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Activity of *Nerium oleander* leaf extract on the larvae of *Crocidolomia binotalis* Zell.

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Abstract

The active compound of *Nerium oleander* L. leaf extract is toxic to insect larvae. It is used to control pests but information on the activity of *N. oleander* leaf extract was not yet known in detail. This research aimed to determine mortality, lethal concentration (LC_{50}), feeding inhibition, larval and pupal fitness weights and imago morphological perfection of *Crocidolomia binotalis* Zell. The research was done at the Laboratory of Pests and Plant Diseases, Faculty of Agriculture, Tadulako University from May 2021 to October 2021. This research used a completely randomized design with five treatments and four replications. Each treatment used 20 tails sample of third instar *C. binotalis* larvae from East Lore Sub-district, Poso Regency. Concentrations for the treatment of *N. oleander* leaf extract were as follows: P0 = 0%, P1 = 1.25%, P2 = 2.5%, P3 = 5%, and P4 = 10%. The results show that the concentrations of *N. oleander* leaf extract that were effective in causing mortality of *C. binotalis* larvae, were from 1.25% to 2.5%. The LC_{50} concentration of *N. oleander* leaf extract, which killed 50% of *C. binotalis* larvae, was 1.915% (0.01915ml/ml), with a minimum concentration of 1.425% (0.01425ml/ml) and a maximum concentration of 2.375% (0.02375ml/ml). The higher the concentration of *N. oleander* leaf extract, the higher the feeding inhibition, causing the decrease in the feeding of *C. binotalis* larvae. In addition, the fitness weights of *C. binotalis* larvae and pupae also decreased, so that fewer larvae developed into imago (adult individuals). A 10% concentration of *N. oleander* leaf extract caused abnormal (rudimentary) morphological forms of *C. binotalis* imago.

Keywords: *N. oleander, C. binotalis*, Lethal concentration (LC_{50}).
Abbreviations: CRD_Completely randomized design; HSD_Honestly significant difference; LC_{50}_Lethal concentration; PPOs_Plant pest organisms; WHO_World Health Organization.

Introduction

Cabbage (*Brassica oleraceae* L.) is widely cultivated by farmers in highland areas of Indonesia (Capinera, 2020). Data obtained from the Central Statistics Agency of the Republic of Indonesia (2020) show that Central Sulawesi cabbage production in 2018 was 14,153 tons, higher than cabbage production in 2019 of 13,064 tons and 2020 of 13,119 tons. This decrease in production was caused by the attack of plant pest organisms (PPOs) which inhibited the production of agricultural products (Lengai et al., 2020). One of the PPOs that attack cabbage plants is *Crocidolomia binotalis* Zell. (Lepidoptera: Pyralidae) which can damage cabbage plants and cause crop failure (Ervinatun et al., 2018).

In efforts to keep up food security, humankind must compete with PPO such as pests, weeds, and microorganisms (Irfan, 2016). This calls a threshold where pest and disease management are needed (Yorobe et al., 2011). The use of chemical pesticides is a method of managing pests and diseases that has been carried out for many years (Mugiastuti et al., 2018). Every year around 2.5 million tons of pesticides are used in agricultural areas, causing worldwide losses of up to $100 billion (Isman, 2020). The World Health Organization (WHO) stated that every year the use of pesticides increases by 10%, and there were 1 to 5 million cases of pesticide poisoning in farmers, most of which (80%) occurred in developing countries (Sari et al., 2016).

Integrated pest control technology is important to meet food safety needs (Setiawati et al., 2016). Pest control using synthetic pesticides must follow the concept of integrated pest control (Fauzana et al., 2019). The continuous and non-selective use of synthetic pesticides could result in several types of PPO becoming immune

Table 1. Percentage of larval mortality of *C. binotalis.*

Treatment	Mortality
P0 (Control)	0.00 (0.71)a
P1 (1.25%)	40.00 (6.36)b
P2 (2.5%)	57.50 (7.61)c
P3 (5%)	70.00 (8.39)d
P4 (10%)	92.50 (9.64)e
HSD	0.61%

Numbers followed by different letters in the same column are significantly different according to the HSD follow-up test at 5% level; the number in brackets is the transformation $\sqrt{x} + 0.5$.

as well as the destruction of natural enemies (parasitoids and predators) and several other types of beneficial insects (Arifin, 2012).

The use of vegetable insecticides is compatible with the Integrated Pest Control system (Untung, 2013) and could overcome the dilemma of using synthetic pesticides (Nicoletti, 2020). Certain plant extracts could be used as vegetable pesticides (Huang et al., 2020). Vegetable pesticides are insecticidal ingredients that are obtained naturally from several plant parts such as roots, stems, leaves, seeds, and so on (Heviyanti and Rusdy, 2016). The use of vegetable pesticides as pest control could be an alternative to chemical pest control (Zowada et al., 2020).

Butter flower (*Nerium oleander* L.) is a plant that is commonly found in several Mediterranean areas and is widely cultivated as an ornamental plant in tropical and subtropical areas. This plant belongs to the Apocynaceae family (Akal and Matrood, 2020). The leaves of *N. oleander* contain oleandrin, which is toxic (Fakoorziba et al., 2015). Oleandrin in this plant is useful as an insecticide as well as an antifeedant (Senthilkumar et al., 2020) and has been used as an effective poison against agricultural pests and rodents (Sivakumar et al., 2020). However, detailed information regarding the use of this plant as a pest control of *C. binotalis* in cabbage plants was not clear. Several studies about *N. oleander* only discussed larvicidal activity (El-Akhal et al., 2015), subacute toxicity (Abdou et al., 2019), and effectiveness (Nasir et al., 2021). So, it was necessary to research the activity of *N. oleander* leaf extract on *C. binotalis* larvae.

Results

Larvae mortality of C. binotalis

The results of observations of mortality of *C. binotalis* larvae after application of *N. oleander* leaf extract are presented in Table 1.

The observation results of mortality of *C. binotalis* larvae in Table 1 show that in the control treatment (P0), the mortality percentage was 0%; in the P1 treatment, the mortality percentage was 40%; in the P2 treatment, the mortality percentage was 57.50%; in the P3 treatment, the mortality of test larvae increased until it reached a mortality percentage of 70.00%; and treatment P4 was the treatment with the highest mortality percentage, namely 92.50%. The higher the concentration of *N. oleander* leaf extract, the higher the mortality percentage

of *C. binotalis* larvae. The results of the Tukey's HSD (honestly significant difference) test at 5% level, mortality of *N. oleander* leaf extract against *C. binotalis* larvae show that the control treatment of *N. oleander* leaf extract was significantly different from all treatments. Likewise, differences between the treatments were significantly different.

LC_{50} (lethal concentration) test

The results of the LC_{50} test of the concentration of *N. oleander* leaf extract on *C. binotalis* larvae are presented in Table 2.

The LC_{50} test results in Table 2 show that the LC_{50} concentration of the *N. oleander* leaf extract was 1.915%. The concentration of this *N. oleander* leaf extract could kill 50% of the *C. binotalis* test larvae with a minimum concentration range of 1.425% to a maximum concentration of 2.375% ml.

Feeding inhibition

The percentage observations of feeding inhibition of *N. oleander* leaf extract against *C. binotalis* larvae are presented in Figure 1.

The results of the feeding inhibition observations of *N. oleander* leaf extract against *C. binotalis* larvae show that the higher the concentration of *N. oleander* leaf extract, the higher the inhibitory power of *N. oleander* leaf extract on the feeding of *C. binotalis* larvae. Increasing the concentration of *N. oleander* leaf extract caused the feeding ability of *C. binotalis* larvae to decrease.

Fitness weights of C. binotalis larvae and pupae

The results of the average fitness weights of *C. binotalis* larvae and pupae after application of *N. oleander* leaf extract are presented in Figure 2.

Figure 2 shows that the higher the concentration of *N. oleander* leaf extract, the lower the weights of larvae and pupae. The control (P0) treatment of *N. oleander* leaf extract resulted in the highest larval and pupal weights. The treatment with 10% (P4) *N. oleander* leaf extract resulted in the lowest larval and pupal weights.

Table 2. LC_{50} test of concentration of *N. oleander* leaf extract on *C. binotalis* larvae.

Concentration (%l)	Replication	Total Larvae	Number of Dead Larvae	Average	Mortality	LC_{50} (ml)		
						Avg	Min	Max
0	0	20	0	0	0			
	1	20	8					
1.25	2	20	8	0.40	40.00			
	3	20	8					
	4	20	8					
	1	20	10					
2.5	2	20	12	0.58	57.50			
	3	20	11					
	4	20	13			1.915%	1.425%	2.375%
	1	20	14					
5	2	20	14	0.70	70.00			
	3	20	15					
	4	20	13					
	1	20	17					
10	2	20	17	0.93	92.50			
	3	20	20					
	4	20	20					

Lethal concentration 50 (LC_{50}) is the concentration in ml needed to kill 50% of *C. binotalis* larvae

Larvae that became imago and morphological perfection in forms of C. binotalis imago

The results of observing the number of larvae that could develop into imago and the morphological perfection in the forms of *C. binotalis* imago after application of *N. oleander* leaf extract are presented in Table 3.

Table 3 shows that the higher the concentration of *N. oleander* leaf extract the more larvae of *C. binotalis* died and the number of larvae developing into imago decreased. The results of observations of the morphological perfection in form of *C. binotalis* imago show that those in the treatments P0, P1, P2, and P3 formed perfect imago morphology. Meanwhile, in the P4 treatment, the pupae that became imago, formed an imperfect morphology with a rudimentary wing shape.

Discussion

The treatment by *N. oleander* leaf extract affected the mortality percentage of *C. binotalis* larvae. The higher the concentration of *N. oleander* leaf extract, the higher the mortality percentage of *C. binotalis* larvae. This happened because the *N. oleander* plant contains the toxic active compound of oleandrin (Fakoorziba et al., 2015), which could cause mortality in *C. binotalis* larvae. This follows the statement that the toxic substance of oleandrin in the leaf extract of *N. oleander* is useful as an insecticide (Senthilkumar et al., 2020).

Oleandrin is an active ingredient in the form of secondary metabolite compounds that plays an important role in protecting plants from disturbances of plant pest organisms and can be used as active ingredients for vegetable pesticides (Nicoletti, 2020). The active compound oleandrin contained in the *N. oleander* leaf extract works as a stomach poison, enters the body and is digested in the middle channel, which is then circulated with the blood (Goktas et al., 2007). Oleandrin accumulated in the insect body acts as a toxicant and is distributed throughout the body's cells through the insect's blood circulation (hemolymph), which results in disruption of the entire circulation in the body. Enzyme secretion is also disrupted, so the digestive process of food is disrupted, and the larvae will lack energy until finally death occurs (Ningsih et al., 2013). Blood-borne toxins affect the nervous systems of larvae and are effective in causing death (Azwana et al., 2019).

With a higher concentration of *N. oleander* leaf extract given, the level of stomach poison that enters the larva's body would increase, resulting in the death of the larvae (Nasir et al., 2021). The large concentration of toxic substances that entered the body and caused death in insect larvae needs to be safe for humans and the environment. The concentration of the toxic substance of oleandrin contained in the *N. oleander* leaf extract, safe for humans and the environment, was 1.915% (0.01915ml/ml).

Table 3. Number of larvae that became imago and morphological perfection in the forms of *C. binotalis* imago (adult individuals).

Treatment	Dead larvae (tail)	Larvae that became imago (tail)	Larvae that became imago (%)	Imago
P0	0	80	100	 Perfect
P1	32	48	60	 Perfect
P2	46	34	42.5	 Perfect
P3	56	24	30	 Perfect
P4	74	6	7.5	 Not Perfect (Rudimentary)

This concentration could kill 50% of *C. binotalis* larvae.

The results of the feeding inhibition observation of *N. oleander* leaf extract against *C. binotalis* larvae are shown in Figure 2, showing that the control treatment (P0) has a percentage of feeding inhibition of 0%. The average feeding inhibition of *N. oleander* leaf extract increased depending on the concentration of *N. oleander* leaf extract. The higher the concentration of *N. oleander* leaf extract, the higher the feeding inhibition of *C. binotalis* larvae. The concentration of *N. oleander* leaf extract in treatment P4 was the highest concentration and the best for inhibiting the feeding activity of *C. binotalis* larvae, reaching an average of 55.13%. This was because the active compound content of *N. oleander* leaves, consisting of glycosides in the form of oleandrin compounds, works as an antifeedant (Senthilkumar et al., 2020). According to Goktas et al. (2007), the nature of *N. oleander* leaf extract as a feeding inhibitor (antifeedant) caused the larvae to lose their appetite so that the body shrank and shortened.

The results of fitness weight observations of *C. binotalis* larvae and pupae in Figure 2 show that the higher concentration of *N. oleander* leaf extract treatment caused the average fitness weights of *C. binotalis* larvae and pupae to be lower. The average fitness weight of the *C. binotalis* larvae and pupae in the treatment of *N. oleander* leaf extract of control (P0) with a concentration of 0% had the highest weight, namely larvae of 0.05 g and pupae of 0.049 g. Furthermore, in the highest *N. oleander* leaf extract concentration of 10%, the average fitness weights of *C. binotalis* larvae and pupae had the lowest weights, namely larvae of 0.020 g and pupae of 0.027 g. This shows that the higher the concentration of *N. oleander* leaf extract applied, the higher the amount of oleandrin toxic substance, which caused the average weight of *C. binotalis* larvae and pupae after eating cabbage leaves with *N. oleander* leaf extract to decrease. According to Goktas et al. (2007), the increasing concentration of *N. oleander* leaf extract has caused decreasing appetite and the body shrank and consequently loss of bodyweight. The toxic substance of oleandrin contained in the *N. oleander* leaf extract reduced the appetite of the larvae to give a similar effect to a decrease in larval weight on survival and insect pupal weight, so that pupal weight also decreased (Sivakumar et al., 2020).

The observation results of the number of larvae that developed into imago and the morphological perfection of *C. binotalis* imago in Table 3 show that in the control treatment (P0) without *N. oleander* leaf extract, all *C.*

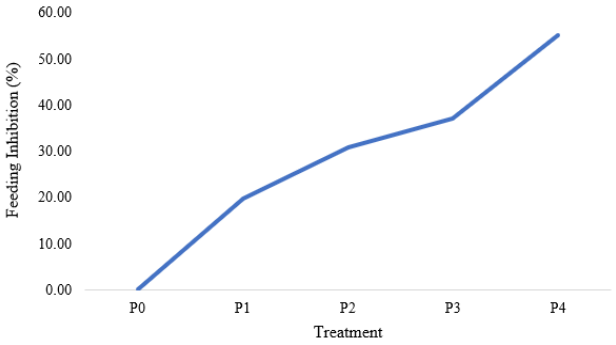

Figure 1. Inhibition by *N. oleander* leaf extract on feeding activities of *C. binotalis* larvae

binotalis larvae developed into imago. In the treatment containing oleandrin, the substance in the *N. oleander* leaf extract, the number of larvae that developed into imago decreased. These results show that the higher the concentration of *N. oleander* leaf extract, the smaller the percentage of larvae that could develop into imago. The observation results of the morphological perfection in form of *C. binotalis* imago show that larvae in treatments of P0, P1, P2 and P3 had perfect imago morphology, while in the P4 treatment, they developed an imperfect imago morphology with a rudimentary wing shape. According to Hasanah et al. (2020), imago wings that are not perfect, do not develop, and do not function properly which also called rudimentary. *Nerium oleander* leaf extract could cause changes from larvae to imago because it could increase the number of pupae deaths prior to the emergence of imago. *Nerium oleander* leaf extract could also cause nutritional deficiencies and result in abnormal morphological forms (Sivakumar et al., 2020).

Materials and methods

Place and time

The research was done at the Laboratory of Pests and Plant Diseases, Faculty of Agriculture, Tadulako University from May 2021 to October 2021.

The research sample of *C. binotalis* was taken from a cabbage plantation in Maholo Village, East Lore Sub-district, Poso Regency.

Research design

This study used a completely randomized design (CRD) with five treatments and four replications, and each treatment used third-instar *C. binotalis* larvae samples totaling 20 tails. The concentration treatments of *N. oleander* leaf extract used were as follows: P0 = Control, P1 = *N. oleander* leaf extract (1.25%), P2 = *N. oleander* leaf extract (2.5%), P3 = *N. oleander* leaf extract (5%), and P4 = *N. oleander* leaf extract (10%).

Construction of N. oleander leaf extract

The procedure for making *N. oleander* leaf extract began with taking butter flower leaves, chopping them into small pieces, and then drying using an oven for approximately 24 hours. After being dried, the butter flower leaves were blended until smooth (as much as 250 g). Furthermore, leaves were soaked using methanol for 2 × 24 hours. The function of methanol itself is as a binder of the active ingredients contained in butter flower leaves. The bath was then filtered using a Buchner funnel lined with filter paper. The filtration results were then evaporated using a rotary evaporator at low pressure to obtain a concentrated extract in the form of a paste, which was then diluted using water (Rimijuna et al., 2017).

The making of treatment concentration solutions was done through dilution using the dilution formula (Sadewo, 2015): $M1 \cdot V1 = M2 \cdot V2$ where M1 = initial concentration (%), M2 = final concentration (%), V1 = initial volume (ml), and V2 = final volume (ml). With a final volume of 50 ml using water as a solvent, the initial volumes of concentrated *N. oleander* leaf extract for each treatment were 0, 2.5, 5, 10, and 20 ml.

Crocidolomia binotalis test larvae propagation

Samples of *C. binotalis* larvae were taken from a cabbage plant garden in Lore Timur sub-district, Poso Regency, and reared and propagated at the Laboratory of Pests and Plant Diseases, Faculty of Agriculture, Tadulako University using cabbage plants that were cultivated and then covered. The imago of *C. binotalis* were added until they bred and laid eggs. The eggs that hatched into larvae were reared until they became third instar larvae, which were used as test larvae.

Application technique

The test was done using the leaf immersion method (Leaf Sandwich). *Crocodolomia binotalis* larvae that had reached the healthy third instar were put into the test jar and fasted for ± 3 hours. Cabbage leaf feed measuring 3 cm × 3 cm was soaked at each concentration for 3 to 5 minutes and then dried at room temperature for ± 10 minutes. Cabbage leaves that had been treated and dried were weighed and put in a test jar (as much as one sheet), and then one third-instar larva of *C. binotalis* was added and left for 24 hours. After 24 hours, the remaining cabbage leaf feed was taken carefully and weighed. Then the *C. binotalis* test larvae were fed with fresh cabbage leaves of the same size, which had been weighed (as much as one sheet), and left for 24 hours, and so on, until the larvae became pupae (Prijono, 1999).

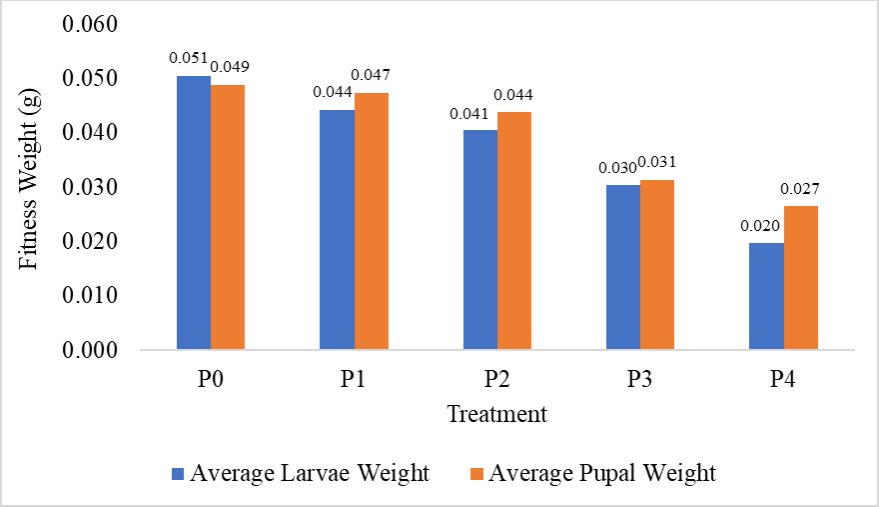

Figure 2. Average fitness weights of *C. binotalis* larvae and pupae.

Observation variables

***Nerium oleander* mortality**

Observation of the concentration of *N. oleander* leaf extract was done by observing the mortality amount of *C. binotalis* larvae exposed to the concentration treatment of *N. oleander* leaf extract. The percentage of larvae mortality was calculated using the following equation (Prijono, 1999):

$$P0 = \frac{r}{n} \times 100\%$$

where P0 = larval mortality, r = number of dead larvae, and n = total number of larvae.

Toxicity of LC50 of N. oleander

Observation of the toxicity of the concentration of *N. oleander* leaf extract was done using the lethal concentration test (LC_{50}) to determine the lethal concentration that could cause the death of 50% of *C. binotalis* larvae based on the probit analysis procedure.

Feeding inhibition

The test of feeding inhibition of *N. oleander* leaf extract on *C. binotalis* test larvae was done to determine the inhibitory power of *N. oleander* leaf extract on *C. binotalis* larval feeding power, which was observed based on the remaining weight of the leaves eaten and calculated by the following formula (Hariri, 2012):

$$PM = \frac{(Bk-Bp)}{(Bk+Bp)} \times 100\%$$

where PM is the food inhibitor (%), Bk is the weight of control leaves eaten, and Bp is the weight of treatment leaves eaten.

Fitness weights of C. binotalis larvae and pupae

The observation of fitness weight of *C. binotalis* larvae and pupae was done after application of *N. oleander* leaf extract concentration treatments by weighing the *C. binotalis* larvae on the fifth day after application and then weighing pupae the day after larvae became pupae.

Larvae that became imago and the morphological perfection in the forms of C. binotalis imago

Observation of the number of larvae that metamorphosed into imago and the morphological perfection in the form of imago was done after the application of *N. oleander* leaf extract treatment.

Data analysis

The research data were analyzed using analysis of variance (ANOVA), and if the treatment had a significant effect, then it was tested post hoc with the 5% level honestly significant difference (HSD) test. The lethal concentration test was analyzed by probit analysis using SPSS 23.

Conclusion

Concentrations of 1.25% and 2.5% *N. oleander* leaf extract were effective in causing mortality of *C. binotalis* larvae. The LC_{50} of *N. oleander* leaf extract on *C. binotalis* larvae was 1.915% (0.01915ml/ml) with a minimum concentration of 1.425% (0.01425ml/ml) and a maximum concentration of 2.375% (0.02375ml/ml). *Nerium oleander* leaf extract with a concentration of 20 ml inhibited the feeding activity of *C. binotalis* larvae up to 55.13%. The higher the concentration of *N. oleander* leaf extract, the lower the fitness weights of *C. binotalis* larvae and pupae. The higher concentrations of *N. oleander* leaf extract caused the percentage of the number of larvae that developed into imago to be smaller, and the highest *N. oleander* leaf extract concentration resulted in imago morphology becoming abnormal. Further research is needed in the field to determine the effectiveness of the use of *N. oleander*

leaf extract in suppressing *C. binotalis* pests attack, especially in cabbage plantations.

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