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EFFECT OF BELUNTAS LEAF (*Pluchea indica*) ETHANOL EXTRACT ON MORTALITY OF INSTAR II LARVAS (*Spodoptera litura* F.) ON LONG BEAN PLANT (*Vigna sinensis* L.)

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Abstract: Low long bean productivity is one of the impacts of *S. litura*. Farmers often rely on chemical fertilizers to control the population of *S. litura* so that this control method can leave residues in the growing media. Therefore, an effort to overcome the problem *of S. litura* is to use natural pesticides from beluntas leaves (*P. indica*), which produce secondary metabolites. This study aimed to determine the effect of ethanol extract of beluntas (*P. indica*) leaves on the mortality of second instar larvae of *S. litura* in long bean plants. This study used a completely randomized design (CRD) consisting of 5 concentrations, namely 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm, as well as control using distilled water with two repetitions. Descriptive statistics analyzed the research data. The results showed that the ethanol extract of beluntas leaves affected the mortality of the second instar larvae *S. litura* by 40.0% at a concentration of 250 ppm. Concentrations of 50 ppm and 100 ppm gave a mortality effect of 10.0%, a concentration of 150 ppm was 15.0%, and a concentration of 200 ppm was 35.0%. Thus it can be concluded that the higher the concentration of the ethanol extract of beluntas leaves, the higher the mortality of the second instar larvae of *S. litura*.

Keywords: *Beluntas, Larvae of S. litura, Instar II, Extract, Mortality*

INTRODUCTION

Local residents can utilize one of the home garden plants, the beluntas plant (*Pluchea indica*) [1]. Beluntas (*P.indica*) grow freely in dry conditions with a hard and rocky soil structure. However, some people also use beluntas leaves as a solution for eliminating body odor [2]. Beluntas leaves (*P.indica*) are known to have benefits as essential oils because they contain essential oils that provide carrying capacity in improving people's welfare [3]. Another potential of beluntas leaves is that they are useful as natural pesticides or biopesticides because they contain bioactive compounds such as alkaloids, alkenyl phenols, flavonoids, saponins, tannins, and terpenoids. Some of these active compounds are secondary metabolites, namely organic compounds that plants in smaller and limited concentrations produce and act as antimicrobial compounds [4-6].

S.litura is a type of pest that plays an important role and is found in horticulture, one of which is a long bean. This type of pest attacks long bean plants on the pods. The flower parts appear hollow due to the attack of the pod borer caterpillar [7]. These attacks have a direct effect on the productivity of long beans. The nature derived from *S. litura* is polyphagous, meaning that *S. litura* has a wide host range, so it has potential as a pest on several horticultural. *S. litura is* a pest with complete metamorphosis. *S. litura* has a life cycle from egg to imago with a time range of 30-60 days [8]. Leaves are the most dominant plant parts damaged by *S. litura* at the larval stage. If the pest attack is not controlled, it will indirectly reduce the productivity of long bean plants.

The results of observations on one of the heads of farmers in the Penanggak Hamlet, Midang Village, Gunungsari District, it was known that the pest that interfered with the long bean plant was the armyworm (*S.litura*) on the long bean fruit. Furthermore, it is said that the population of this pest is very large, and generally, to overcome the attack, farmer groups use Phonska brand of chemical fertilizer. Phonska fertilizer is a group of compound fertilizers containing nutrients with each concentration of 15%. The chemical fertilizer used by a number of farmers in Penanggak Hamlet is because the long bean seeds are hybrid seeds with a short lifespan. The rules for using fertilizer doses are 3 grams per plant in the first 3 days, then 10 grams on days 7 and 10 until a period of 1 month, still using a dose of 10 grams per plant. However, not all farmers use Phonska fertilizer because the fertilizer has a high price value.

The farmers outsmart the losses from their crops by focusing on the use of insecticides as an effort to overcome them. The research results by [9] show that the pesticides used by farmers in dealing with pests and weeds aim to obtain high agricultural products. On the other hand, the use of pesticides harms biodiversity. The use of broad-spectrum pesticides can kill target

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pests and kill non-target living things such as bees, pollinating insects, earthworms, and carrion insects [9]. Indications of excessive use of pesticides on vegetable agricultural commodities [10]. The behavior of tomato farmers shows that farmers use chemical pesticides for various reasons: (1) Farmers' perception states that the higher the quantity of non-biological pesticides used, the higher the risk, the lower the risk. Resistance of the cultivar structure of a plant; (2) The perception of the lower the knowledge and understanding of farmers, the higher the quantity of non-biological pesticides used [10].

Based on the description above, in the study to suppress the armyworm population *(S. litura)* , the beluntas plant *(P.indica) leaves were used* as vegetable insecticides. In addition to its use as a vegetable insecticide, many people still do not pay attention to other uses of the plant. They tend to prune and discard and even burn the leaves. Therefore, the authors took the initiative to test the toxicity of beluntas leaves on the mortality of armyworm *(S. litura)* to increase the productivity of long bean plants.

RESEARCH METHODS

The research was conducted at the Laboratory of Chemistry and Biology, Faculty of Teacher Training and Education, University of Mataram. The type of research is experimental research. The method for extracting beluntas leaves is maceration using 96% ethanol as solvent. The experimental design was a completely randomized design (CRD) using a control group and an experimental group. This study used the second instar larvae of armyworm *(S.litura)* as test animals. The concentrations used consisted of 5 different concentrations, namely 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm, with 100 ml of water at each concentration.

Implementation

Procedure The initial extraction procedure is to mix dried beluntas leaves that have been mashed using a blender in a ratio of 1:5 (100 grams dry weight of beluntas leaves: 500 ml of 96% ethanol solvent, then allowed to stand at room temperature for ± 72 hours (3 days) with occasional homogenized by stirring using a spatula. It was then filtered using filter paper until a residue-free filtrate solution was obtained. The filtrate solution is evaporated using *a rotary evaporator* at a temperature of 65°C to separate the solution from the solvent to produce beluntas extract containing secondary metabolites. The beluntas extract is allowed to stand at room temperature until the extract is obtained in the form of a paste and continued with dilution for each concentration using the following equation:

$$M_1 \times V_1 = M_2 \times V_2$$

Description:
M_1 : Initial concentration
M_2 : Initial volume
V_1 : Concentration end
V_2 : Final volume

The process of administering leaf ethanol extract beluntas on the second instar larvae of armyworms through the contamination method and the mortality rate of the larvae was observed. It can be analyzed using the equation according to Abbot [11] to determine this study's percentage value of mortality.

$$P_0 = r/n \times 100\%$$

Information:
P_0 : mortality Armyworm
r : Number of larvae mortality
n : Total number of larvae

Observation of the body: The second instar larvae of S. litura that experienced mortality were carried out under a stereo microscope that had previously been coated with millimeter block paper.

Tools

The tools used include a blender, knife, *rotary evaporator*, stereo microscope, 600 ml plastic jar, net, beaker, measuring flask, stirrer, 250 ml plastic bottle, scale, analytical balance, tweezers, and a glass funnel.

Materials

The materials used include 500 grams of beluntas leaves, armyworm instar II larvae (*S.litura*), 96% ethanol solvent, 10% honey solution, mustard leaves, aquades, gauze, filter paper, rubber, and the label paper, millimeter block.

RESULTS AND DISCUSSION

The Effect of Ethanol Extract of Beluntas Leaves on Mortality of Instar II Caterpillar Larvae (*S.litura*)

The results were obtained based on the administration of ethanol extract of beluntas leaves (*P.indica*) through the maceration method with different concentrations. The effect of giving ethanol extract of beluntas (*P.indica*) leaves on the mortality of second instar larvae of armyworm (*S.litura*) is presented in Table 1.

Table 1. Mortality of Larvae Instar II of Grayak Caterpillar (*S.litura*)

No	Concentration (ppm)	Number of Larvae	Mortality		Average	Percentage
			I	II		
1	0	10	0	0	0	0.0%
2	50	10	1	1	1	10.0 %
3	100	10	2	0	1	10.0%
4	150	10	3	0	1.5	15.0%
5	200	10	6	1	3.5	35.0%
6	250	10	3	5	4	40.0%

Table 1 shows that the highest mortality percentage is at a concentration of 250 ppm at 40.0%, while the lowest mortality percentage at a concentration of 50 ppm and 100 ppm is 10.0%. These results showed that the concentration of 250 ppm of beluntas leaf extract had the greatest effect on the mortality of the second instar larvae of *S.litura* compared to the ppm concentration below it. However, the 250 ppm concentration has not been able to cause complete mortality in *S. litura* larvae.

Mortality in *S. litura* occurred as a result of the administration of ethanol extract of beluntas leaves, which was toxic to the larvae. This activity is an effort that is needed in developing types of vegetable pesticides. The high mortality rate proves a plant material's effectiveness in eradicating larvae [12]. The presentation of the research results in Table 1 shows that the concentration of 250 ppm has the highest effect on the mortality of the second instar larvae of *S. litura* compared to other concentrations. A toxicity test of *P.indica* combined with *C.odora* larvae *Plutella xylostella* showed that high mortality was obtained from the concentration level of the extract, which was directly proportional to the number of active compounds [13]. Research [14] showed that the highest concentration of active compounds also resulted in the highest mortality because more toxins accumulated in the highest concentration resulted in high mortality.

Mortality in larvae can occur due to the influence of active compounds in plants, such as tannins, saponins, alkaloids, and flavonoids [5]. In addition to internal factors in the form of active compound content, it turns out that external factors, namely the environment, also contribute to the growth and development of larvae [12]. A good temperature for the growth and development of the larval and pupal phases is 20°C [15]. So it can be said that if the temperature of the environment where the larvae live is not supportive, it will impact the growth and development and even death of the larval life phase.

The ethanol extract of the leaves of beluntas (*P.indica*) worked as a stomach poison against the mortality of *S.litura* [12]. This mechanism of action damages the larvae's digestive system when the toxic active compound is ingested and gradually impacts the subsequent development of *S. litura*. Mortality at a concentration of <250 ppm is included in the low mortality rate because that concentration indicates the second instar larvae of *S. litura* can still develop until the next instar larval stage. At a concentration of <250 ppm, the behavior of the larvae, on average, showed behavior away from feed contaminated with ethanol extract of beluntas leaves (*P.indica*) by hiding under a layer of tissue and rolling their bodies, and their movements were less active and would be active when touched or external stimuli. The mortality of P. xylostella caterpillars *Antifeedant* showed that there were compounds that could increase the feeding power of *P. xylostella* without feeding inhibition *)* [12]. Tolerant insects will neutralize active compounds to become inactive through the metabolism of foreign compounds so that the larvae can adapt to exposure to active compounds [12]. The ability of larvae to adapt to the amount of compound concentration can help the larvae go to the next life phase, namely pupae.

Morphological Symptoms of Mortality of Larvae Instar II Grayak Caterpillar (*S.litura*)

leaves *P.indica* to contain active compounds in saponins, flavonoids, tannins, alkaloids, and essential oils [13]. A number of these active compounds have their respective functions in influencing the mortality *of S. litura*.

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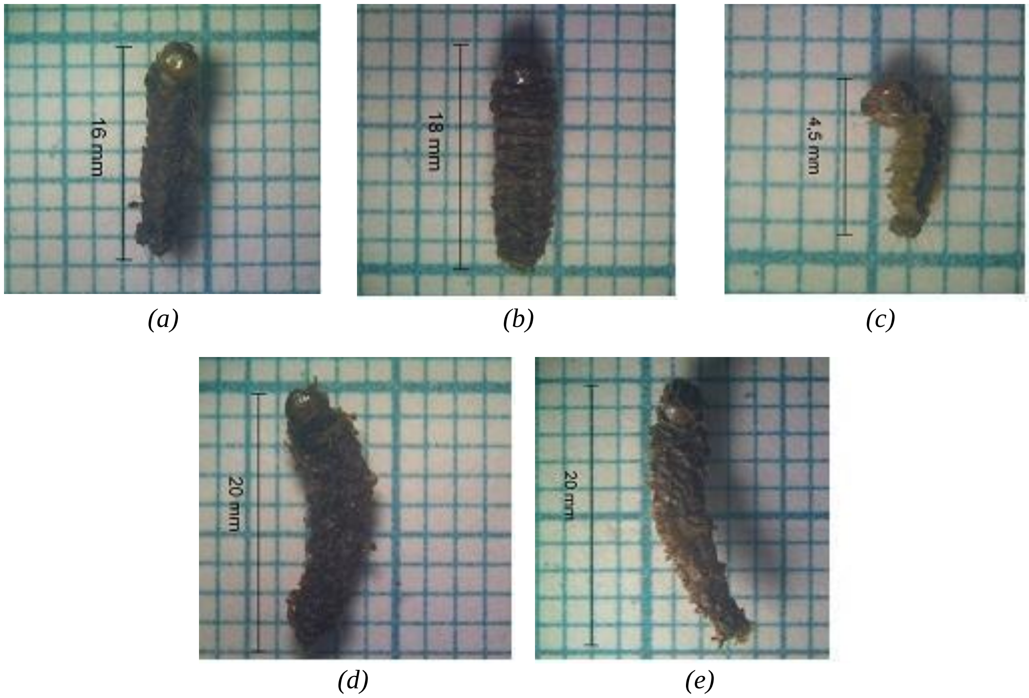

Figure 1. The effect of each concentration of beluntas leaf extract on the morphology of the second instar larvae of *S.litura* (a) 50 ppm; (b) 100 ppm; (c) 150 ppm; (d) 200 ppm; and (e) 250 ppm.

Morphological symptoms of second instar larvae of *S.litura* due to the administration of ethanol extract of beluntas leaves are a change in body color to blackish brown and body shape tends to bend (Figure 1). Changes in the larval body are influenced by tannin compounds that change and bind to insect epicuticle proteins until the tissue degenerates. Meanwhile, tannin compounds also provide an anti-feeding so that there is a decrease in feeding activity in *S. litura*. It impacts reducing the nutritional requirements for the growth and development of *S. litura*. The activity of the protease enzyme also decreased due to the tannin compound, so the process of protein synthesis and ATP formation could not occur, leading to the larvae's death. Similar morphological symptoms were found in the mortality of second instar larvae *kirinyuh* leaf ethanol extract [16]. The active compounds from the beluntas leaf extract were absorbed and elicited a response in the larvae causing death. The mechanism of action of the active compound predominantly acts as a stomach poison. It is because the larvae *S. litura* directly consume feed contaminated with the ethanol extract of the leaves of the beluntas, which is eaten from the mouth to the midgut [17].

In general, the symptoms of mortality in larvae are the same [18]. Physiological symptoms in second instar larvae of *S.litura* after administration of ethanol extract of beluntas leaves were the slow movement of the larvae and even did not move if not given external stimulation. Some of the larvae rolled their bodies and hid under tissue away from mustard feed contaminated with the extract. The process begins with the attachment of poison to the cuticle and penetrates the integument larva *S. litura* followed by physiological changes, especially reproduction [18]. This mechanism causes a decrease in the rate of metabolism and secretion of digestive enzymes. So the energy that should be needed for larval growth decreases.

Alkaloid compounds also act as *antifeedants* that can reduce larvae' appetite so that the larvae will die slowly. The same thing was also found in a study [19] using the extract of the fruit of the god's crown (*Phaleria papuana* Warb.) against symptoms of poisoning, which are characterized by lack of appetite and sluggish movements, and symptoms of death which are characterized by an unpleasant odor, producing mucus, and shrinking body parts.

The presence of tannin compounds causes the bitter taste of P.indica. This compound is an *antifeedant* against larvae. In addition, tannins also play a role in inhibiting the work of protease enzymes through the mechanism of protease enzyme inhibition, namely binding to proteins that enzymes will catalyze. Thus, the bond between the tannins and the protein causes disturbances in the digestive system of the larvae and eventually causes the death of the larvae. Tannins are toxic polyphenolic compounds which can disrupt the digestive system of larvae by binding to proteins, carbohydrates, vitamins, and minerals in the digestive tract of larvae [20].

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CONCLUSION

The low productivity of long bean (*Vigna sinensis* L.) is caused by the armyworm (*S.litura*), which attacks the pods. One of the efforts to overcome this problem is using natural pesticides, namely the ethanol extract of the leaves of beluntas (*P.indica*). The effect of the extract was shown through larval mortality. The results showed that using beluntas leaves as a natural pesticide affected the mortality of the second instar larvae of *S.litura* at a concentration of 250 ppm by 40.0%.

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