectinicornis*

Based on the equation above, it can be determined that the optimal X point was 14.66. The inverse quadratic pattern indicated that providing fertilizer doses less than or greater than 14.66 g will extend the pupal age. This demonstrated that the accuracy of NPK fertilizer dosage determines the length of the life cycle of *S. pectinicornis*.

Research on pupal age was conducted by (Bansode & Purohit, 2013a), which reported

Sesamia calamistis (Lepidoptera: Noctuidae), pupal age was not influenced by nitrogen application to the plants. In contrast, in the case of the insect *S. frugiperda* (Lepidoptera: Noctuidae), the longest pupal period was observed when no nitrogen fertilization treatment was provided (Bansode & Purohit, 2013b). Referring to research outcomes involving fertilizer treatments, it can be observed that without NPK fertilizer application, the pupal age becomes longer compared to treatments with fertilizer application. Larvae consuming suboptimal quality food can lead to a decrease in their size (Song et al., 2020), which can further impact their pupal stage duration.

Adult of *S. pectinicornis*

The parameters observed for the adult stage were included survival ability, lifespan (age), the number of produced eggs, and the calculation of the number of males and females that emerge. The survival ability of *S. pectinicornis* adults under different NPK fertilizer dosage treatments can be seen in the graph below (Figure 9). From the graph, it was evident NPK fertilizer dosage treatment of 15 g provided a relatively good result (66.67%) in terms of survival ability.

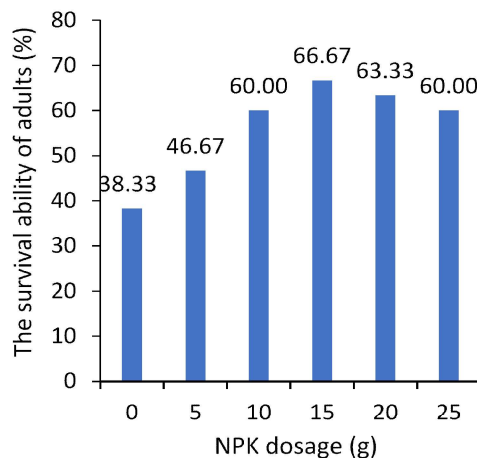


Figure 9 The survival ability of *S. pectinicornis* adult

The research findings of (Bansode & Purohit, 2013b) suggested that the influence of supplied fertilization to host plants can impact the growth and development of insects at the pre-adult stage, but sometimes this influence might not be reflected in the adult stage of those insects. Fertilization, particularly with nitrogen (N), can enhance the fecundity of *Sesamia calamistis*, yet according to (Brewer et al., 1987), the number of eggs laid by insects such as *Choristoneura occidentalis* can decrease with both too low and too high N fertilization. The preference of female adults can also be influenced by other stimuli, such as allelochemical compounds, which may change due to the application of fertilization to the host plants.

The results of the polynomial regression analysis indicated a significant effect of fertilizer dosage on the survival ability of *S. pectinicornis* adults, following a linear regression pattern (Figure 10). Based on this figure, it can be stated that an increase of fertilizer dosage was directly proportional to the survival ability of adults.

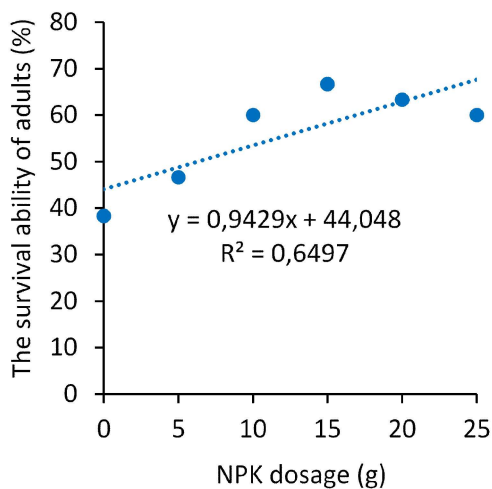


Figure 10 Polynomial regression analysis of the survival ability of *S. pectinicornis* adults

Historically, within the order Lepidoptera in general, it can be stated that the optimal survival of adults is greatly dependent on the nutrition consumed by the larvae. Based on the obtained results, it can be observed that without the application of fertilizer treatments, the developmental time of insects is longer compared to when NPK fertilization is applied. Short developmental time and high average reproduction can indicate a better suitability of the insect towards its host (Saeed et al., 2010).

CONCLUSION

The survival ability of *S. pectinicornis* larvae, pupae, and adults was higher when NPK fertilizer was applied to *Pistia stratiotes* compared to the control group. The age of *S. pectinicornis* at each developmental stage was shorter compared to the control group, indicating better insect growth and develop. It is recommended to use an NPK fertilizer dosage of 15g/20 L of water for the cultivation of *Pistia stratiotes* as a food source during the propagation of *S. pectinicornis*.

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