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The Effect of Noni (*Morinda citrifolia L.*) Fruit Extract and Time of Application to Control *Crocidolomia binotalis* Zell in Cabbage Plants

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ABSTRACT

Crocidolomia binotalis Zell is a significant pest in the vegetable plant Brassicaceae. Bioinsecticides are one of the potential alternatives for controlling these pests. This study aims to determine the time of application of Noni fruit extract and determine the Effective concentration of Noni fruit extract as a bioinsecticide in controlling C. binotalis larvae in cabbage plants. This study used a Completely Randomized Design (CRD) consisting of two treatment factors and four replications. The first factor is extract concentration (5 ppm, 10 ppm, 15 ppm, and 20 ppm) and the second factor is the time of application of biopesticides before the pest is invested (W1) and after the pest is invested (W2). So in total, there are 32 experimental units and 4 more are added as the control unit. The results showed that noni fruit extract had no significant effect on mortality of C. binotalis larvae. The highest mortality rate is found in the concentration of K2 (10 ppm) at 100% at the time of application before the pest is invested (W1). Interactions have a significant effect on plant stover wet weight but have no significant impact on pest mortality, attack intensity, percentage of pupae formed, and rate of imago that appears.

INTRODUCTION

In cabbage cultivation, several obstacles must be overcome, namely the presence of OMOs (Plant Pest Organisms) which are the limiting factors for the yield of vegetable crops. One of the pests that attack cabbage plants is the caterpillar pest (Crocidolomia binotalis Zell.). The larval stage of *C. binotalis* is an essential pest in cabbage plants (Widiana and Zeswita, 2012). These insects are very greedy pests in its larval stage. Larvae can attack both young and old leaves. The part of the leaf that has been eaten by a group of young larvae is usually left unused and then perforated after the epidermis layer is dry. An attack from a caterpillar pest can cause crop failure if intensive control measures are not taken. This pest attack can result in cabbage yield loss of 65.8% (Uhan and Sulastrini, 2008). Until now, the focus of pest control on cabbage plants still relies on excessive synthetic pesticides both in terms of doses and types of pesticides. (Oka, 1995; and Sembel, 2010).

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But the use of synthetic pesticides that aren't a wise choice since it damages the environment and not suitable for human health. This is happening because not all synthetic pesticides used to manage to hit the targeted pest. About 30% of pesticides are wasted on the ground in the dry season, and 80% in the rainy season, which will then be spent into the waters (Suryaningsih dan Hadisoeganda 2004).

Side effects due to excessive use of synthetic pesticides are (a) death of non-target organisms (b) occurrence of resistance and resurgence of target pests and (c) insecticide residues on food ingredients (d) and second pest explosions. Likewise, the appearance of environmental pollution both in the lithosphere, hydrosphere, and atmosphere. It minimizes the emergence of negative influences from the use of insecticides, and it is necessary to look for other alternatives, namely, by using bio insecticides (Oka, 1995 and Sembel, 2010). Bioinsecticides are an alternative to replace synthetic insecticides because bio insecticides don't have adverse effects on humans, livestock, and the environment. In general, bioinsecticides are interpreted as a pesticide whose necessary ingredients come from plants that are easily made. This type of insecticide is readily biodegradable and doesn't pollute the environment since its residue will quickly be lost (Dinas Pertanian dan Kehutanan, 2002). To control *C.binotalis* many other ingredients can be used as pesticide ingredients. Such as the result of Hasnah and Purnama (2013) research that reported the application of M. charantia leaf extract to control *C.pavonana* in mustard plants. The use of *M.charantia* leaf extract with a concentration of 20% was able to control *C. pavonana* up to 60%. Mujib *et al.* (2014) The administration of vegetable pomegranate leaves, babadotan leaves, and soursop leaves have significantly affected the mortality of *C. pavonana* caterpillar.

The concentration of 80 g/50 ml lemon grass extract can be used as a botanical insecticide to control C. binotalis pests in laboratory conditions because at this concentration it can lead to death by 95% (Henny and Deflly, 2011).

One of the plants that can be used as bioinsecticides is Noni (*Morinda citrifolia* L.). Noni contains essential oils, alkaloids, saponins, flavonoids, polyphenols, and anthraquinones. Other ingredients are terpenoids, ascorbic acid, scopoletin, serotonin, damnacanthal, resins, glycosides, eugenol, and proxeronin. (Hayani and Fatimah, 2004) From the results of the phytochemical screening, the analysis showed that in noni seeds, there are alkaloids, saponins, tannins, and glycosides. Current research on bio insecticides is getting more frequent and earn dedicated attention. Noni fruit insecticide is easily decomposed, so it doesn't pollute the environment and is relatively safe for humans. (Bangun and Sarwono, 2002).

Based on the description above, it is necessary to examine the concentration of effective Noni fruit extract as a bioinsecticide in controlling *C. binotalis* larvae . This study aims to : Determine the timing of application of Noni fruit extract and determine the concentration of Noni fruit extract that is effective as a bioinsecticide in controlling *C. binotalis* larvae in cabbage plants at the screen house.

MATERIALS AND METHOD

The research is conducted at Jl. Merpati 11 Kelurahan Rawa Makmur Kecamatan Muara Bangkahulu, Kota Bengkulu, from April 2016 to June 2016.

The design used in this study was a Completely Randomized Design (CRD) consisting of two treatment factors and four replications. The first factor is the extract concentration (K), and the second factor is the time of application (W) of biopesticides before the pest is invested, and after the pest is invested so that there are 32 experimental units and 4 more experimental units as controls.

The first factor is the concentration treatment ml/L in a solution :

K1 = 5 ppm (0,5 ml Noni fruit extract + 99,5 ml aquades), K2 = 10 ppm (1 ml Noni fruit extract + 99 ml aquades), K3 = 15 ppm (1,5 ml Noni fruit extract + 98,5 ml aquades)

K4 = 20 ppm (2 ml Noni fruit extract + 98 ml aquades)

The second factor is the time of application of bio pesticides: W1= Before pest is Invested , and W2= After pest is Invested.

Maintenance of *C. binotalis* Zell Larvae

C. binotalis Zell is obtained from cabbage plants in Kepahyang and maintained on Jl. Merpati 11 Kelurahan Rawa Makmur. Larvae are maintained on cabbage plants that are alive and given a bamboo frame and gauze enclosure. Larvae are maintained to form imago. Imago then maintained to produce eggs, and the eggs are kept until they hatch and form 3 instar larvae which are used for research. The number of 3 instar larvae used in the study is 180.

Seed Preparation and Nursery

The seeds used are cabbage seeds of Hybrid F1 varieties. Nurseries are carried out with a second transplanting method, ie, the seed was sown first in a tray containing soil mixed planting media, and husk charcoal in a ratio of 1: 1. After 7 days the grown plants are transferred into 10 x 10 polybags containing soil mixed planting media, and manure with a ratio of 2: 1, then the plants are maintained until they are 2 weeks old. Seeds that are 2 weeks old, then transferred into a 5 kg volume bag containing soil and manure in 2:1 ratio

Plant maintenance includes watering, weeding, and fertilizing. Watering is done twice a day (morning and evening). Weed control is done manually by removing weeds using a hand. Weeding is carried out every time there weeds shown up during the study, so that the condition of the plants is free weeds free. Fertilization carried out includes additional fertilization with a dose of 100 kg urea / ha, SP -36 250 kg / ha and 200 kg KCl / ha. For each plant, 4 g of urea is needed + SP-36 9 g, and 7 g of KCL is given at the age of plant 3 MST (Balai Penelitian Tanaman Sayuran, 2007).

Making of Noni Fruit Extract

Nonies are obtained in Bengkulu city. The making of noni fruit extract is carried out at the Plant Protection Laboratory of the University of Bengkulu. 1 kg of cooked noni fruit is washed with running water and then dried. Making the extract is done by chopping noni fruit into small pieces then the fruit is made into a purée and left for 24 hours. The simplicia is then macerated with 95% ethanol so that this polar solvent can attract all the compounds contained in the noni fruit. Furthermore, macerate was put into the oven at 40^{o} C for 3 x 24 hours until a crude extract was obtained. The oven solution is then filtered using filter paper to obtain a solution with a concentration of 100%. This extract is then made into various concentrations according to the treatment. The solution that has been obtained is then added Adjuvant as much as 1 drop as adhesive.

Pest Investigation

The larval investment was carried out when cabbage plants are 4 weeks old. The cabbage is each given 5 instar larvae 3 sample plants. Then on the surface of the soil polybags are given a white paper to accommodate dead larvae.

Extract Application

Application of extracts was carried out by spraying the extracted liquid on each plant according to the concentration tested. The extracted liquid is sprayed evenly. The first spray (W1) was carried out 1 hour before investment in the third instar *C. binotalis* larvae. The second spraying (W2) is carried out 1 hour after the pest is invested according to the concentration of treatment.

Observation Variable

Pest Mortality. Observations were made 24 hours after application, and if there were *C. binotalis* larvae that survived, they were kept until the larvae became imago. Each dead larva is recorded, and the percentage of death is calculated using the equation :

$$M = \frac{a}{b} \times 100\%$$

Information:

M = Mortality

a = Number of dead test insect

b = Total number of test insect

If the average mortality of test insects in the control group is between 5-20%, then actual mortality is corrected using the Abbot formula in Rosenheim and Hoy, (1989) as follows :

$$P = \frac{P0 - C}{100 - C} \times 100\%$$

Information:

P = corrected mortality (%)

P_0 = mortality of observations in each treatment (%)

C = mortality in control (%)

Crop damage intensity. Observation of plant damage due to attack by *C.binotalis* is carried out 1 day after the first spraying until the beginning of the preproduction period (instar 5). By calculating the percentage of damage to cabbage plants from each replication and if larvae attack the plant until the growing point is considered to have a 100% damage rate. With the equation as follows :

Damage intensity (%)

$$= \frac{\sum \text{damage leaf}}{\sum \text{total leaf}} \times 100\ \%$$

Percentage of pupae formed. Observations were made by counting the number of pupae formed from C.binotalis larvae that survived after the extract was applied. The percentage of insects created is calculated from one day the larvae enter the prepupa phase until the pupa is formed. The rate of pupae formed is calculated using the following formula:

Percentage imago formed (%)

$$= \frac{\sum \text{Formed image}}{\sum \text{early larva}} \times 100\%$$

Percentage of imago formed. Observations were made by calculating the number of imagoes formed from C.binotalis larvae that survived after the extract was applied. The percentage of imago that appears is calculated using the following formula :

Percentage imago formed (%)

$$= \frac{\sum \text{Formed image}}{\sum \text{early larva}} \times 100\ \%$$

Wet weigh of plant stover. After completing the observation, each sample plant was removed and separated from the plant roots and plant stover, and then the plant stover was weighed using digital scales in units of grams. Each weight of each treatment is recorded in a table..

Data analysis

Data obtained on the percentage variable of pupae formed and the percentage of imago developed was transformed using a formula $x=\sqrt{(x+0,5)}$ and analyzed statistically by analysis of variance (F test level of 5%). If the treatment shows a real effect, then further statistical analysis is carried out using DMRT.

RESULT AND DISCUSSION

Analysis Observation Variables Variant

The results of variance analysis showed that the interaction had a significant effect on the wet weight of plant stover, but singularly it had no significant impact on pest mortality, crop damage intensity, percentage of pupae formed, and rate of imago formed.

Mortality and Symptoms in *C. binotalis* Larvae

The average percentage of *C. binotalis* larvae mortality in each treatment of noni fruit extract with varying concentration and application time is presented in Table 2:

From the data on the percentage of mortality of C. binotalis larvae, (Table, 2) it can be seen that the concentration (K1) has been able to produce pest mortality by 90%. Larvae mortality tends to occur at its peak in treatments with level (K2) at the time of application before the pest is invested (W1) not at higher concentrations.

It is suspected that the chemical compounds of noni fruit such as alkaloids, saponins, flavonoids, polyphenols and anthraquinones, terpenoids, ascorbic acid, scopoletin, serotonin, damnacanthal, resins, glycosides, eugenol, proxenies and other ingredients in noni seeds are alkaloids, saponins, tannins and glycosides which are capable of being toxic to C. binotalis. It is said that because the concentration (K1) is thought to have been able to control C. binotalis by 90%.

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Table 1. Results F calculated the effect of concentration and application time on pest mortality, crop damage intensity, percentage of pupae formed, rate of imago established and wet weight of plant stover.

Variable	F-value		
	Interaction	Concentration	Application time
Pest Mortality	0.42^{ns}	0.50^{ns}	0.25^{ns}
Crop damage intensity	0.32 ^{ns}	0.55 ^{ns}	0.68 ^{ns}
Percentage of Pupae formed	0.50 ^{ns}	0.23 ^{ns}	0.02 ^{ns}
Percentage of Imago formed	0.47 ^{ns}	0.22 ^{ns}	0.01 ^{ns}
Plant stover wet weight	3.66*	0.92 ^{ns}	1.43 ^{ns}

Note: * : significant effect on the F level test of 5%, ns : no significant effect on the F level test of 5%

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Mumford and Norton (1984) explain that a pesticide is considered to be effective if it can kill at least 80% of test insects, so the concentration of K1 (5ppm) is effective in controlling C. binotalis because it can cause C. binotalis mortality rate by 90%. The highest level of lethal C. binotalis is K2 (10 ppm) at 100% so, the concentration that can be considered useful in controlling C. binotalis is the K2W1. It is suspected that the chemical compounds contained in Noni fruit extract directly seep or stick to the plant when sprayed directly before the pest is invested (W1) extract which is applied directly to the plant and eaten by the larvae into the body of the larvae through the mouth or as stomach poison so the residue of noni fruit extract in the shape of the larvae at the W1 application time treatment more than the W2 application time. According to Rusdy (2009), if an insect eats an active insecticide compound (acts as a stomach poison), as a reaction to insects that cannot bear death, otherwise tolerant insects will survive until they can follow the next stage into pupa or imago. For insects that are resistant to these active compounds, before they die, insects can survive by maximizing the use of energy sources in their bodies.

As a consequence of this situation, the larvae will experience barriers to growth and development. Saponins and alkaloids are stomach poisoning or stomach poisons. When these compounds enter the insect's body, the digestive tract will be disrupted. Alkaloids are also able to inhibit the growth of insects, especially the three main hormones in insects, namely brain hormone (brain hormone), hormone edition, and growth hormone (juvenile hormone). The lack of development of these hormones can cause the failure of metamorphosis.

The treatment of application time after the invested pest (W2) can cause mortality rate by 95% at concentrations of 10ppm (K2) and 15ppm (K3). This is lower than the application before the pest is invested (W1) because the chemical compounds of noni fruit such as tannins, steroids, alkaloids, saponins, and terpenoids are stomach poisons, if the mixture enters the body of the larvae of C. binotalis, it will interfere with its digestive organs (Agazali and Prastowo, 2015). The compounds of tannin and saponin cause minimal food consumption and reduce the activity of digestive enzymes

Tabel 2. Average Percentage of Mortality of *C. binotalis* Larvae with Various Concentration Treatment and Application Time

Concentration	Average larvae mortality (%)	
	W_1 (Before pest is Invested)	W_2 (After pest is Invested)
K_1 (5 ppm)	90	90
K_2 (10 ppm)	100	95
K_3 (15 ppm)	90	95
K_4 (20 ppm)	95	85

and absorption of food, resulting in a decrease in the population of C. binotalis pests naturally and functioning of plant defenses by blocking insects from digesting food. Steroids and terpenoids are secondary metabolites that act as plant defenses by inhibiting insect feeding.

These metabolites are also toxic. Whereas at the time of application after the pest is invested (W2) the extract is immediately sprayed after the pest is invested in contact poison, such as Flavonoids which can inhibit the insects' appetite (antifeedant). When these compounds enter the insect's body, the digestive tract will be disrupted. This compound also works by inhibiting the taste receptors in the insect's mouth area. This results in insects failing to get a sense stimulus, so they are unable to recognize the food. Resulting in the larvae lost its appetite and ate less, the larvae don't die immediately until the pre-pupae stage, but the pupae that are established become deformed. This shows that the toxic effects of Noni fruit extract not only occur in the larval stage but also up to the pupal stage.

At the beginning of the investment, C. binotalis larvae are still very active and immediately eat young leaves of cabbage plants. After the application of Noni fruit extract, the symptoms shown by larvae poisoning such as sluggish movement and inactivity, unlike the healthy counterpart. After 24 hours of use, dead larvae have shown up and remain attached to the leaves and cabbage crops. C. binotalis larvae that were poisoned from Noni fruit extract were initially pink in color, and over time the larvae became brown and then blacked all over their bodies. When pressed, the larvae emitted a light brown and foul-smelling liquid (Picture 1). This is following the study (Candra, 2016) which said poisoned larvae with krinyuh extract and extracts of continued creep combination were initially pink, then turned brown which eventually died with the middle part turned black and the larva's body becoming softer and foul-smelling.

Crop damage intensity

The application of Noni fruit extract has no significant effect on damage to cabbage plants. At the concentration of K0, the percentage of damage caused by *C. binotalis* is (78.07%), higher than the concentrations of K1, K2, K3, and K4 (Table 3) because the K0 treatment wasn't sprayed that the *C. binotalis* larvae remained healthy after consuming cabbage leaves and caused considerable damage.

Based on the average intensity of crop damage (Table 3.) K2 concentration (10ppm) on the application of Noni fruit extract before the pest was invested (W1) can reduce the percentage of damage caused by *C. binotalis* to 52.57%. This indicates that Noni fruit extract has insecticidal properties that work quickly. The active compounds of Noni fruit extract include *saponins, flavonoids, polyphenols* which are antifeedant that affect the nervous system, effecting in reduced appetite (Hasna Dan Nasril, 2009). This is appropriate when compared with pest mortality, because the highest percentage of mortality is at the concentration of 10 ppm. (K2)

Picture 1. A) healthy larvae, B) unhealthy larvae after noni fruit expl

The application of Noni fruit extract at K2 concentration (10 ppm) when judged from the average intensity data of crop damage can be considered good enough to reduce the level of damage to the plants. The existence of allelochemical compounds that are toxic to insects will affect the amount and rate of

Table 3. Average Intensity of Plant Damage Due to C. binotalis Attacks on Cabbage Plants With Various Concentration and Application Time Treatments

Concentrtion	Average crop damage intensity (%)	
	W_1	W_2
K_1 (5 ppm)	65.16	64.92
K_2 (10 ppm)	52.57	63.71
K_3 (15 ppm)	62.41	65.47
K_4 (20 ppm)	59.02	60.00

consumption, so it will also affect the rate of development, and the final weight of the larvae. If the food consumed contains toxic compounds, then the larvae will not reach the crisis weight to become pupae (Simpson, 1990).

Although at a concentration of 10 ppm (K2) noni frut extract it manage to reduce it by 52.57%, crop damage intensity is still considered high because the larvae directly attacked the plant's growing point and were categorized as 100% attacked. The crop damage intensity is still high because the observations were carried out for 7 days. Even though the average data on K2W1 pest mortality shows 100% pest mortality, in other treatments there are still pests that live up to day 6.

Pupa and Imago Formaed Percentage

The average percentage of pupae formed in each noni extract extract dose and application time is presented in Table 4.

Table 4. Mean percentage of pupa and imago formed from *C. binotalis* larvae in cabbage plants with various concentration and application time treatments

Application time	Concentration	presentation (%)	
		Pupa percentage	Imago percentage
W_1	K_1 (5 ppm)	5	0
	K_2 (10 ppm)	0	0
	K_3 (15 ppm)	10	0
	K_4 (20 ppm)	5	0
W_2	K_1 (5 ppm)	10	0
	K_2 (10 ppm)	5	0
	K_3 (15 ppm)	0	0
	K_4 (20 ppm)	10	0

The results of pupa formation in treatment K0 show a different result number wise, but statistically, the result were not significantly different from treatments K1, K2, K3 and K4, the highest number of pupae formed was in the treatment of K0 at 85% and the lowest in treatment K1 , K2, and K4 at 5%. In the controlled units, all test larvae succeeded in forming a perfect pupa. The number of pupae formed is closely related to the percentage of larval mortality if the larval mortality is high, then the number of pupae is low. In a treatment that application time is done after the pest have been invested (W2) can cause a pupa formation of 10% at higher concentrations of K1 and K4 when compared to the application before the pest is invested because the larvae become pre-pupae soon after eating the plants that have been sprayed with Noni fruit extract. However, many of the pupa produced from that pre-pupa are defective. Pupa is a defect due to *C. pavonana* larvae which consume food directly form pre-pupa, it is suspected that the larval digestive tract has been poisoned by compounds from Noni fruit extract, resulting in damage to the digestive tract even though the larvae are in pre-pupa form. This is indicated by the surface of the body of the pupa that is formed will deflate on the abdomen, allegedly due to damage to the digestive tract. (Fig 2).

Rosyidah (2007) states that *flavonoid* and *saponin* compounds can cause wilting of the nerves and damage to spiracles which results in

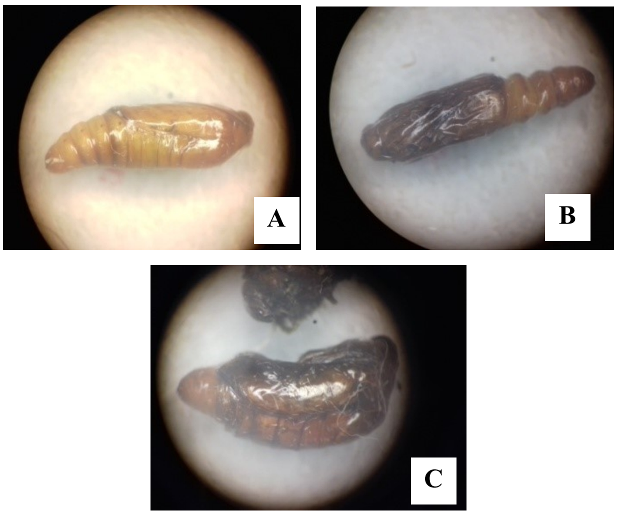

Figure 2. A) Healthy *C. binotalis* pupa B dan C) deformed pupa

insects being unable to breathe and eventually die. *Saponins* are toxic and antifeedant in fleas, larvae, beetles and various other insects. The metabolic process requires a lot of energy. The energy used for detoxification is obtained from the energy that is supposed to be for development, as a result of which insect growth will be disrupted (Ferrar *et al.*,1989).

The percentage of imago that appeared on treatment K0 on average differed numerically but were not statistically there is no significant different from treatments K1, K2, K3 and K4, the highest number of pupa was found in the treatment of K0 at 85% and the lowest result is from all treatment (K1, K2, K3 and K4) is caused by a defective pupa that can't become an imago. In this case there are larvae that are still able to survive up to a certain period of time and there are even larvae that can live up to the pupa stage but soon die. This happens because the higher the level of toxins that are in the body of an organism, the more difficult it is for an organism to inhibit or neutralize toxins in its body. According to Wigglesworth (1974), when entering the pupa stage, there are a lot of glycogen reserves and proteins needed for cocoon formation, thus biochemical activity is directed more towards the formation of these compounds, so that metabolic activities to inhibit or neutralize toxins inside the body becomes decreased and as a result the formed pupa finally dies (cannot live to the imago stage).

Treatment	Plant fresh weight (g)	
K_1W_1	328.75	ab
K_1W_2	407.50	a
K_2W_1	473.75	a
K_2W_2	351.25	ab
K_3W_1	428.75	a
K_3W_2	245.00	b
K_4W_1	318.75	ab
K_4W_2	381.25	ab

From the results of the average pest mortality and crop damage intensity it can be seen that the treatment of Noni fruit extract is still optimal to control *C. binotalis*. But it can be tried by combining noni fruit with other bio pesticide ingredients such as research that has been done (Candra, 2016) wherein the 15% concentration of combination of chirinyuh extract (*Chromolaena odorata*) and crevice (*Mikania micrantha*) is effective against *Crocidolomia pavonana* with a percentage mortality of 56, 66% and can reduce the percentage of imago by up to 50%.

Effects of Interaction

Plant fresh weight.

The results of the DMRT found that the highest wet weight of the plant stover was found in the K2W1 treatment of 473.75 g and the lowest in the K0 treatment of 200 g. The 10 ppm treatment given before the pest was invested was able to maintain the wet weight of the plant stover from the attack of *C. binotalis*, this was supported by data on pest mortality in the K2W1 treatment mortality rate at 100% (Table 2). The interaction between the treatment of 10 ppm (K2) and the time of application before the pest is invested (W1) is effective in killing pests before the level of attack. Resulting in damage especially to the plant stover. Whereas in the treatment of K0 without spraying the lower stover with noni fruit extract, the low weight of plant stover was caused by the larvae growing and developing damaging the cabbage plants so that it would inhibit the formation of cabbage krob as a result of decreased plant weight supported by treatment data K0 on the lowest average pest mortality data.

CONCLUSION

The treatment of K2W1 is effective in controlling *C. binotalis* because it manage to produce the highest plant stover wet weight of 473.75 g. From the combination of Concentration treatments (K) and Application Time (W) K2W1 treatment tends to produce good result due to its ability to produce a mortality percentage of 100%. The combination of Concentration treatment (K) and Application Time (W) K2W1 treatment tends to produce good result because it can reduce the intensity of crop damage. The lowest damage intensity is measured at 52.57%.

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