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Toxicity Test of Methanol Extractof *Rhinacanthus nasutus* L.Kurz. Leaf to *Spodoptera litura* F.

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Abstract: *Spodoptera litura F. is one of a pest insect species in cultivated plants, including the Brassica juncea L. The control management of this species could be done by using bioinsecticide. In this research, bioinsecticide tested for toxicity to Spodoptera litura F. are methanolic extract of Rhinacanthus nasutus L. Kurz concentrations of 1%, 2%, 4%, 8%, and 16%. This research used a complete randomized non-factorial design. The toxicity of this extract was observed for 24 hours period by counting the number of instar II larvae that dead after having treatment. The data was analyzed by using Analysis of Variance and LSD test. The result of statistical analysis showed that the methanol extract of Rhinacanthus nasutus L. Kurz leaf is a strong toxicant to instar II larvae of Spodoptera litura F.*

Keywords- *Bioinsecticide, Rhinacanthus nasutus, Spodoptera litura.*

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I. Introduction

Brassica juncea is avegetable crops that are simply cultivated in Indonesia. There are many people like and utilize it. In addition to get sufficient nutritional content for the needs of the human body ([1]) *Brassica juncea* is also useful for treating several kinds of diseases ([2]). The cultivation of this plant is often constrained by plant-disturbing organisms in the form of pest and disease. One of the frequent pest of *Brassica juncea* is *Spodoptera litura*. This species is one of insect pests that potentially attacks horticulture plant and vegetables in Indonesia ([3]). *Spodoptera litura* is pholyphagic and affects about 112 other plant species, such as Tobacco, soy, mustard greens, cabbage, peanuts, potato, chili,onion, and another vegetables plant([4]).

To control the pest, the farmers generally intensively use chemical insecticide. The effects of chemical insecticide usage such astriggering the symptoms of resistance, killing natural enemies, increasing number of chemical residue in plant, contaminating environment and disrupting health. Therefore, management control of *Spodoptera litura* needs a friendly environment safer way to control this species, including the utilization of natural enemy and the use of bio-insecticide vegetable ([5]).

Rhinacanthus nasutus is one of plant that produces natural insecticide. This plant is called *nagamalli* (Tamil) and is a nutritions crop widely spread and cultivated in Southern China, Taiwan, India and Thailand ([6]).

As a natural insecticide, the methanol extract of *Rhinacantus nasutus* leaf is a very effective substance for controlling pests. According to Siripong *et al.,* (2006) the methanol extract of *Rhinacanthus nasutus* contaeins flavonoids, steroids, terpenoids, antraquinone, lignins, naphthoquinone ,and naphtoquinon analog. *Rhinacantus nasutus* contains many active ingredients such as flavonoids, benzenoid, coumarine, anthraquinone, quinone, glycoside, triterpen, sterols, and lupeol ([7]). The methanol extract of *Rhinacanthus nasutus* leaf beside very potential to control *Aedes aegypti* and *Culex qiunquefasciatus* ([8]) the extract also use to medicates cancer ([9]).

This research use leaf extract of*Rhinacanthus nasutus* as a bio insecticide because the leaf of this species contains abundant secondary metabolites compared to its flower, stem, or root. Therefore it is necessary to do further research to test the potentiality of bio insecticides derived from extract of *Rhinacanthus nasutus* leaf to *Spodoptera litura* larvae by determining the concentration of the extract that may affect the mortality of *Spodoptera litura* larvae.

II. Methods

2.1 Location and Time

The research was done on July 29th to September 4th 2017 in FKIP University of Mataram start from drying the leaves until testing the extracts to *Spodoptera. litura*.larvae

2.2 **Material**

The material used in this research is instar II larvae of *Spodoptera litura*, leaf of *Rhinacanthus nasutus*, aquadest, methanol, leaf of *Brassica juncea*, and honey.

2.3 **Testing Method**

This research used non-factorial Random Complete Design. The factor was examined is the toxicity of *Rhinacanthus nasutus* leaf extract in six levels of concentration.

Treatment of *Rhinacanthus nasutus* extract consist of six levels concentration that is:

Treatment 1 using the extract of *Rhinacanthusnasutus* concentration of 0%
Treatment 2 using the extract of *Rhinacanthusnasutus* concentration of 1%
Treatment 3 using the extract of *Rhinacanthusnasutus* concentration of 2%
Treatment 4 using the extract of *Rhinacanthusnasutus* concentration of 4%
Treatment 5 using the extract of *Rhinacanthusnasutus* concentration of 8%
Treatment 6 using the extract of *Rhinacanthusnasutus* concentration of 16%

Each treatment used 10 instar II larvae repeated three times per each so that the number of samples in this study is 180 larvae.

2.4 **Research Timeline**

2.4.1 **Insect Breeding**

Insect breeding was done by collecting imago in area of rice fieldin Suralaga District and then acclimatized in the Laboratory using a plastic container. 10% of honey was provided as a daily food for the insect. After copulate the imago will put their eggs on gauze or at the wall of container. The eggs from the container is transferred into a petri dish for hatching. The larvae is transferred into other plastic box containing *Brassica juncea* leaves. The larvae continued to be nourished by feeding *Brassica juncea* leaves which were replaced daily until the instar II larvae.

2.4.2 **Preparing *Rhinacanthusnasutus* Leaf Extract**

To prepare methanol extract of *Rhinacanthu snasutus* leaf, 2000 grams leaf collected from near by of Masbagik, Lombok Timur washed with tap water and then washed again with aquadest.Leaf is cut into a small pieces and then dried at room temperature. After having dried, the leaves are crushed into smallest pieces by using a blender.500 grams of *Rhinacanthus nasutus* leaf so aked into methanol 1:10 (w / v) for 24 hours period ([10]). Filtering using Bunchner funnel is conducted after 24 hours soaking step.The results of extraction usingthe solvent is evaporated and concentrated with *freeze dryer* to produce coarse extract. Coarse extract is saved in refrigerator until used.

2.5 **Statistical Analysis**

The data is analyzed by using Analysis of Variance and LSD test.

III. Results

The effect of treatment on tested animals is showed in table 1. This table is showed that the increase of extract concentration is followed by the increase of tested animal mortality.

Table 1. Toxicity test of methanol extracts of *Rhinacanthus nasutus* leaf to *Spodoptera litura* larva in 24 hours period

Treatment %	Number Tested Animal	Number of Dead Animals Each Repeat			Total Dead Animals
		I	II	III	
1%	30	0	0	1	1
2%	30	2	3	3	8
4%	30	4	5	5	14
8%	30	7	8	6	21
16%	30	9	10	10	29

The results of the analysis of variance is presented in table 2. Result showed that the treatment has a very significant impact to tested animal mortality. A very significant impact of treatment showed from value of F_{count}t that is much more bigger than F_{table}. This condition means that the methanol extract of *Rhinacanthus nasutus* leaf is a strong toxicant to instar II *Spodoptera litura* larvae.

Table 2: Analysis of variance methanol extract of *Rhinacanthus nasutus* leaf to *Spodoptera litura* in *Brassica juncea*

The source of diversity	Df	SS	MS	F_{count}	F_{tab}	
					95%	99%
Between group	4	156,067	39,767	85.214	3.48	5.99
Error	10	4,667	0.467			
Total	14	163,733				

Table 3: LSD test of methanol extract of *Rhinacanthus nasutus* leaf to *Spodoptera litura* larvae

Treatment	The mean of Total Dead Larvae	Notation	Mortality
1%	0.333	1.576240251	3.34 [a]
2%	2,666	3,909240251	26.67 [b]
4%	4,666	5.909240251	46.67 [c]
8%	7	8.243240251	70 [d]
16%	9,666	10,90924025	96.67 [e]

Description: The numbers followed by the same letters are not significantly different from the α 0.05 LSD
The result of LSD test as seen in table 3 shows that each treatment gives a different effect to tested animal mortality.

IV. Discussion

Result shows that the highest tested animal mortality is 96.67% occurred after having treatment with a highest exract concentration whereas the lowest mortality is 3.34% occured in a lowest extract concentration treatment. Table 1 shows that the higher concentration is the higher the mortality of the tested animals. This condition could be related to the level secondary metabolite content wich is rise by rising the extract percentage.

The observation conducting after treatment showed that *Spodoptera litura* changes in some parameter. After having treatment lomotion of larva become slower, body size become smaller, and green color of the body become dark brown and finally died. The similar result is also obtained by Herminanto and Sumarsono ([11]).

According to Siripong *et al.,* ([6]) the methanol extract of *Rhinaacanthus nasutus* leaves contain secondary metabolites such as flavonoids, steroids, terpenoids, antraquinone, lignin and analog naphthoquinone. Those metabolites have impact on both mortality and feeding activity of *Spodoptera litura* larva.

According to Reddy *et al.,* ([12]) insects will face two things to initiate feeding activityi.e, 1) *feeding stimulant* and 2) the detection of the presence of foreign substances *(foreign compound)* which is can inhibit the feeding activity. Foreign compound will drive feeding activity shorten or terminated.

According to Budianto and Tukiran, ([13]) the compounds which is likely to play a role in decreasing animal feeding activity is terpenoids. Rotenon which is one of the content of extract of *Rhinacanthus nasutus* leaf is a phenolic,a group of flavonoid which isacts as a toxicant in inhibiting the metabolism and nervous system. This toxicant works slowly. Insects die of starvation due to paralysis of the mouth ([14]). Flavonoid compounds can also reduce the ability of insects in digesting food, to reduce protease and amylase activity therefore. This condition disturbing insect growth ([15]).

Triterpenoid is one of *antifeedant* compounds because the bitter taste of the substance will drive insect refuse to eat. The presence of these compound working by stimulating chemoreceptor and then affect nervous system to drive insect to terminate their feeding activity ([13]).

V. Conclusion

The methanol extract of *Rhinacanthus nasutus* leaf is a strong toxicantto instar II *Spodoptera litura* larva.

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