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# Biological features of *Spodoptera litura* fed on three vegetable host plants under controlled laboratory conditions

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**Abstract.** *Melanie M, Hermawan W, Kasmara H, Yunitasari F, Panatarani C, Joni IM. 2024. Biological features of* Spodoptera litura *fed on three vegetable host plants under controlled laboratory conditions. Nusantara Bioscience 16: 297-303.* Mass-rearing insects is necessary for biological control research, supporting the insect populations for effective in-vitro bioassay evaluations. The success of mass culture depends on the quality of insect feeding and environmental conditions. *Spodoptera litura* (Fabricius, 1775) larvae are notorious as major pests in horticultural crops. This study investigates the feeding preferences and developmental outcomes of *S. litura* larvae when fed on 3 different host plants (water spinach, spinach, and cabbage) with distinct nutritional compositions. The larvae were reared in a controlled insect-rearing cabinet, with parameters observed host-plant nutrition, total consumption, larval weight gain, and developmental duration from larvae to imago. The experimental design employed a completely randomized design with 3 host plants and 9 replications, analyzed with ANOVA and Duncan's multiple-range test. Results indicate that 4$^{th}$-5$^{th}$ instar larvae showed highly consumed water spinach leaves compared to early larvae, which significantly preferred cabbage leaves. Larvae fed on water spinach leaves achieved the highest average weights for larvae, pupae, and imago, along with accelerated developmental times. However, no significant differences were observed in weight gain or developmental duration to imago among all feeding treatments. In conclusion, water spinach is an appropriate host plant for the controlled mass rearing of *S. litura*.

**Keywords:** Controlled insect-rearing, development time, host plants, *Spodoptera litura*, total consume, weight gain

## INTRODUCTION

*Spodoptera litura* (Fabricius, 1775) is recognized as one of the economically significant polyphagous pests affecting crops, known for its resistance to various synthetic insecticides (Babu and Singh 2023). Recent studies on *Spodoptera* have spanned from molecular laboratory investigations to field-scale applications of biological control (Supartha et al. 2022). Establishing mass cultures of beneficial insects is crucial for effective biological control programs (Baratella et al. 2017). This requires the availability of mass-cultured insects at low cost and in healthy conditions suitable for bioassay preparations. Previously, We developed a controlled insect-rearing cabinet that promotes suitable physical environment conditions for insect culture, including air-conditioning systems, temperature and humidity, and lighting (Hermawan et al. 2017). In the successful mass culture of insects, the regulated environmental conditions and optimal nutritional feed sources play a crucial role (Belluco et al. 2023). Nutrition significantly influences the growth, development, reproduction, fecundity, and longevity throughout the life cycle of insects (Blackburn et al. 2016). For this reason, it is important in insect mass culture to fulfill appropriate feed with sufficient nutritional content (Carasi et al. 2014).

Several artificial diets to support insectary mass culture feed resources have been studied to supply nutrients needed and acceptable for insects including *S. litura* larvae. The soybean-based artificial diet effectively fed *S. frugiperda* (J.E.Smith, 1797) and affected pupal survival, sex ratio, and fecundity, but did not significantly influence survival or larval-pupal longevity (Thamrin et al. 2022). Research on the advantages of an artificial diet that contains gelatin, corn, wheat flour, yeast, and several chemical additives as a substitute feed for *S. litura* found that the survival rate of *S. litura* reached only 74.8% (Taufika et al. 2022). These facts show that fresh food as a host plant remains the best feed for *S. litura* culture.

Phytophagous insects depend on the host plant as their nutrition source (Kalaisekar et al. 2017). For instance, *S. littoralis* larvae demonstrated improved growth when fed castor beans with elevated nitrogen and phosphorus content (El-Refaie et al. 2024). Conversely, *Helicoverpa armigera* (Hübner, 1808) larvae displayed the slowest growth when fed red beans, which have the lowest protein and carbohydrate levels among the bean cultivars (Namin et al. 2014). The *S. litura* larvae are reported as polyphagous insects with a wide range of host plants, i.e., food crops, vegetables, fruit, and plantation, including soybeans, eggplant, chili, tomatoes, cabbage, potatoes, peanuts, corn, tobacco, sugar cane, onions, cotton, and mustard (Patil et al. 2014; Rao et al. 2014; Fand et al. 2015; Ullah et al. 2016; Srivastava et al. 2018; Subiono 2020; Ramzan et al. 2021; Taufika et al. 2022; Ginting et al. 2024). The study of *S. frugiperda* feed sources in South America recorded 76 host plants, including the genera *Brassica*, *Amaranthus*, and *Ipomoea* spp. (Montezano et al. 2018). In Indonesia, cabbage plants (*Brassica oleracea* var. *Capitata* L.) have long been identified as host plants for *S. litura*. In contrast, water spinach (*Ipomoea reptans* (L.) Poir. ex G.Don.) and spinach (*Amaranthus hybridus* L.) are not primary hosts for this species. However, local farmers note that *S. litura* larvae can feed on water spinach and spinach. Several studies to investigate the influence of food plant sources on the biological characteristics of *S. litura* larvae have been carried out (Bayu and Krisnawati 2016; da Silva et al. 2017; Narvekar et al. 2018; Montezano et al. 2018; Subiono 2020; Taufika et al. 2022). According to available literature, no records exist of the consumed preference, growth, and development of *S. litura* larvae fed on *I. reptans*, *A. hybridus*, and *B. oleracea* var. *capitata* under controlled conditions in the insect-rearing instrument.

This paper aims to investigate the effects of different host plants on the mass culture of *S. litura* larvae in a controlled rearing cabinet for a suitable physical environment. The investigation focused on the evaluation of three types of host plants: *I. reptans*, *A. hybridus*, and *B. oleracea* var. *capitata* on *S. litura* larvae by determining the preferred consumed feed, the growth (increase the weight gain of larvae, pupa, and imago), and the development (accelerate the time development of larvae up to imago emergence). The nutritional content of the host plants related to their performances was discussed. This study is an important investigation to support sustainable pest control research by addressing biological and ethical considerations of test animals.

## MATERIALS AND METHODS

### Equipment and materials

The main equipment for rearing is a rearing cabinet (63.5×64×186.2 $cm^3$ equipped with an automatic control system for the humidity, temperature, and light and dark (L:D) (Figure 1) (Hermawan et al. 2017). The feeds under investigation for the *S. litura*. were water spinach leaves (*I. reptans*), spinach leaves (*A. hybridus*), and cabbage leaves (*B. oleracea* var. *capitata*) (Figure 2). The 10 % honey solution was used for imago feed.

### Mass-culture *S. litura* in controlled rearing cabinet

The initial population of *S. litura* was obtained from the Indonesian Vegetable and Crops Research Institute (BALITSA) Lembang, West Java, Indonesia. It was reared from egg to hatch into larval stages ($1^{st}$-$5^{th}$ instar larvae), developed into pupation and emergence as imago, under controlled laboratory conditions (Figure 3). The larvae were preserved in a container (14×5.5 $cm^2$) with a tissue pad. The feed plant leaves were washed before feeding to the larvae. When the larva reached the pupa phase, the pupa was inserted into a separate container enclosure with tissue. The female imago lays its eggs in host-plant leaves placed inside the imago cage. Finally, the larval and imago were placed in a rearing cabinet equipped with a temperature setting at 23°-25 °C, humidity at 70%, and photoperiodism (12 L:D) (Carasi et al. 2014). Moreover, the developmental period from oviposition to hatching was recorded. The population of $1^{st}$ instar larvae for each treatment was 100 hundred larvae. Each group of larvae was fed according to the treatment groups; water spinach leaves, spinach leaves, and cabbage leaves, respectively (Table 1). The feeds were given in ad-libitum and replaced every day. The weight of the feeds before and after daily consumption was weighed to obtain the average daily consumption. The weight of each larval instar phase was weighed up to the imago stage. When the pupa emerged into imago, the pupa was placed in a special cage provided with soil as pupation substrate, fed with 10% honey solution, and equipped with leaves overlap.


**Figure 1.** Insect-rearing cabinet with specified dimensions and controlled temperature, humidity, and lighting settings (Hermawan et al. 2017).


**Figure 2.** Host plants for feeding *Spodoptera litura*: A. Cabbage leaves (*Brassica oleracea* var. *capitata*), B. Water spinach leaves (*Ipomoea reptans*), C. Spinach leaves (*Amaranthus hybridus*)

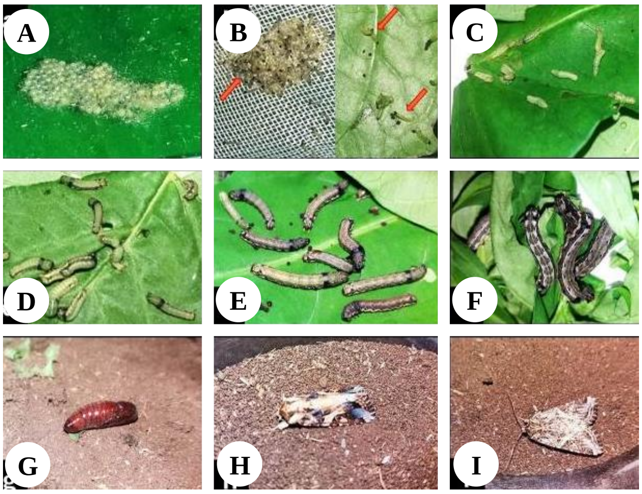


**Figure 3.** The life cycle of *Spodoptera litura* reared under controlled conditions from larval to imago stages: A. eggs, B. first instar larvae, C. second instar larvae, D. third instar larvae, E. fourth instar larvae, F. fifth instar larvae, G. pupae, H. ♂ imago, I. ♀ imago

### Analysis of host plant nutritional content

A proximate analysis was conducted on the host plants to determine their water, ash, and nutritional contents. Water, ash, fat, carbohydrate, and protein contents were analyzed according to the SNI 01-2891-1992 standard test method (SNI 1992). The water content was determined by evaporating the leaves in an oven at a temperature of 100-105°C until the weight loss stabilized, indicating complete evaporation of the water content. The ash content was obtained by burning the leaves in a furnace at a temperature of 600°C for 5 hours, to remove all the main elements of the organic compounds (C, H, O, N). This ash represented the total mineral content in the leaves. The determination of crude protein content was based on the nitrogen content in the raw material. The fat content was obtained by extracting the leaves with a fat solvent, such as ether and acetone, using a Soxhlet apparatus for 16 hours. The extracted fat accumulated in the solvent (Soxhlet flask) was then separated from the solvent using a centrifuge and heated in an oven at a temperature of 105°C.

### Data analysis

The experiment's design was completely randomized design with three types of feeding treatment on *S. litura* larvae. The data were analyzed by one-way ANOVA and Duncan's multiple ranges test ($p<0.05$). The parameters are the development time of larvae to imago; the weight of larvae, the weight of pupae and weight of imago, and the weight-average of feed consumed, respectively. Development of *S. litura* was observed in the population from the eggs hatched until imago. In contrast, growth was observed in the average weight of the *S. litura* population at each developmental phase. The feed consumed by insects was determined based on the dry weight of the leaves and was calculated using the formula in equation (1).

$$CPC = \sum_{i=0}^{j} BB_i \, x\%BK - BKS_i \qquad (1)$$

Where:
*CPC* : percentage of the dry weight of feed (%)
$BB_i$ : wet weight of feed (g)
*BK* : the dry weight of feed (g)
*BKSi* : the dry weight of residual feed (g)

The feed consumption was obtained by measuring the weight of the remaining feed which did not consume and was calculated using equation (2):

$$KMT = BB_i \, x\%BK - BKS_i \qquad (2)$$

Where:
*KMT* : total feed consumption (g *BK*/individual)
*BKSi* : the dry weight of residual feed (g).

This study is an important investigation that addresses the biological and ethical considerations of test animals, supporting sustainable pest control research.

## RESULTS AND DISCUSSION

### The nutritional composition and benefits of *Spodoptera litura* host plants

The analysis of the nutritional content by proximate analysis of *S. litura* host plants reveals that each feed source has a diverse composition, described in Table 1. Comparatively, among the three types of feed, spinach leaves exhibited the highest carbohydrate and protein contents, although spinach had the lowest water content. Cabbage leaves had the highest water and fat contents compared to the others but had the lowest protein and carbohydrate levels. However, water spinach shows a more balanced composition among the three feed types. The notable variation shows in protein content among the 3 host plants (water spinach, spinach, and cabbage), while the contents of carbohydrates and fats are relatively the same (Table 1).

**Table 1.** The composition of nutrients, fiber, water, and ash contents in feeds for *Spodoptera litura*

| Host plants | The nutrition content of fresh feeds | | | | | |
|---|---|---|---|---|---|---|
| | Protein (%) | Carbohydrate (%) | Fat (%) | Fiber (%) | Water (%) | Ash content (%) |
| Water spinach (p1) | 2.67 | 3.97 | 0.34 | 2.85 | 91.91 | 1.11 |
| Spinach (p2) | 5.05 | 4.12 | 0.44 | 2.11 | 88.39 | 2 |
| Cabbage (p3) | 1.69 | 3.16 | 0.45 | 4.24 | 94.21 | 0.49 |

Spinach is one of the vegetables rich in protein according to the USA Food Center database (2021), spinach is included in the 10 groups of vegetables with the highest protein content, which protein accounts for 50% of its calories. Cabbage (*B. oleracea*) from the Cruciferae or Brassicaceae family group is characterized by its high water, fat, and fiber content (Ashfaq et al. 2018). Cabbage is a plant that requires a high water supply and a minimum rainfall of 500 mm. Consequently, cabbage has a high water-holding capacity (Huđ et al. 2023). Although water spinach grows on land, it can adapt well to muddy soils and slightly water-logged areas. Like cabbage and spinach, water spinach stems also exhibit a high water-holding capacity (Lakitan and Kartika 2020). The plant provides all the essential nutrients that herbivorous insects require; however, the specific amounts and ratios of these nutrients and water composition, especially macronutrients, can vary significantly (Deans et al. 2022).

*Spodoptera litura*, a polyphagous insect, relies on different host plants to meet its nutritional requirements. These plant nutrients are crucial for its development, growth, immune system, and survival (Vengateswari et al. 2020). This means that polyphagous insects like *Spodoptera* specialize in a diverse nutritional intake, and their adaptation to different feed sources supports their ability to regulate balanced water and nutrition (Deans et al. 2022). In general, insect herbivores can manage their nutritional intake. When confronted with an imbalanced diet, they adjust their nutrient consumption to address deficiencies and eliminate excesses, thus optimizing efficiency and survival. For instance, insect herbivores effectively utilize surplus proteins and fats for energy reserves. However, excessive protein can hinder optimal growth, so insects must effectively meet their protein requirements (Deans et al. 2022).

Macro and micronutrients play a crucial role in supporting the longevity of insects (Bayu and Krisnawati 2016). Carbohydrates promote general vitality, activity, and growth in insects (Le Gall and Behmer 2014). In the structure of glucose and glycogen, carbohydrates constitute the main energy source during metabolism. Proteins and lipids also provide alternative energy sources for insects (Bala et al. 2018). The protein content serves as an energy reserve and supports insect growth. Additionally, the amino acid composition of their diet influences their growth and reproduction (Kröncke and Benning 2023). Essential amino acids are crucial for forming cell membranes, enzymes, and hormones that regulate insect growth and development. A small amount of fat or sterol in the diet is sufficient to positively affect the growth and development of Lepidopteran larvae (Jing and Behmer 2020). In addition to being part of all cellular membranes, sterols are precursors for hormone regulation in insects. Insects require a high water intake for normal growth, development, and biological functions. Water content prevents dehydration and maintains the osmotic balance of cells, tissues, and organs (Benoit et al. 2022). The need for fiber in insects is primarily related to their digestive function.

**Influence of different host plants on total *Spodoptera litura* consumption**

Insect food preferences were measured quantitatively based on the amount of food consumed by the larvae. Total consumption was assessed by the average weight of feed consumed by *S. litura* larvae at each instar stage (Figure 3). Water spinach and cabbage had the same average weight consumed by 1st to 2nd instar larvae (0.004 g/larva). However, 2nd to 3rd instar larvae consumed the most cabbage, as indicated by the highest average weights of cabbage consumed compared to other feeds (0.035 g/larva). 3rd to 4th instar larvae consumed the most water spinach, with average weights of 0.072 g/larvae, and 4th to 5th instar larvae consumed 0.094 g/larvae (Duncan's multiple range test, $p<0.05$).

It is widely recognized that *Spodoptera* larvae consume larger quantities of feed with a softer surface texture and higher water content (da Silva et al. 2017), such as water spinach and cabbage, compared to spinach. The increased water content enhances food assimilation and promotes higher food intake. During the fourth and fifth instar stages, larvae favor water spinach over cabbage leaves. These stages also need significant nutritional reserves, obtained from water spinach, to prepare for pupation. As a polyphagous insect, *S. litura* demonstrates notable tolerance to various types of food with differing nutritional contents, whether high or low. This adaptability is supported by the larvae's ability to balance the nutrition requirements, ensuring survival through developmental phases (Le Gall and Behmer 2014). Test results showed that the highest carbohydrate and protein content in spinach limited larval food preferences, due to lower overall consumption during the instar stage compared to the other two feeds. Higher carbohydrate and protein intake for *S. litura* larvae can function as a large source of energy (calories) but can limit larval digestive efficiency. The feed with low carbohydrate and protein concentrations presents an opportunity to optimize digestive efficiency (Deans et al. 2022). Thus, insects do not require excessive intake if the nutritional content meets their needs. This is likely to cause variations in preferences and the quantity of food consumed by the *S. litura* larvae for their survival and development.

**Influence of different host plants on insect growth in terms of larval, pupal, and imago weights**

The qualitative assessment of larval growth was performed by measuring the average weight gain during each instar phase. Larvae fed with water spinach exhibited the greatest weight gain during the instar stages, followed by those fed with spinach, while the smallest weight gain was observed in larvae fed with cabbage (Figure 4). Water spinach leaves significantly enhanced larval weight gain, with average weight increments of 17.33, 155.11, 460, and 813.33 mg per larva for the 1st to 5th instar *S. litura* larvae. In comparison, the weight gains for the same instar stages with spinach and cabbage leaves were 12.22, 92.67, 288.44, and 667.56 mg per larva, and 8.22, 28.43, 137.04, and 295.28 mg per larva, respectively (Duncan's multiple range test, $p<0.05$). The type of feed similarly influenced the average weight of pupae. Water spinach resulted in the highest average weight gain (309.78 mg per pupa), followed by spinach (303.56 mg per pupa), and cabbage, which produced the lightest weight gain (234.17 mg per pupa) (Figure 5.A). However, imago growth showed no significant difference in average weight among the 3 host plants—water spinach, spinach, and cabbage (ANOVA test, p>0.05), as shown in Figure 5.B.

The larval stage is a critical phase in the life cycle of Lepidopteran insects (El-Refaie et al. 2024). During this stage, larvae feed and grow significantly, accumulating essential nutrients and energy needed for metamorphosis into the imago stage. The pupal phase is characterized by a period of rest and no feeding. Therefore, the mass gain of pupae depends on the nutrient intake during the larval stage. The efficiency of nutrient utilization as a reserve affects the pupal mass gain. During the pupal phase, various processes occur, including developing new organs, leading to the imago stage. Feed consumption influences the intake of macronutrients, particularly carbohydrates and proteins (Le Gall and Behmer 2014; Blackburn et al. 2016).

Three groups of *S. litura* larvae fed with water spinach, spinach, and cabbage, showed no significant difference in weight gain among the imago stages. This suggests that the larvae adapt to variations in food sources and consume large amounts, reflecting efficient feeding behavior that optimizes nutrient use for imago growth. Insects require complete nutritional content in their feed for normal development. While normal growth can be maintained by addressing nutritional deficits or imbalances, herbivorous insects may face challenges due to suboptimal nutrition over time (Le Gall and Behmer 2014). Future studies could explore how insects compensate for nutritional deficiencies during development.

**Influence of different host plants on insect development time**

The viability of insect survival is indicated by the normal or shortened development period from larvae to imago. The results showed that the three types of food plants had significantly different effects on the average development time of *S. litura* larvae. Larvae fed with water spinach had the shortest average total development time from larvae to imago emergence, at 27.27 days (Duncan's multiple range test, p< 0.05) (Figure 6). This result aligns with earlier evidence that larvae fed water spinach achieved the highest food consumption and weight gain.

Optimal food intake ensures that *S. litura* larvae absorb essential nutrients in the midgut, and then circulate through the hemolymph system. The hemolymph carries these nutrients throughout the body, significantly impacting insect development. Proper nutritional content is crucial for metabolic processes (Bala et al. 2018). Carbohydrates and proteins are essential for effective growth and development (Le Gall and Behmer 2014; da Silva et al. 2017; Bala et al. 2018) Carbohydrates provide necessary metabolic energy and play a role in regulating developmental hormones. Proteins are vital for the secretion of Juvenile Hormone (JH), which governs the metamorphosis process, particularly during instar transitions (Le Gall and Behmer 2014). Therefore, *S. litura* larvae fed on water spinach, with their optimal levels of carbohydrates and proteins, benefit from improved nutrient intake that positively influences their development period. Our study found that *S. litura* takes approximately 27.27 days to develop from larvae to imago when fed water spinach, 28.62 days with spinach, and 33.8 days with cabbage. These durations are within the typical range for *S. litura* development from the first instar to imago emergence when using the main host plant as a food source (Ramzan et al. 2021). All emerging imago displayed normal sex ratios and morphological characteristics. This indicates that *S. litura* has effectively adapted to variations in the nutritional composition of its food intake, ensuring its survival and longevity.

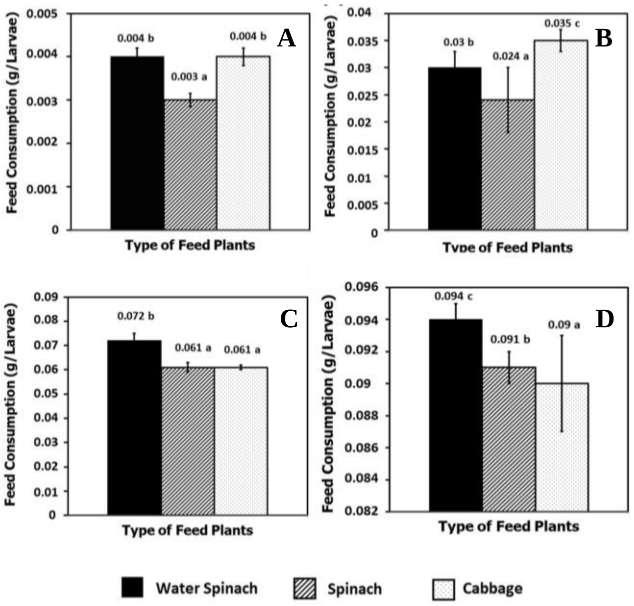


**Figure 3.** Total feed consumption of *Spodoptera litura* at different larval stages: A. 1st-2nd Instar, B. 2nd-3rd Instar, C. 3rd-4th Instar, D. 4th-5th Instar

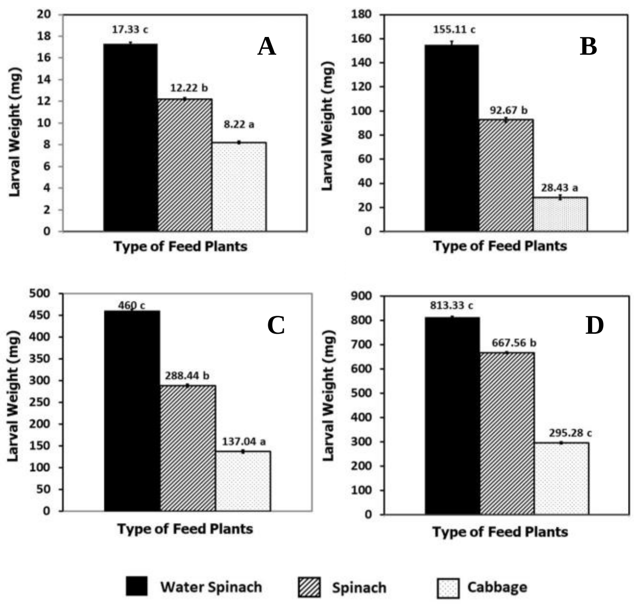


**Figure 4.** Average weight gain of *Spodoptera litura* larvae at various stages fed different host plants. A. 1st-2nd Instar, B. 2nd-3rd Instar, C. 3rd-4th Instar, D. 4th-5th Instar

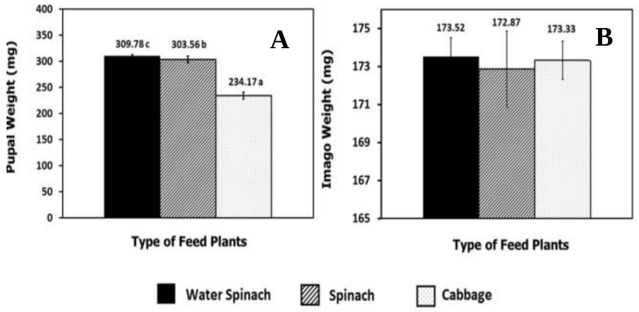


**Figure 5.** Average weight gain of *Spodoptera litura* pupae and imago at various stages fed different host plants. A. Pupal stage, B. Imago stage

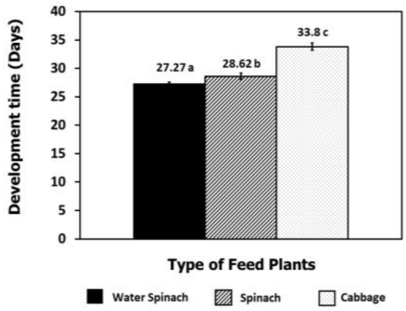


**Figure 6.** Development time from larvae to imago in *Spodoptera litura* influenced by different host plants

In conclusion, the nutritional content of food plants is essential for the growth, development, and survival of *S. litura* in a controlled rearing environment. Among the three host plants tested, water spinach offers a more balanced composition of carbohydrates, proteins, and water than spinach and cabbage. During the 4$^{th}$ to 5$^{th}$ instar stages, larvae exhibited a higher consumption of water spinach, while early instar 2$^{nd}$ to 3$^{rd}$ larvae showed a significant preference for cabbage leaves. The least consumed is spinach even though its protein composition is the highest. This demonstrates that *S. litura* as a phytophagous insect can adapt to various host plants with different nutritional compositions due to efficiently consumed behavior. The 4$^{th}$ to 5$^{th}$ instar *S. litura* larvae require sufficient carbohydrates and proteins from water spinach to support critical developmental stages leading to pupae and imago. The optimal nutrition from water spinach leads to higher average weights for all larval and pupal stages and shortens the development time. Despite these findings, no significant differences were observed in weight gain or developmental duration to imago across all feeding treatments. The total development period, sex ratio, and morphology of the surviving imago were all within normal ranges. Based on our findings, we recommend the three types of food plants as alternative main hosts for the mass rearing of *S. litura* in a controlled environment. Water spinach is preferred and demonstrates the finest growth and development from larvae to pupae relative to spinach and cabbage. Furthermore, water spinach provides an optimal nutritional composition that enhances the longevity of *S. litura*. Its benefits include ease of cultivation, adaptability to tropical and subtropical climates, widespread availability, and cost-effectiveness.

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