

Antifeedant Effect of *Moringa oleifera* (L.) Leaf and Seed Extract on Growth and Feeding Activity of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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Abstract

This study was conducted to evaluate the effect of *Moringa oleifera* leaf and seed extract on length, weight, and feeding activity of *Spodoptera litura*. Leaf and seed were extracted by maceration method in 70% ethanol. Bioassays were conducted by using five concentrations (0%, 5%, 10%, 15%, 20%) on 5 larvae by leaf-dip method with 5 times replication. The result showed that leaf and seed of *Moringa* extract contained alkaloid, flavonoid, and saponin which gave antifeedant effect to growth and feeding activity of larvae. The increased extract concentrations are lead to weight loss and the length shrinkage of larvae. The extracts promoted prolongation of the larval instar. Reduction of food intake of the larva is in line with the concentration increase of extracts. The antifeedant effect also affects time to stop feeding and give mortality of larvae.

Keywords: antifeedant, leaf, moringa, seed, *S. litura*

INTRODUCTION

Agriculture is one of the largest sectors for the economy in developing countries, including Indonesia. One of the efforts made in improving the quality and quantity of productivity is pest control. Insect pests are known to cause significant damage to crops and affect agricultural productivity because they eat the crops as their food [1].

Insect pests from order Lepidoptera are found throughout the world [2]. *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) which is known attack many types of crop, such as cotton, soybean, tobacco, onion, chili, corn, and rice [6]. The survey showed *S. litura* was found in 16 regencies in East Java with leaf damage rate above 12.5% [3]. The *S. litura* has a wide range of host, feeding nearly on 112 species worldwide [4]. It is causing considerable economic loss to many vegetable and fruit crops. Pest control use chemical insecticides has been a fundamental tool for pest control, but leads to serious consequences such as intoxication of people and animals, contamination of water, air, and soil, residues on food, high persistence in the environment, resistance in pest, and impact on beneficial insect [5] or non-target insect, and secondary pests explosion [6]. This impact encourages to use biological control methods

without damaging the environment or harming other organisms. The holistic approach towards insect pest management which includes the use of botanicals such as plant products to pest control and combat resistance problem [12]. This is in line with the government program in the effort to protect crops productivity which applies the Integrated Pest Control technique in accordance with Presidential Instruction No 3 of 1998 [7].

Several types of plants have been known to have potential as botanical pesticides because of the content of phytochemical compounds that have an antifeedant impact and mortality on insect pests. Earlier studies have indicated that antifeedant compound derived from leaf, seeds, flowers, fruit, and root of the plants can be used as botanical pesticides to against the growth and metamorphose of the noxious insects [12]. This compound came from processed organ plants into various forms, such as raw materials in the form of flour, extras, or resins which are the result of extracting liquid secondary metabolites from plant parts.

Phytochemicals are a group of non-nutrient compounds that are naturally synthesized by plants. These compounds are in flowers, tubers, leaves, fruit, roots, tree bark, and in the rhizome. Phytochemicals act as a defensive mechanism for the plants against disease and many external attacks. Phytochemicals are the results of primary or secondary metabolites of plants [8]. These substances include saponins, tannins, alkaloids, flavonoids, and terpenoids. *Moringa* is a plant that contains active compounds alkaloids,

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flavonoids, saponins, steroids, and tannins [8]. In the study [9,10] Moringa seeds proved to be larvacidal against *Anopheles stephensi* and Moringa flowers had a larvacidal effect on the *Aedes aegypti*. Moringa seed extract contains high concentrations of flavonoids, and leaves contain flavonoids, alkaloids, saponins, and tannins [8]. Based on the results of previous studies, *Moringa oleifera* can be used as a candidate for botanical pesticides, especially in *S. litura*. The purpose of this study was to determine the antifeedant effect of *M. oleifera* leaf and seed extract on growth and feeding activity of *S. litura* larvae.

MATERIAL AND METHOD

The culture of *S. litura*

Spodoptera litura larvae were obtained from the Research Institute for Sweeteners and Fiber Plants (BALITTAS), Malang. Larvae are collected at the second instar stage and are ready for use at the third instar. The larvae are stored in jam bottles (5 larvae each bottle) that have been given mustard leaves as feed. The bottle is closed using a cloth. Feed is replaced every day and dirt cleaned using a brush.

Extraction of Moringa Leaf and Seed

Moringa leaves and seeds are powdered into simplicia. Total 500 grams of simplicia was extracted with 3750 mL of ethanol using the maceration method. The maceration vessel is closed and left for 3-4 days, protected from direct sunlight. The immersion was stirred in several times, so the diffusion process of dissolved compounds in ethanol more effective [11]. The immersion was filtered using a cloth. The resulting filtrate was then evaporated using a rotary evaporator [13] to evaporate the solvent. The resulting extracts were then stored in the refrigerator ($\leq 4^{\circ}\text{C}$) until ready to used. The extract use for bioassay studies in five concentrations (0 %, 5%, 10%, 15%, and 20%).

Total Phytochemical Assay for Moringa Leaf and Seed

The tested phytochemicals were flavonoids [29], alkaloids, and saponins [30]. The total phytochemical content test was carried out by the spectrophotometric method. Each extract was tested in duplicate.

Bioassay of extract

The third instar *S. litura* larvae are exposed for 1-2 hours before testing. Each of the concentrations of the extracts was prepared and

put in a small basin container. Mustard leaves that are still fresh and not contaminated with chemical pesticides are dipped in each concentration for ± 10 seconds. Testing is done by the deep-in method and then placed on a tray to be air dried. The leaves are inserted into all jam bottles which contain *S. litura* larvae [6]. Each concentration used 5 larval and repeated 5 times. The total number of *S. litura* larvae used was 250 individuals.

Observation of Antifeedant Effect

The development of larvae was observed starting from the 3rd instar larvae to the instar 5. Observation variables included the length, weight, feed residue, development time, observed at each turn of the larval instar stage, and the stopped eat time which was observed at 4, 8, and 12 hours after the application (HAA).

RESULTS AND DISCUSSION

The result of the total phytochemical compound of leaves and seeds showed that all samples positive contained flavonoids, alkaloids, and saponins (Table 1). In each extract, it was known that the content of flavonoids and alkaloid was relatively higher compared to saponins. These results are in line with the study [14], which showed that flavonoid content ($3.46 \pm 0.03 \text{ g.100g}^{-1}$) and alkaloid ($3.30 \pm 0.00 \text{ g.100g}^{-1}$) in *M. oleifera* was higher than saponin content ($0.95 \pm 0.03 \text{ g.100g}^{-1}$).

Table 1. The total compound of phytochemical of *M. oleifera*

Extract	Total (mg.100g ⁻¹)		
	Flavonoid	Alkaloid	Saponin
leaf	3157.095	153.711	106.159
	3205.656	154.542	108.814
seed	2075.002	205.134	65.553
	2041.744	209.240	63.703

The three secondary metabolites gave antifeedant effect to the development of *S. litura* larvae including length, weight, larval development time, food residue, and time to stop eating. Secondary metabolites in plants have an impact on growth, development, and reduced digestive capacity in insects [15].

Flavonoids, alkaloids, and saponins on Moringa leaves and seeds give significant different to the length of *S. litura* larval on various instars. The increased concentration of Moringa leaf and seed extract declined the mean length of *S. litura* larvae (Fig. 1). The 3rd instar larva on both treatment significantly different between control (0%) and with all

concentrations, except 5%. The 4th instar and 5th instar were significantly different at all concentration. The shrinkage of larvae length is higher in larva treated by Moringa seed extract than the leaf. This is due to the higher alkaloid in Moringa seeds than Moringa leaves. Alkaloids become inhibitors of several metabolisms. Alkaloid inhibits the work of sucrose in the intestine in digestive metabolism and affects the growth of larvae [16].

The treatment of Moringa leaf and seeds extracts also affects the weight of larvae of *S. litura*. The increase of extract concentration in line with losing weight of larvae (Fig. 1). Alkaloids cause lose weight and a decrease in food intake

of insects [17]. In the study [18] reported that the alkaloid from *P. harmala* was studied in *S. greraria* and it caused weight loss. Based on the previous research, it was explained that the presence of rapid neurotoxicity symptoms hampered larval feeding activity [17]. The weight of each larval instar growth has decreased which higher in the treated diet of Moringa seed extract. The weight of the 3rd instar larvae with both extracts was not significantly different in 3rd instar and 4th instar between control and 5% concentration. Alkaloids for insects act as stomach and contact toxic. Alkaloids in the form of salt can be degraded cell membranes in the digestive tract to enter and damage cells.

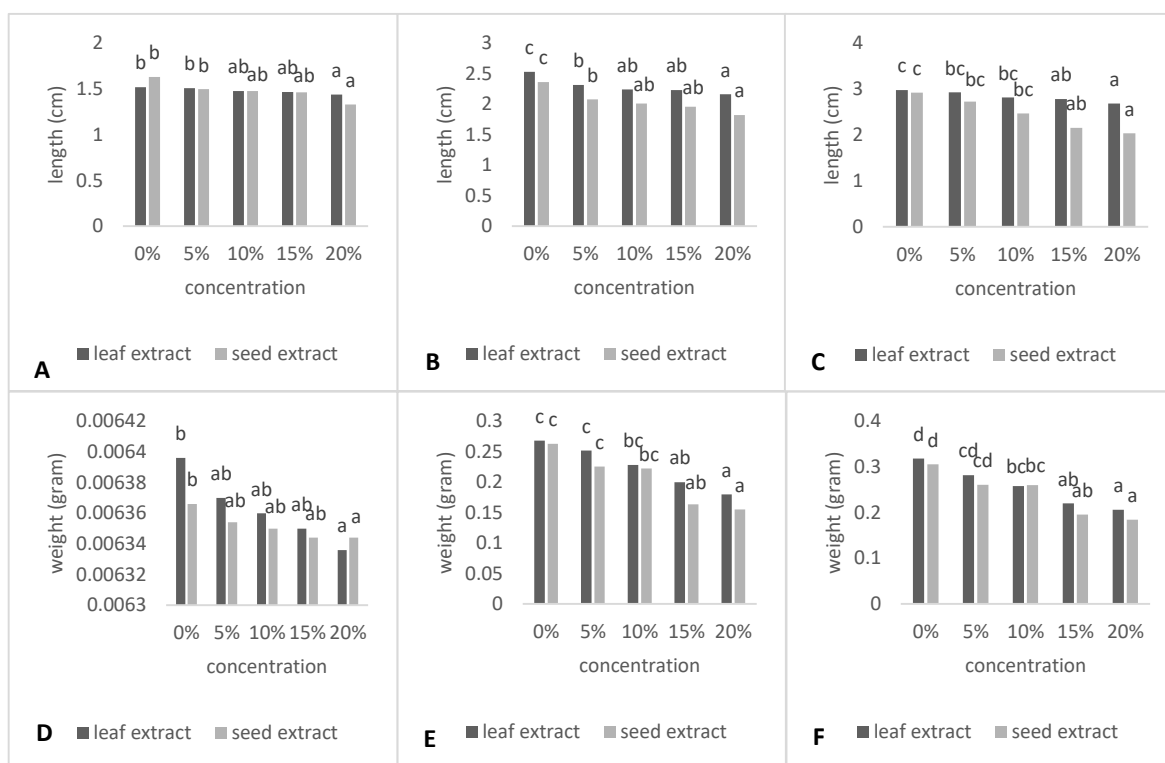


Figure 1. Mean length of *S. litura* on diet treated by moringa extract in 3rd instar (A), 4th instar (B), 5th instar (C); and mean weight of *S. litura* on diet treated by moringa extract in 3rd instar (D), 4th instar (E), 5th instar (F).

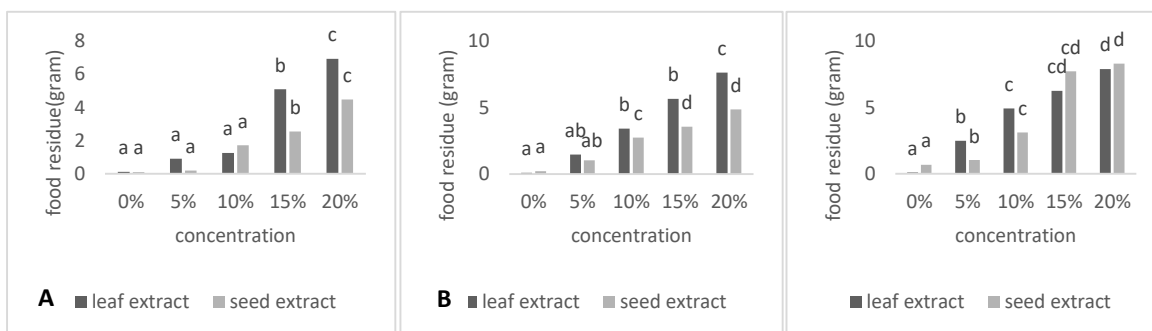


Figure 2. Mean food residue of *S. litura* on diet treated by moringa extract in 3rd instar (A), 4th instar (B), 5th instar (C)

These results indicate that alkaloids interfere with neuroendocrine control by inactivating acetylcholinesterase in larvae [19]. Alkaloids from some plants have a similar effect to blocking the acetylcholinesterase enzyme. This enzyme cannot work in the body to continue sending the signal to larval digestive [20]. Higher alkaloid content in Moringa seed extract caused weight loss in larval higher than larvae given Moringa leaf extract.

In addition to alkaloids, flavonoid content also affected the length and weight of larvae. Flavonoids affect molting, reproduction, feeding, and insect behavior, including cutworms [21]. Flavonoids in plants can reduce digestive ability or can even be toxic [22]. This is related to food residue of *S. litura* larvae (Fig. 2). Food intake in control larvae was higher than the treatment of both extracts. In Figure 3, it can be seen that the higher concentration of the extract reduced the food intake of larvae. Feeding with the addition

of leaf extract and seed extract caused a decrease in larval feed residue for each increase in concentration.

Alkaloid, flavonoids, and saponins give antifeedant effect in insects. Antifeedant compounds affect eating behaviour through the direct action of insect taste organs. The insect's reaction was refusing to eat or eating in small amounts. Increased feed residue at each increase in extract concentration because the food is not suitable and stops eating temporarily or permanently. This feeding inhibitory activity is related to the sensitivity of gustatory neurons and thus inhibits insect growth [27].

The activity of inhibiting eating by extracts of Moringa leaves and seeds is also shown by the results of the time to stop eating. In Figure 4, the time to stop eating of *S. litura* after feeding with the addition of Moringa leaf and seed extract increased.

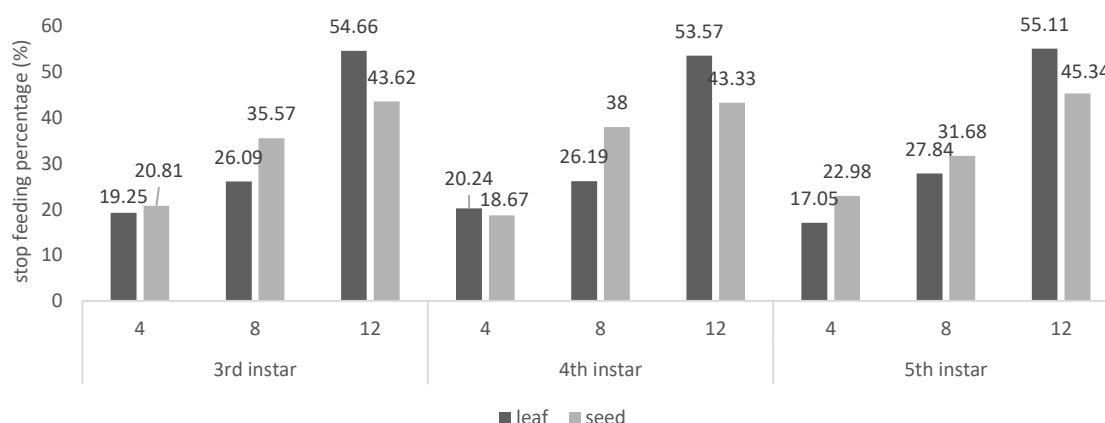


Figure 3. Percentage of stop feeding of *S. litura* on diet treated by moringa extract in 3rd instar (A), 4th instar (B), 5th instar (C) at 4 hours after application (HAA), 8 HAA, and 12 HAA

On the 3rd, 4th, and 5th instar, treatment of Moringa leaf and seeds extract caused the percentage of time to stop feeding at the 4th, 8th and 12th hours after application (HAA) increased. At 4, 8, and 12 HAA, the percentage of time to stop feeding with Moringa leaf extract treatment was higher than the seeds extract on the third and fifth instar. On the fourth instar, the percentage of time to stop eating was higher in Moringa seed extract treatment at 4 and 12 HAA. Leaf and seeds extract containing bioactive substances from secondary metabolites that inhibited larval activity, marked by slow motion, no response to the motion, and stop feeding [6].

The secondary metabolites also affect the duration of development of larvae. In Figure 4, it

can be seen that the larval development duration did not differ significantly in the development of the 4th instar larvae in both extracts and 5th instar with the of Moringa leaf extract treatment. Plant secondary metabolites can disturb development, lead to malformations or malfunctions, extend the duration of developmental stages or act as repellents [23]. Diet treatment on larval gives development time prolonged compared to control, although statistically not significantly different. The extent of development time is due to a disruption in the insect hormone development, namely brain hormone (BH), juvenile hormone (JH), and the ecdysone hormone. This disruption caused by saponin in the extracts.

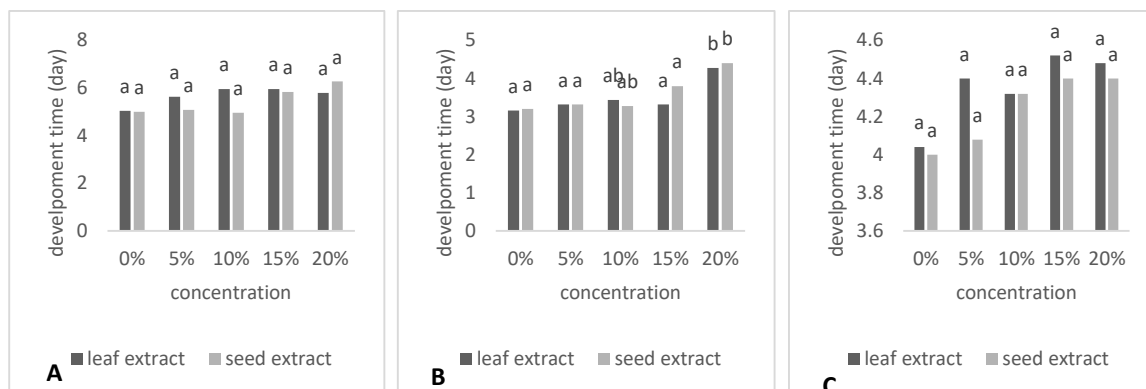


Figure 4. Mean development time of *S. litura* on diet treated by moringa extract in 3rd instar (A), 4th instar (B), 5th instar (C)

Saponins are terpenoid compounds. The activity of saponins in the insect's body is to bind free sterols in the digestive tract of food. Sterols are substances that function as precursors of the ecdysone hormone [25]. Larvae growth is affected by JH. As long as there is JH, the molting that occurs under the control of ecdysone hormone will only produce an immature stage of larval. If the concentration of JH is relatively higher compared to the ecdysone hormone, it

will stimulate the larvae and prevent the formation of pupae [28]. It was reported that the saponin content of *S. litura* growth resulted in prolongation of the development stage, including at the larval stage [26]. In addition to giving prolongation of the development stage, the secondary metabolites in Moringa leaf and seed also causing mortality to larval. Mortality is characterized by reduced in eating activity and passive movements.

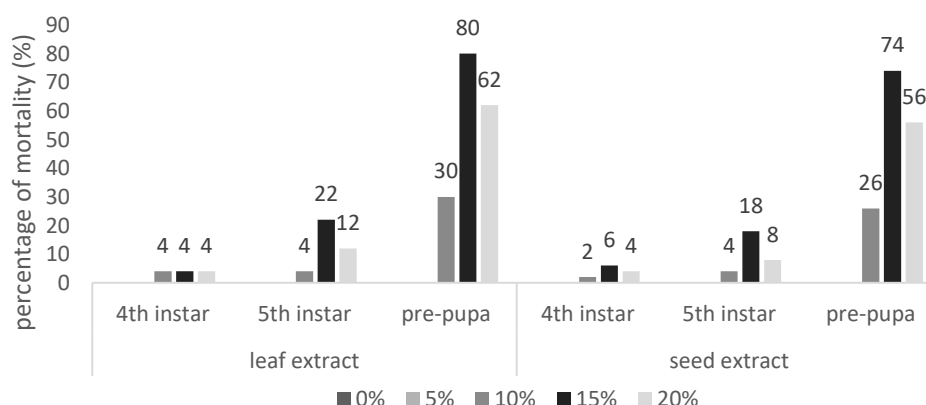


Figure 5. Percentage mortality of *S. litura* diet by moringa leaf and seed extract

Mortality began in 4th instar diet by 0%, 15%, and 20% concentration of extract (Fig. 5). The highest mortality occurred in 15% extract in pre-pupa phase. Mortality caused by quality and quantity of diet on stage larvae. The secondary metabolites in both extracts are toxic and the toxicity in larvae also increase due to feeding activities, the larvae were unable to neutralize toxins in the body. These toxic reduce metabolic activity resulting in mortality. The insect that is exposed to insecticides in lethal concentrations affected the physiological and behavioral

changes, resulting in stunted growth and failure in pupae formation [31].

CONCLUSION

The treatment of leaf extract and Moringa seeds affect the length shrinkage, lose weight of *S. litura* larvae. Both extracts also caused longer larval development in instar growth but reduce food intake. The number of larvae that consume feed that has been given extract also decreases with every increase in time. This antifeedant effect gives mortality to the larvae.

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