

Effectiveness of tuba root extract (*Derris elliptica* L.) against antifeedant of *Crociodolomia binotalis* caterpillar on mustard plant (*Brassica juncea* L.)

Alfrits Komansilan¹, Ni Wayan Suriani², Helen Joan Lawalata³

¹ Department of Physics, Faculty of Mathematics and Natural Sciences, Manado State University, North Sulawesi, Indonesia

² Department of Chemistry, Faculty of Mathematics and Natural Sciences, Manado State University, North Sulawesi, Indonesia

³ Department of Biology, Faculty of Mathematics and Natural Sciences, Manado State University, North Sulawesi, Indonesia

*Email: alfritskomansilan@gmail.com

Abstract— The application of tuba root bioactive extract (*Derris elliptica* L.) as a natural insecticide on the *Crociodolomia binotalis* caterpillar on mustard plants (*Brassica juncea* L.) was carried out. The test results showed that there were significant differences in antifeedant activity of the *Crociodolomia binotalis* caterpillar on mustard (*Brassica juncea* L.) plants at various concentrations. The research was carried out in several stages, starting from the tuba root extraction stage, phytochemical testing, preparation of caterpillar tests, testing of antifeedant activity. As the treatment is the level of methanol concentration of 50 ppm, 100 ppm, 500 ppm and 1000 ppm. The parameter observed was the percentage of Feeding Reduction (FR). The test results showed that the best antifeedant activity of *Crociodolomia binotalis* was at concentrations of 500 ppm and 1000 ppm because it was able to inhibit feeding power or Feeding Reduction of test caterpillars by 15.35% and 32.33%, was able to inhibit the feeding activity of *Crociodolomia binotalis*.

Keywords— *Derris elliptica* root, antifeedant, Natural Insecticide, Mustard, Feeding Reduction.

I. INTRODUCTION

Tuba root plant is a type of plant commonly used as fish poison. Plant roots tuba potential as biopesticide is in addition found in almost all regions in Indonesia also found in Africa, Southeast Asia and some islands in the Pacific (Novian, 2004).

The use of insecticides unwisely will have a bad influence on the environment and public health, especially farmers. Farmers in general overcome pest caterpillars by using synthetic chemical pesticides. In terms of pest population suppression, the results of chemical control with pesticides are quickly felt, especially in large areas. Until now, pest control of mustard greens which is commonly done by farmers is chemically using synthetic pesticides. Soewadi (2002) suggested that the application of synthetic chemical pesticides which are not wise and not in accordance with Integrated Pest Management (IPM) can have various negative impacts such as the occurrence of pest resistance, the emergence of secondary pests, the killing of non-target organisms, the presence of insecticide residues on food ingredients, pollution environment, and dangerous for consumers. As an alternative,

the use of plant material to be used as a vegetable pesticide is now being developed.

To get an effective, efficient and safe insecticide, it is necessary to have a comprehensive and directed study so that a formulation that is ready to be used by agricultural actors will be produced. Making a simple plant insecticide formulation is expected to be the forerunner to the development of an environmentally friendly plant-based insecticide industry on a large scale and will be able to compete with insecticidal formulations made from synthetic active conditions provided that the plant-based insecticides have efficacy and competitive prices, practical in use, and the most important is safe for human health users. Plants that have been isolated by researchers contain active compounds of plant-based insecticides on *Aedes aegypti* mosquito larvae are soursop seeds (*Annona muricata*) with Lethal Concentration $LC_{50} = 117.27$ ppm (Komansilan *et al.* 2012), and Hutun seeds (*Barringtonia asiatica* Kurz) with Lethal Concentration $LC_{50} = 35.72$ ppm (Komansilan and Suriani. 2016).

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found in almost all regions in Indonesia also found in Africa, Southeast Asia and some islands in the Pacific (Novian, 2004).

Crocidolomia binotalis is an important pest in cabbage plants. This caterpillar attacks plants from the *Brassica* family such as mustard greens, radish cabbage and radish, these pests attack mainly on the inside of the plant until it reaches the point of growth (Pracaya, 1999; Kalshoven, 1981). Control measures that can be applied to these pests are regulation of cropping patterns, farming techniques, utilization of natural enemies, physical, mechanical control and use of vegetable pesticides. The use of tuba root extract as a vegetable pesticide to kill insect pests has not been widely reported, therefore it needs to be studied and examined how the effect of extracts from the plant *Derris elliptica* L. on *Crocidolomia binotalis* which is one of the important pests in mustard plants.

Antifeedant is a substance that can stop eating insects or other animals permanently or temporarily depending on the strength of the substance (Garson, 2010). The potential of antifeedant substances has long been known, because it has become one of the alternatives in food crop protection.

The process of food crop production is often hampered due to insect pests that cause crop failure. The development and spread of insect pests that disturb agricultural crops, now require serious attention. To overcome this, farmers generally use synthetic pesticides in pest control. The use of synthetic pesticides in the process of agricultural production can result in the presence of pesticide residues in agricultural products (Untung, 1996). Residues of a number of chemicals such as pesticides can be left through various cycles directly or indirectly, so that it reaches humans and enters the digestive tract with food and drinking water (Tjokronegoro, 1987).

Seeing the negative impact of pesticides, the development of a biorational pest control agent needs to be done. This antifeedant substance has good prospects to be developed into a biopesticide agent (Mayanti, *et al.*, 2005). This research will examine the potential of food activity inhibition for pest insects with different inhibitory activities.

II. RESEARCH METHODS

A. Research Location and Time

This research was conducted at the Laboratory of Integrated Sciences, Laboratory of Physics and Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Manado State University, in Tondano. The study was conducted from May to August 2019, starting from the sampling phase, phytochemical screening extraction and testing of *antifeedant* activity.

B. Materials and tools

The material used is tuba roots sampled from Bulo plantation in Bulo village, Mandolang sub-districts, Minahasa Regency. The materials used are 70% ethanol and 95% for maceration of tubal root samplings, technical methanol acetic acid, sulfuric acid, chloroform, 5% FeCl_3 % solution, Dragendorff reagent, Meyer reagent, tissue, cotton, whattman filter paper no. 42, aluminum foil, plastic samples, mustard leaves, and caterpillars *Crocidolomia binotalis*. The tools used are analytical scales, petri dishes, vial tubes, Erlenmeyers, goblets, measuring cups, volumetric pipettes, fillers, test tubes and tube racks, drop pipettes, 50 mL and 100 mL measuring flasks and rotary vacuum evaporators (Heidolph-Laborota 4000/4001 efficient).

C. Experiment Design and Data Analysis

This study uses a Completely Randomized Design (CRD) as a treatment that is the concentration level of methanol solvent 50 ppm, 100 ppm, 500 ppm and 1000 ppm and negative control 0 ppm. Each treatment was repeated 3 times. The parameters observed were percentage of *Feeding Reduction* (FR) or % *antifeedant* and phytochemical screening / screening test for tubal root ethanol extract. The data obtained were analyzed using one-way analysis of variance (ANOVA). If the treatment has a significant effect on the inhibition of eating *Crocidolomia binotalis* on mustard plants, then further testing of LSD or LSD at 5% significance level.

D. Research procedure

Tuba Root Extraction (*Derris Elliptica* L.)

Tuba root samples obtained from the Bulo plantation in the village of Bulo, Mandolang sub-district, Minahasa Regency. The extract material used in this study was the roots of the tuba plant which grew around Bulo, the village of Bulo, Mandolang sub-district, Minahasa Regency. Making the root extract tuba done by weighing 1,2 Kg of tuba root dried at room temperature, then soaked in ethanol (maceration) for 1 x 24 hours in maserator. Maceration is done several times until the extracts run out. The maceration solution is then filtered using Whattman filter paper No. 42. The filtrate obtained was then put into a vacuum evaporator at 40°C until the ethanol solvent evaporated to obtain a thick ethanol extract. Furthermore, the extraction results obtained were weighed using analytical scales. To make the test solution, a dilution was carried out using technical

methanol solvents. The concentration of the tuba root extract used in this study was 50; 100; 500 and 1000 ppm. While the control (0 ppm) is in the form of methanol solution. Each treatment was repeated three times.

E. Preparation of Test Larvae / Caterpillars

Pupa *Crocidolomia binotalis* instar to III obtained by way of setting up a mustard that has not been sprayed synthetic pesticides. A 1 x 2 m dark culture box is prepared. Larvae prepared by way of taking pupae of agricultural acreage of mustard incorporated into the plastic bottle and then hung in the breeding box. At the top of the culture box will be hung cotton that is tied to a rope and has been dipped in a mixture of 1 mL of honey with 10 mL of water. Honey solution serves as a food source for the *Crocidolomia binotalis* imago. The next pupa hatches and becomes a moth after two days. Moths will reproduce and lay their eggs on mustard plants. The eggs will hatch into larvae instar I to instar III. Furthermore, third instar larvae will be used in *antifeedant* activity testing.

F. Antifeedant Activity Testing

The test was carried out using the leaf disc method according to (Atta, Choundary, & Thomson, 2001). On sterile petri dishes are placed wet filter paper / tissue and gauze and the filter paper is coated with transparent plastic that has been perforated. Leaf discs are made with a circle the size of a petri dish on mustard leaves that have not been given synthetic pesticides. Leaf discs to be made are the same in size, shape and thickness. Leaf discs were dipped in each extract sample and compared with positive control. The study was conducted with three repetitions. Leaf discs are dipped / applied for 5 minutes then aired for 5 minutes. After aerating, the leaf disc will be weighed and put into a prepared petri dish.

Caterpillars *Crocidolomia binotalis* included 1 caterpillar on each petri dish, the petri dish that already contains leaves and caterpillars test discs will be observed caterpillars avoidance response to the leaf discs that had been given each extract concentration. Observations are made after 24 hours. Antifeedant activity testing is done by looking at the nature of the *Feeding Reduction* of the sample. The parameter to be observed is the weight of the remaining leaves that are not eaten by the larvae or *Feeding Reduction* (FR). Leaf discs were then weighed, to find out the weight of mustard leaf discs eaten by the

Crocidolomia binotalis caterpillar, the percentage of *Feeding Reduction* (% FR) was used. The percentage value of *Feeding Reduction* is measured by the formula (Atta *et al.*, 2001):

$$\% FR = \left\{ 1 - \frac{\text{Weight of the treated leaf eaten}}{\text{Control weight of the leaf eaten}} \right\} \times 100\%$$

G. Phytochemical Screening

Phytochemical Test Work Procedures (Ayoola, *et al.*, 2008 & Farnsworth, 1966)

A certain amount of viscous extract was carried out by phytochemical tests which aimed to determine the class of compounds contained in the roots of the tuba. Phytochemical tests were carried out on the group of Alkaloids, Flavonoids, Phenols, Saponins, Triterpenoids, Steroids, Terpenoids, and Tannins.

a. Alkaloid Test

One gram of ammonia extract was added to 10% and then extracted with chloroform and added 1 N hydrochloric acid. The extraction results will be divided into two layers. The upper layer (acid layer) is divided into two tubes. In one tube Meyer reagent was added, while in the other tube Dragendorf reagent was added. Yellow indicates a positive alkaloid.

b. Flavonoid Test

Two methods are used to test Flavonoids.

1. Dilute ammonia (5 mL) is added to the aqueous filtrate portion of the extract. Then concentrated sulfuric acid (1 mL) is added. A missing yellow indicates flavonoids.
2. A portion of the extract is heated with 10 mL ethyl acetate which has been evaporated for 3 minutes. The mixture is then filtered and 4 mL of the filtrate is shaken with the addition of 1 mL of aqueous ammonia solution, the formation of a yellow color indicates the presence of flavonoids.

c. Phenol Test

To one gram of extract was added 1% iron (III) chloride. Green / red / purple / blue / black colors indicate positive phenols.

d. Saponin Test

One gram of extract is added to water then boil in a water bath for 5 minutes, after which it is shaken vigorously. Saponin is positive if foam forms stable for \pm 30 minutes.

e. Triterpenoid and Steroid Test

Anhydrous acetic acid was added to the extract until it was submerged; leave for \pm 15 minutes. After that, add 1 drop

of concentrated sulfuric acid. Green / blue deposits indicate steroids, while red / orange deposits indicate triterpenoids.

f. Terpenoid Test

A number of extracts were added with 2 mL chloroform. Then carefully added concentrated H₂SO₄ (3 mL) to form a layer. The formation of a brownish red color indicates terpenoids.

III. RESULTS AND DISCUSSION

Insects will face two things to start eating activities, first there are stimuli to initiate feeding activities (*feeding stimulants*) in plants that provide input signals for the introduction of food types and maintain eating activities. The second is the detection of the presence of foreign substances (*foreign compounds*) which act as inhibitors to eat so can shorten or terminate the feeding activity feeding activity at all. Based on the results of interviews with farmers spraying mustard area where pest control caterpillars *Crociodolomia*

binotalis still relies on the use of chemical pesticides. Spraying intervals with chemical pesticides are carried out for 5-6 days, while the recommended use of pesticides should ideally be once a month. This results in faster selection of insect resistant to insecticides. The use of botanical insecticides was also not carried out because given the vast land area making it less practical to apply. According to Danar Dono *et al*, insect resistance to synthetic insecticides can be broken using botanical insecticides, due to the different mechanism of action of the two insecticides. In addition, one of the advantages of botanical insecticides is that it is difficult to cause an immune (*resistant*) reaction on the target pest so it is safe for the balance of the ecosystem.

Based on research results the influence of tuba root ethanol extract produces data that is food activity / food inhibitors (*Feeding Reduction*). The results of barriers to eating *Crociodolomia binotalis caterpillars* can be seen in Table 1.

Table 1. Effect of tuba root extract on decreased feeding activity of *Crociodolomia binotalis* caterpillar on mustard plants.

Treatment	U	Area of leaves eaten (gr) within 24 hours	Percentage of Food Obstacles (%)	Average
P1 (50 ppm)	1	2.21	8	6.66
	2	2.17	10	
	3	2.37	2	
P2 (100 ppm)	1	1.79	26	27.73
	2	1.70	29.2	
	3	1.75	28	
P3 (500 ppm)	1	1.68	30	30.16
	2	1.80	25	
	3	1.55	35.5	
P4 (1000 ppm)	1	1.11	54	44.0
	2	1.50	38	
	3	1.45	40	
control	1	2.40	0	0

Based on table 1 , eating activity data can be seen from the percentage of food barriers at a concentration of 50 ppm for replications 1, 2, 3 in succession of 8, 10 and 2 while for 100 ppm concentrations of 26, 29.2 and 28 . At concentrations of 500 ppm and 1000 ppm the percentage of food barriers increased by 30, 25 and 35.5, while for the

concentration of 1000 ppm amounted to 54, 38 and 40 . The higher the value of eating barriers means a decrease in the eating activity of the *Crociodolomia binotalis caterpillar* on mustard plants. The average percentage of food resistance (*Feeding Reduction, %*) results in the following diagram:

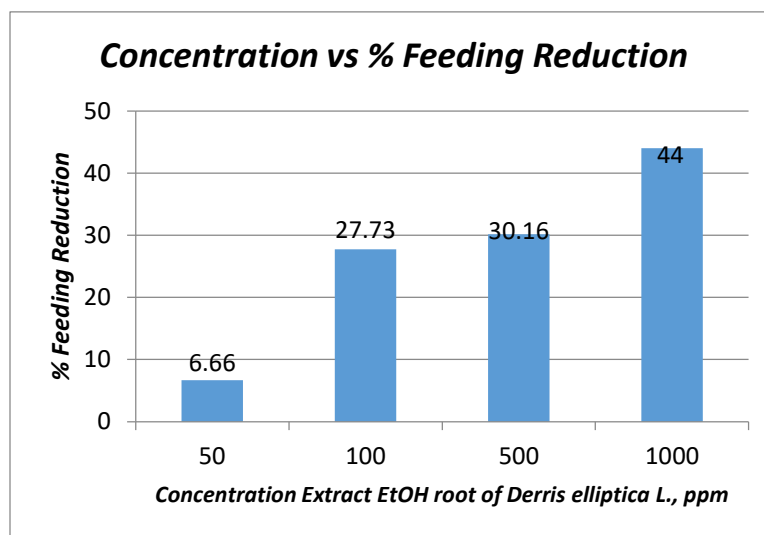


Fig.4.1 Diagram of average decrease in feeding activity (% Feeding Reduction) of caterpillar *Crociodolomia binotalis*

Based on the results of the test the influence of tuba root ethanol extract with three repetitions gives a value of eating resistance that is different from each concentration. The higher the concentration, the higher the percentage of eating obstacles will be and this means a decrease in eating activity. The highest value of eating resistance is at a concentration of 500 ppm and 1000 ppm.

Normality test is a test used to determine the distribution of data obtained is normal or not. The normality test is a prerequisite for the one-way ANOVA test. If the number of samples >50 used is Kolmogorov-Smirnov, whereas if the number of samples <50 then what is used is Shapiro-Wilk. The results in table 2 are then tested for normality as follows:

Table 2. Test for normality of feeding activity of *Crociodolomia binotalis* caterpillars on mustard plants.

Tests of Normality						
Treatment	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistics	df	Sig.	Statistics	df	Sig.
Antifeedant 1.00	.292	3	.	.923	3	.463
2.00	.232	3	.	.980	3	.726
3.00	.179	3	.	.999	3	.948
4.00	.343	3	.	.842	3	.220

a. Lilliefors Significance Correction

If in the Shapiro-Wilk column the value of Sig. > 0.05 then the data for each treatment is normally distributed, whereas if the value of Sig. < 0.05, then each treatment data is not

normally distributed. The conclusion of the normality test on the above eating activity data meets the normal requirements because the Sig. > 0.05 for each treatment.

Table 3. Homogeneity test of feeding activity of *Crociodolomia binotalis* caterpillars on mustard plants

Test of Homogeneity of Variances

Antifeedant

Levene Statistics	df1	df2	Sig.
2,922	3	8	.100

Data for each treatment is said to be homogeneous if the Sig value > 0.05 and vice versa the treatment data is said to be homogeneous if the Sig value < 0.05. Based on the homogeneity test table above the activity data of each treatment was declared homogeneous because the Sig value > 0.05 so that the ANOVA test could be performed. ANOVA test table can be seen in Table 4.

After ANOVA test (variance analysis) at a 5 % confidence level, the results showed that the treatment had a significant influence on the antifeedant of the *Crocicidolomia binotalis caterpillar* on mustard plants. can be seen in the Table 4 below:

Table 4. ANOVA test of feeding activity of the *Crocicidolomia binotalis caterpillar* on mustard plants

ANOVA					
Antifeedant	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2138,789	3	712,930	23,085	.000
Within Groups	247,060	8	30,883		
Total	2385,849	11			

If the Sig . Value < 0.05, the treatment was stated to have a significant effect. Based on the ANOVA test table above shows that there is a significant effect of tuba root ethanol extract on the feeding activity of the *Crocicidolomia binotalis caterpillar* on mustard plants.

treatment data can be further tested to find out more specific effects. Further tests used were those with the smallest significant difference (LSD) or LSD (Least Significant Different) to show differences between each treatment individual.

Based on the ANOVA test results above, the

Table 5. Average% of leaves eaten and decreased feeding activity of tubal roots against the *Crocicidolomia binotalis caterpillar* on mustard greens *Brassicca juncea*.

Concentration of tuba root ethanol extract , ppm	Average food resistance (%), Feeding Reduction (%)	Average food resistance at 24 hours after application (ppm) \pm SD	Average food resistance at 24 hours after application (ppm) \pm SEM
P1 (50)	6.66	6.66 \pm 4.16	6.66 \pm 2.40 ^a
P2 (100)	27.73	27.73 \pm 1.61	27.73 \pm 0.93 ^a
P3 (500)	30.16	30.16 \pm 5.25	30.16 \pm 3.03 ^b
P4 (1000)	44.00	44 \pm 8.71	44 \pm 5.03 ^c

Note: the treatment followed by the same letter shows no significant difference and the treatment followed by different letters shows significantly different

Phytochemical Test Results

Table 6. Phytochemical screening test results of ethanol extract (EtOH) from tuba root plants

No.	Group	Observation result	
1	Alkaloids	Hager (-)	Meyer (+)
2	Flavonoids	(-)	
3	Phenol	(++)	
4	Saponin	(+++)	
5	Steroids / triterpenoids	(++)	
6	Terpenoids	(+))	

Note: +++ = Compounds that are contained a lot
++ = Medium contained compound
+ = The compound contained is small
- = The compound contained does not exist

Table 7. Phytochemical Testing of ethanol extract (EtOH) of tubal roots (*Derris elliptica* L.)

Phytochemical Test	Results (color)	Standard Result (color)
Saponin	Shaped foam (+++)	Formed foam \pm 15 minutes stable
Phenol	Purple color (++)	Bluish purple
Steroids / triterpenoids	Green or blue (++)	Brown sediment
Terpenoids	Chocolate (+)	Reddish brown deposits
Flavonoids	Yellow (-)	Chocolate
Alkaloids	Light Brown (+)	Brown sediment

Based on the results of phytochemical tests, tuba root ethanol extract belongs to the saponin, steroid, phenol, and alkaloid classes. Saponins are generally bitter and also toxic to some cold-blooded animals such as fish and amphibians. The use of saponins as an antidote to predator attacks, the media to fight over the scope, and help the process of reproduction (Liang & Guo, 2013). The bitter taste issued by saponins is thought to inhibit the feeding activity of test larvae.

The content of triterpenoid compounds in ethanol extract was characterized by the formation of reddish brown deposits in the extracts tested. The terpenoid compounds contained in the tuba roots function as fish poisons to fight predators that threaten their survival (Handayani *et al.*, 1997). Alkaloids can inhibit the response of cyanogenic glycoside sugars, which are sugars formed from bonds between sugar and toxic compounds stored in plants so that the toxic compounds are lost in toxicity.

IV. CONCLUSIONS AND SUGGESTIONS

Based on the results of further tests BNT at 5% level showed significantly different from the administration of ethanol extract to the feeding activity of the *Crocidolomia binotalis caterpillar* on mustard greens *Brassicca juncea* L. In the treatment P1 was not significantly different from the treatment P2, but significantly different from P3 and P4. In the P2 treatment also not significantly different from P1 but significantly different from P3 and P4. In the treatment of P3 significantly different from P4, also significantly different from P1 and P2. While the P4 treatment was significantly different from P1 and P2 also significantly different from P3. The best antifeedant activity against *Crocidolomia binotalis* is at concentrations of 500 ppm and 1000 ppm because it is able

to inhibit feeding power or Feeding Reduction of caterpillars test 30.16% and 44.00%.

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