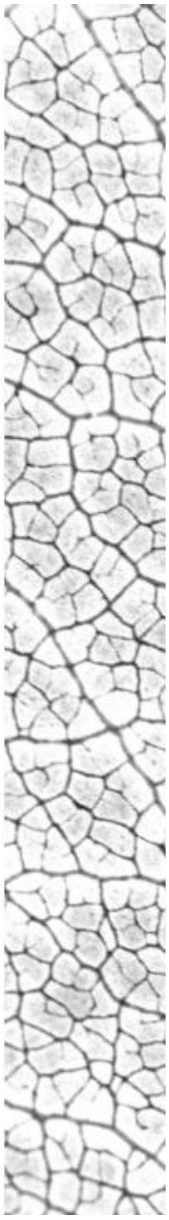

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Toxicity and feeding inhibition of neem leaf extract (Azadirachta indica) against Plutella xylostella larvae on cabbage

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Abstract
Cabbage (*Brassica oleraceae* L.), one of the *Brassicaceae* family, was widely planted in Central Sulawesi and was favored by the community. One of the main pests that attacks the Brassicaceae family is *Plutella xylostella* and it can cause damage to cabbage plants ranging from 85% - 100%. Neem leaf extract contains azadirachtin compound which is toxic and can affect the development of live larvae of *P. xylostella* larvae in test insects. The objective of this research was to determine the toxicity, feeding inhibition and morphological changes in larvae after the application of neem leaf extract. This research was done at the laboratory of pest and plant disease, faculty of agriculture, Tadulako University from September 2018 to February 2019. This research used the Sandwich method, consisted of 5 treatments, namely 0% concentration (control), 15%, 25%, 35%, and 45% which were repeated 3 times each, and the number of larvae for each treatment used 20 tails (3 instar larvae were used). Variables observed were larvae mortality, feeding inhibition, and morphological changes of larvae. The results showed that neem leaf extract could cause mortality of *P. xylostella* larvae by 50% with LC_{50} value of 20.73%, and, at 45% concentration, larvae mortality reached 95%. Moreover, it could inhibit the activity of feeding larvae with a value of R^2 0.96 or 96% and affect the morphological changes of larvae, which turned from bright green to pale green with a body shape that was short and looked swollen at the abdomen.

Keywords: cabbage plant, extract, *azadirachta indica, plutella xylostella*

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INTRODUCTION

The cabbage plant of *Brassica oleraceae* L., one of Brassicaceae family, is a type of commercial vegetable plant that has a high enough economic value and is favored by the community as a fresh or processed food (Sousa et al. 2008; Ahuja et al. 2010; Santos et al. 2018). The cabbage was widely planted in Central Sulawesi and had greater productivity than other vegetables (BPS 2019). The cultivation of cabbage plants was highly susceptible to pest attacks and diseases. One of the main pests that attack cabbage plants is *Plutella xylostella*, an oligophagous insect pest (Sarfraz et al. 2006). *P. xylostella* tends to distribute at temperatures between 6 and 31^0C and in proportion to the availability of host plants (Furlong et al. 2013).

P. xylostella pests have high reproductive rates, high adaptation rates, and can travel long distances (Furlong et al. 2013; Philips et al. 2014). In addition, since they exhibit rapid resistance to new insecticides, it was difficult to control them chemically, and ecological control became important (Grzywacz et al. 2010; Newman et al. 2016).

Farmers in Central Sulawesi, in controlling cabbage plant pests, still used chemical insecticides with relatively high concentrations and doses. About 80% of cabbage farmers still believed that chemical pesticides could lower yield losses and quickly control pests and diseases (Shahabuddin and Anshary 2010). If this was allowed to continue, it would result in a number of negative impacts such as pest resistance, pest resurgence, and pollution (Shahabuddin and Anshary 2010). This practice was a serious problem that caused an increase in production costs due to insecticide applications and production losses. Besides, the use of chemical insecticides is harmful to human health and agroecosystems (Azevedo et al. 2013).

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Indonesian government regulation number 6 of 1995 article 3 stipulated that plant protection was done through the Integrated Pest Control system. Furthermore, in article 19, it is stated that the use of pesticides in the context of controlling plant-disturbing organisms is the last alternative and the impacts caused must be minimized. Therefore, it was necessary to find effective ways of controlling targeted pests that were safe for non-target organisms and the environment. One of the pesticide groups that meets these requirements are pesticides derived from plants (vegetable insecticides) (Isnaini et al. 2015).

In general, the vegetable insecticide is defined as an insecticide whose basic ingredients are from plants and which is relatively easy to make with limited ability and knowledge. The nature of vegetable insecticides makes them generally not harmful to humans or the environment and easily biodegradable compared to chemical insecticides (Azevedo et al. 2013).

One of the natural ingredients that can be used is neem leaf. Neem leaf (*Azadirachta indica* A. Juss) has pesticide and therapeutic properties (Kuma and Navaratnam 2013). Based on phytochemical content analysis, there are compounds in the neem leaf vegetable pesticide that have insecticidal ability, namely alkaloids, flavonoids, tannins, and kuinons (Javandira et al. 2016). According to Ardiansyah et al. (2002), neem leaf extract was toxic in mulberry snail tillers with a mortality rate of tillers reaching 98.35% at a concentration of 27.5%. The greater concentration of neem leaf extract caused greater mortality of mulberry snail tillers. The 24 hours LC_{50} value of neem leaf extract against mulberry snail tillers was 25.65%. According to Prates et al. (2003), neem leaf extract could be as effective as chemical insecticides and could overcome insecticide resistance. Neem leaf extract has been shown to be effective against larvae and cocoons of fruit flies (Di Ilio et al. 1999; Singh 2003; Alvarenga et al. 2012).

This research aimed to analyze the toxicity, feeding inhibition, and morphological changes of larvae (color, shape) after the application of neem leaf extract on *P. xylostella* larvae. This information would help predict the concentration of neem leaf extract in controlling *P. xylostella* larvae. This finding could result in a decrease in the use of chemical pesticides by farmers, which could then reduce production costs and environmental pollution as well as increase the quality of cabbage and competitiveness in the market.

MATERIALS AND METHODS

Study areas

This research was done at the laboratory of pest and plant disease, faculty of agriculture, Tadulako University Palu, from September 2018 to February 2019. The tools used in this research were 7 cm × 20 cm polybags, scales, shakers, filters, beaker glass, markers, blenders, knives, label paper, Buchner funnels, rotary evaporators, gauze, wire, tissue paper, stirring rods, plastic containers measuring 36 cm x 9.5 cm, measuring cups, stationery, and cameras. The ingredients used were aquades, methanol, 10% honey, three instar *P. xylostella* larvae, neem leaf, and mustard plants.

Research procedure

Toxicity test and feeding inhibition of neem leaf extract: The toxicity test and the feeding inhibition of neem leaf extract against *P. xylostella* larvae were conducted using the Sandwich method or feed dip method. The feed used was fresh mustard leaves measuring 5 cm × 5 cm dipped in each concentration for ± 5 minutes then air-dried for ± 2 minutes. The test was done using a plastic container measuring 36 cm wide and 9.5 cm high. The larvae used were the second generation (F2) of three instars from the test insect rearing result.

Propagation of *P. xylostella* larvae: *P. xylostella* larvae were propagated by taking as many as possible *P. xylostella* larvae from the farmer's field (cabbage plantation land). The larvae were kept until they became imago. Imago (male and female) were put into insect care boxes that contained mustard plants in polybags; as imago, the feed given was 10% honey feed dripped on cotton. Imago were kept until laying eggs. Then, the eggs produced by the imago were transferred to a rearing container and kept until they hatched into larvae. The larvae were kept by being given mustard leaves feed until the three instar larvae, the second generation (F2), were produced and used a test insects.

Extract making: Making of the neem leaf extract was modified from Shahabuddin and Anshary (2010). The neem leaf was cleaned and then dried in the oven for 24 hours at 40 ^{o}C. The neem leaf, after drying in in the oven, was then blended to become powder. Once the neem leaf powder weighed as much as 250 grams, it was then soaked using methanol solvent for 2 × 24 hours. The immersion results of neem leaf extract were then filtered using a Buchner funnel that was fitted with a filter paper and evaporated using a rotary evaporator so that the concentrated extract was produced. The concentrated extract was then diluted simultaneously with a water solvent according to the tested concentration (treatment).

Observation variable

1. *P. xylostella* larvae mortality. Observation of larvae mortality was done for 7 days after treatment.

2. Feeding inhibition of larvae. Percent of feeding activity inhibiting of larvae was calculated using a formula without choice (Mendes et al. 2016).

$$F = \frac{W-D}{W} X100\% \quad (1)$$

Where:

F = feeding inhibition (%)

W = weight of eaten control leaf (g)

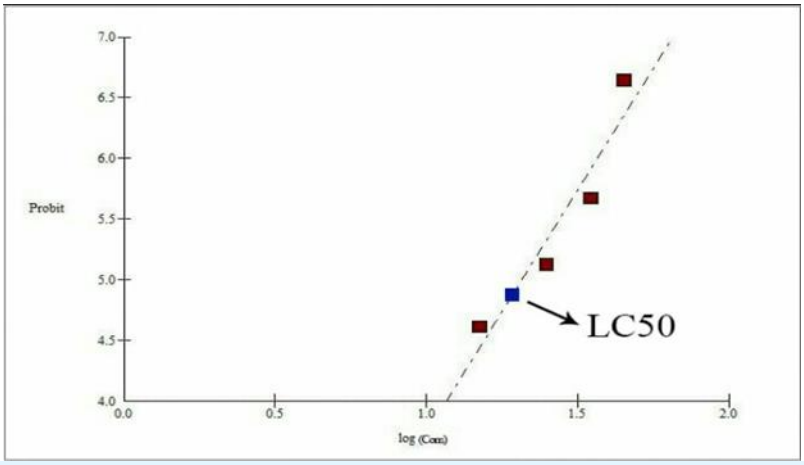

Fig. 1. *P. xylostella* larvae mortality probes with various concentrations of neem leaf extract

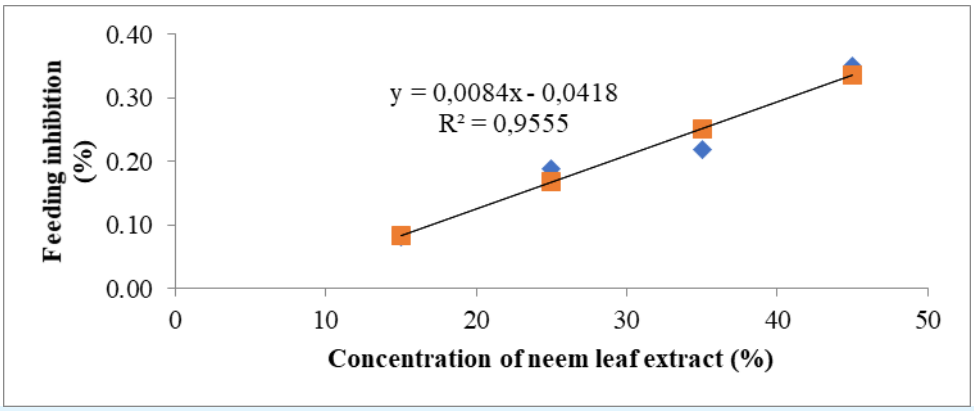

Fig. 2. The relationship between the concentration of neem leaf extract on the feeding inhibition of *P. xylostella larvae*

D = dried weight of eaten treatment leaf (g)

3. Morphology of larvae (color, shape, and behavior). Morphological observation of the test larvae was done by observing changes in the color, shape, and feeding behavior of the larvae after given treatment (application).

Data analysis

The observation result of feeding inhibition was tested using regression analysis. To find out the relationship of the concentration of neem leaf extract to the feeding activity of *P. xylostella* larvae and the observation result of *P. xylostella* larvae mortality, probit analysis was used by using Hsin Chi application (Chi, 1997).

RESULTS

Toxicity of neem leaf extract on the mortality of P. xylostella larvae

The observation results of toxicity of neem leaf with a concentration of 0%, 15%, 25%, 35%, and 45% against the mortality percentage of *P. xylostella* larvae on the first day to the seventh day showed the value of LC_{50} (Lethal Concentration) was 20.73% with a confidence interval 95%. LC_{50} values of neem leaf extract were found in concentrations between 15% and 25% with larvae mortality percentage of 35% to 55%, whereas, at the highest concentration of 45%, larvae mortality reached 95% (**Fig. 1**). The value of the regression equation between the concentration log of the neem leaf extract and the larvae mortality probit is Y = 0.185 + 3.656X. This shows that the concentration treatment of neem leaf extract had a strong effect on the mortality of *P. xylostella* larvae.

The feeding inhibition of neem leaf extract against P. xylostella larvae

The test results of feeding inhibition show that the concentration of neem leaf extract used had a very close relationship to the feeding activity of *P. xylostella* larvae. Based on the results of the regression analysis obtained, the regression equation model Y = 0.0084X - 0.0418 with a correlation value (R) of 0.98 (**Fig. 2**). This shows that there was a strong relationship between the concentration of neem leaf extract and the feeding inhibition of *P. xylostella* larvae. The determinant coefficient value (R2) 0.96 (96%) shows that the feeding inhibition of *P. xylostella* 96% larvae was affected by neem leaf extract.

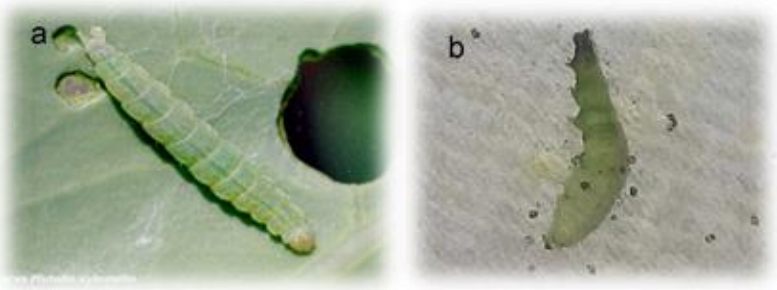

Fig. 1. (a) *P. xylostella* larvae before the application of neem leaf extract, (b) *P. xylostella* larvae after the application of neem leaf extract on the second day.

Morphological changes of P. xylostella larvae after the application of neem leaf extract

The results of the analysis show that the use of neem leaf extract could affect changes in color and shape, decrease feeding activity, and change larvae motion activity. The difference of larvae before and after the application of neem leaf extract can be seen in **Fig. 3**.

DISCUSSION

The observation results show that the mortality rate of *P. xylostella* larvae reached 95% at 45% concentration with LC_{50} of 20.73%. This result shows that neem leaf extract was toxic to *P. xylostella* larvae. The higher the concentration of neem leaf extract, the more active ingredients contained in it, so the more effective the killing power on the tested larvae. This is in line with the findings of Ardiansyah et al. (2002) and Setiawan (2010), that the higher concentration of neem leaf extract caused the death of mulberry snail tillers, and the warehouse pest insects of *Sitophilus oryzae* Linn were getting bigger. This mortality was caused by the active ingredient of azadirachtin in neem leaf which could affect the development of larvae life (Boeke et al. 2004; Alvarenga et al. 2012). Toxins contained in neem leaf affected the process of food digestion, inhibiting intestinal contraction so that the process of food digestion could not take place (Ardiansyah et al. 2002). The use of neem leaf extract was a promising tactic in the integrated pest management program of *P. xylostella*.

The administration of neem leaf extract generally disrupted the process of development of *P. xylostella* larvae because the toxins contained in neem leaf would cause disruption of feeding activity and larvae behavior. Disruption of larvae feeding activity began to appear after 1 × 24 hours of administration of neem leaf extract. The results of the regression analysis with the determinant coefficient value of (R^2) 0.96 (96%) with a correlation value (R) 0.98 means a positive and very strong correlation. This means that the higher the concentration the greater the percentage of feeding inhibition of larvae. Decreasing the feeding activity of larvae slowly would cause death. According to Dewi et al. (2017), deaths experienced by all larvae occurred as a result of the presence of secondary metabolites. In the neem plant, which functions as an insecticide, that is Azadirachtin which is formed naturally in the form of substances that are included in the class of organic molecule of tetranortriterpenoids. Compound of Azadirachtin neem leaf is one type of compound that is quite active but does not directly kill insects; it does so eventually through a mechanism of refusing food, disrupting the growth and reproduction of insects (Sumaryono et al. 2013). In the neem leaf extract, there is an Azadirachtin compound which is an appetite suppressant and inhibitor of growth hormone, so, eventually, the insect will die.

The observation results show that there were differences in the characteristics of *P. xylostella* larvae before the application and after the application. Before the application of neem leaf extract, the larvae motion was very active, and, within 1 × 24 hours after the application of neem leaf extract, the larvae motion activity became slower and, eventually, the larvae remained silent and did not eat. Morphological changes of *P. xylostella* larvae after the application of neem leaf extract were characterized by changes in skin color, initially colored bright green to pale green, and changes in body shape, that swelled then shrank and become destroyed. According to Dewi et al. (2017), treatment with neem leaf of 25% concentration was able to give a feeding repellent effect so that the death occurred of all larvae on the 7th day. Azadirachtin in neem leaf could inhibit the ecdysone hormone in insects. The ecdysone hormone in insects helps in the formation of new cuticles and their enzymes affect the exfoliation of the skin. If there were insects that were exposed, then the ecdysone hormone would be inhibited; this hormone is very important for the growth and development of insects. When the insect is ready to change but the hormone to molting is not formed, eventually the insect's life cycle is disrupted, and, usually, a failure in this process often also results in the death of the insect. According to Debashri and Tamal (2012), neem leaf contained four natural chemical compounds that were active as pesticides, namely azadirachtin, salanin,

meliantriol, and nimbin. Azadirachtin, that did not directly kill insects, but, through the mechanism of refusing food, disrupted the growth and reproduction of insects. Salanin worked as an inhibitor of feeding insects; nimbin worked as an anti-virus and meliantriol as an insect repellent.

CONCLUSION

Neem leaf extract was toxic to *P. xylostella* larvae test insects and could cause mortality of 50% with LC_{50} value of 20.73%; at a concentration of 45% larvae mortality reached 95%. Application of neem leaf extract with feed dip method (Sandwich) was able to inhibit the feeding activity of *P. xylostella* larvae up to 96%. In addition, it could also disrupt the motion activity of *P. xylostella*, as larvae became slow, and cause changes in the color and shape of larvae body size.

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