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Effectiveness of Bitter melon Leaf and Fruit Extract (*Momordica charantia* L.) and its Combination on Antifeeding Activity and Mortality of *Plutella xylostella*

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Abstract
Plutella xylostella is a pest that attacks plants from the Brassicaceae family. Bioinsecticides from bitter melon leaf and fruit extracts are one alternative to control *P. xylostella*, because they contain alkaloids, flavonoids, saponins, tannins, and the main compound is momordicin. This study aims to determine the effectiveness and interaction between the type and concentration of bitter melon leaf and fruit extracts and their combinations that have the most effect on antifeeding activity and mortality of P. xylostella. The research method used a two-factor Completely Randomized Design (CRD), namely 20%, 25%, and 30% extract concentrations, negative control (distilled water), positive control (permethrin), and types of bitter melon leaf and fruit extracts and their combinations. Data were transformed into Arcsin, then analyzed using Kolmogorov-Smirnov, homogeneity, Two Way ANOVA, and Duncan tests. The results showed the effect of extract type, extract concentration, and their interaction on anti-feeding activity and mortality of *P. xylostella*. The most effective treatment was the 30% combination extract because it produced a mortality value of 83.33% and inhibition of feeding activity reached 37.67%. This research can be a new alternative bioinsecticide that is environmentally friendly to control *P. xylostella.*

Keywords: bioinsecticide; bitter melon extract; *Plutella xylostella*; protected flora
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INTRODUCTION

Cultivation of cabbage plants (Brassicaceae) often experiences problems, one of which is the presence of pest attacks. Pests are disturbing organisms that can attack and cause mild or severe plant damage until crop failure (Safirah *et al.*, 2016). One pest that attacks cabbage plants is the tritip caterpillar (*Plutella xylostella*) (Awalliyah *et al.,* 2022). Pest *P. xylostella* is oligophagous because it attacks several species of plants in the same family (Susniahti *et al.*, 2017).

P. xylostella feeds on the leaves of its host plant. Early instar larvae eat the lower part of the leaf to leave the epidermal layer, resulting in holes after the epidermal layer dries up (Awalliyah *et al.*, 2022). *P. xylostella* pests cause plant damage, up to 100% crop failure (Lestariningsih *et al.*, 2020). Damage to cabbage plants caused by *P. xylostella* attacks usually occurs in the dry season (Lina, 2016).

Generally, pest control of *P. xylostella* is carried out using chemical insecticides because they have the advantage of being able to kill pests in a fast period. However, chemical insecticides can cause environmental pollution and health problems, make non-target animals die and become extinct, and destabilize the ecosystem on agricultural land (Dhiaswari *et al.*, 2019). Thus, it is necessary to develop other alternatives, one of which is using insecticides from natural ingredients or so-called bioinsecticides. The advantages of using vegetable insecticides are that they only kill the target organism and do not pollute the environment because the residue produced quickly decomposes in nature (Wulandari *et al.*, 2012).

All plants produce secondary metabolites. One of the plants that produce secondary metabolite compounds and can have potential as a bio-insecticide is a bitter melon (*Momordica charantia* L.) both in the leaves and fruit. Active compounds present in bitter melon leaves are alkaloids, flavonoids, saponins, momordicin, phenolic acid, and carotenoids.

Alkaloid, flavonoid, and saponin compounds are toxic compounds that can cause mortality in larvae (Rama, 2018). Bitter melon leaf extract can control *C. pavonana* pests up to 70% (Hasnah *et al.*, 2013). Bitter melon contains secondary metabolite compounds in the form of alkaloids, flavonoids, saponins, steroids, tannins, essential oils, triterpenoids (cucurbitacin), momordica acids (momordicin and momordin), charantin, polyphenols and lipids that can function as natural insecticides (Kumar *et al.*, 2010). The bitter melon extract can cause the mortality of *S. litura* by 62.87% (Lidyawatie, 2016).

Bitter melon leaves and fruits each contain secondary metabolite compounds that act as antifeedants and are toxic so that they can inhibit feeding power and cause mortality of *P. xylostella.* Alkaloids can reduce appetite in larvae (Octaviana *et al.,* 2020). Alkaloids can damage cell membranes and affect the nervous system by inhibiting the work of the enzyme acetylcholinesterase (Prakoso *et al.,* 2017). Saponins act as antifeedants as it can irritate the stomach of insects (Desiyanti *et al.,* 2016). Tannins can inhibit protease enzymes, disrupting larval digestive system (Sari & Isworo, 2020). On the other hand, flavonoid compounds can cause mortality in larvae (Kasman *et al.*, 2020), while momordicin is an antifeedant that acts as stomach poisoning (Syam & Pawenrusi, 2015).

Bioinsecticidal potential of single extract of bitter melon leaves and bitter melon fruit has been studied prior. Bioinsecticidal extracts from bitter melon leaves (*M. charantia* L.) with methanol solvent at a concentration of 25% can control *C. pavonana* pests up to 70% (Hasnah *et al.*, 2013). However, the combination of leaf extracts with bitter melon fruit has never been studied. Combining several plant parts with toxic properties for pests can increase the effectiveness of bioinsecticides. Using natural insecticides in the form of a combination of two or more types of synergistic plants can optimize the use of plants and reduce dependence on one type of plant (Syahroni & Prijono, 2013). Synergistic effects can be raised because the content of compounds between bitter melon leaves and bitter melon fruit has similarities, such as alkaloid compounds, flavonoids, saponins, and triterpenoids. Although they have similar compound contents, the levels of compounds in bitter melon leaves and fruits are different. According to previous research, flavonoid levels contained in bitter melon fruit was up to 17.702 mg/g (Sugiyanto & Anisyah, 2022), while in bitter melon leaf was 2.75% (Azizah *et al.,* 2018).

Previous research on the combination extract between bitter melon fruit and avocado leaves effectively killed *A. aegypt* larvae was the comparison of P1: E1 with an average Wallis crucial test level of 9.88 and an LC50 value of 1189.54 ppm (0.119%). Compounds found in bitter melon fruit are more influential than compounds in avocado leaves (Sajati *et al.*, 2020). Using bioinsecticides can prevent adverse effects on non-target organisms and reduce the possibility of pest resistance (Hendrival *et al.*, 2017).

This study aims to test the effectiveness of bitter melon (*M. charantia* L.) leaf and fruit extracts and their combination at various concentrations on the anti-feeding activity and mortality of *P. xylostella* larvae, determine the most effective concentration and compare the effectiveness of secondary metabolite compounds from two different organs in one plant species.

MATERIALS AND METHODS

This research is an experiment using a completely randomized design (CRD) with two treatment factors: extract concentration and extract type. The extracts used were bitter melon leaf extract, bitter melon fruit extract, and a combination of both. The concentrations used were 20%, 25%, and 30%. There are two observation parameters: observation of anti-feeding activity for 24 hours and observation of mortality of *P. xylostella* for 7 days. The research was conducted in Plant Physiology Laboratory C14 and Basic Biology Laboratory C10, Department of Biology, FMIPA, Unesa, for 6 months from January to June 2024.

This study consisted of stage I, which was the observation of the anti-feeding activity of *P. xylostella* 24 hours after treatment, and stage II, which was the observation of mortality of *P. xylostella* for 7 days. Each study stage had 33 sample units consisting of 27 extract treatment units, three negative control units (distilled water), and three positive control units (permethrin). The application method used was the mustard leaf feed-dipped method. This study used three replicates, and each replicate consisted of 10 test containers. Each test container contained 1 *P. xylostella* instar III larvae.

This study began with the collection of fresh samples of bitter melon leaves and fruits taken from plantations in Made Village, Sambikerep, Surabaya. Then, the samples were washed, dried, and mashed into powder. Powder was macerated for 3 days in a ratio of 1:3, 1:2, and 1:2 using 96% ethanol. The filtrate obtained from the maceration results evaporates using a rotary vacuum evaporator to produce a thick extract. The evaporated extract was diluted using distilled water before application. Dilution of single extracts of bitter melon leaves and bitter melon fruit for a concentration of 20% consists of 20 grams of extract and 80 ml of distilled water, 25% concentration consists of 25 grams of extract and 75 ml of distilled water, and for 30% concentration consists of 30 grams of extract and 70 ml of distilled water.

The combined extracts were diluted by mixing bitter melon leaf extract and bitter melon fruit in a ratio 1 by 1. The 20% concentration consists of 10 grams of bitter melon leaf extract and 10 grams of bitter melon fruit extract, the 25% concentration consists of 12.5 grams of bitter melon leaf extract and 12.5 grams of bitter melon fruit extract, the 30% concentration consists of 15 grams of bitter melon leaf extract and 15 grams of bitter melon fruit extract. The amount of distilled water is the same as the single extract dilution.

The calculation of stage I is obtained from the weight of the remaining leaves that have been weighed after 24 hours of treatment. The observation results of the anti-feeding activity parameters were analyzed using the following formula (Baskar *et al.*, 2010).

$$A = \frac{C-T}{C} x\ 100\%$$

Description :
A = percentage of *Antifeeding* activity (%)
C = initial weight of leaves before treatment (g)
T = final weight of leaves after treatment (g)

Mortality of *P. xylostella* larvae is indicated by changes in the visual characteristics of the larvae, such as body color and shape. Data from the observation of mortality parameters is the number of P. xylostella deaths averaged in each treatment and repetition. Mortality of *P. xylostella* can be calculated using the following formula (Siahaya & Rumthe, 2014).

$$M = \frac{d}{N} x\ 100\%$$

Description :
M = percentage of Pest Mortality (%)
d = number of dead pests
N = total number of pests tested

The data obtained in this study were the percentage value of anti-feeding activity and the percentage of mortality of *P. xylostella.* The data were transformed into Arcsin form and then analyzed using SPSS 23.0. The data was analyzed statistically using Kolmogorov-Smirnov normality test, homogeneity test, Two Way ANOVA test, and Duncan test at the 0.05 level to determine whether the data were statistically significantly different.

RESULTS

The results of this study are in the form of observation data on anti-feeding activity and mortality of *P. xylostella*. In stage I, data on the percentage of anti-feeding activity was obtained by calculating the weight of the remaining leaves eaten by *P. xylostella*. The initial weight of the feed leaves given was 0.1 grams. In stage II, mortality percentage data were obtained by calculating the number of *P. xylostella* that died every day, observed every 24 hours for 7 days.

Data on anti-feeding activity is typically distributed as evidenced by sig > 0.05 in the Kolmogorov-Smirnov test. The Two Way ANOVA test results obtained a significance value <0.05. Based on the significance value, it can be stated that there is an effect of the extract and extract concentration, and there is an interaction between the type of extract and extract concentration, with the best results being in the treatment of combined extracts with a concentration of 30%.

Table 1. Effect of various types of extracts on the anti-feeding activity of *P. xylostella* 24 hours after application

Treatments	Anti-feeding activity 24 hours after application (%) ± SD		
	20%	25%	30%
Bitter melon leaf extract	7.67 ± 0.01[b]	8.67 ± 0.02[bc]	12.33 ± 0.02[cd]
Bitter melon fruit extract	10.33 ± 0.02[bcd]	13.00 ± 0.02[d]	14.67 ± 0.01[de]
Combination extract	18.00 ± 0.02[e]	32.67 ± 0.06[f]	37.67 ± 0.04[f]
K- (aquadest)	-	0.00 ± 0.00[a]	-
K+ (permethrin)	-	100.00 ± 0.00[g]	-

Description : Letter notation shows a significant difference based on the results of Duncan's test (α = 0.05).

Table 1 shows the results of Duncan's test observing anti-feeding *P. xylostella*. Duncan's test showed that all treatments had a significantly different effect from the K- and K+ treatments as indicated by different letter notations from all treatments. In the treatment of 20% and 30%

concentrations, bitter melon leaf extract was not significantly different from bitter melon fruit extract but significantly different from the combination extract. At 25% concentration, all treatments were significantly different. The combination extract at 25% concentration was not significantly different from the combination extract at 30% concentration. The lowest percentage of antifeedant activity was the 20% bitter melon leaf extract treatment, which amounted to 7.67%, and the highest was the 30% combination extract treatment, which amounted to 37.67%. An extract is said to be active as an antifeedant if the percentage is above 25% (Mandana *et al.*, 2013).

Based on the result of statistic test, there was an effect of the type and concentration of extracts on antifeeding activity, with the best results from extract combination at concentration 30%. There was an interaction between extract type and concentration of bitter melon, resulting in extract combination had higher antifeeding activity of *P. xylostella*.

Table 2. Test results of various types of extracts on mortality of *P. xylostella* for 7 days

Treatments	**Percentage of Mortality *P. xylostella* (%) ± SD**		
	20%	**25%**	**30%**
Bitter melon leaf extract	36.67 ± 0.58^{b}	40.00 ± 1.00^{bc}	46.67 ± 0.58^{bc}
Bitter melon fruit extract	43.33 ± 1.15^{bc}	46.67 ± 0.58^{bc}	$53.33 \pm 0.58c^{d}$
Combination extract	60.00 ± 1.00^{d}	73.33 ± 0.58^{e}	83.33 ± 0.58^{e}
K- (aquadest)	-	0.00 ± 0.00^{a}	-
K+ (permethrin)	-	100.00 ± 0.00^{f}	-

Description : Letter notation shows a noticeable difference based on the results of Duncan's test (α = 0.05).

Table 2 shows the results of Duncan's test on the observation of mortality of *P. xylostella*. Duncan's test showed that all treatments had a significantly different effect from the K- and K+ treatments as indicated by different notations from all treatments. In the treatment of 20%, 25%, and 30% concentrations, bitter melon leaf extract was not significantly different from bitter melon fruit extract but significantly different from the combination extract. The combined extract of 25% concentration was not significantly different from the combined extract of 30%.

The lowest mortality percentage was the bitter melon leaf extract treatment with a concentration of 20% at 36.67%, and the highest was the combined extract treatment with a concentration of 30% at 83.33%. The higher the concentration, the higher the mortality percentage value. An extract can be said to be effective as a bioinsecticide if it can cause mortality of test larvae with a mortality percentage of 80-90% (Supriadi, 2013). Pest control efforts are carried out by controlling the pest population to obtain a mortality value that is higher than the level of plant damage. Prevention of plant damage is more important than killing 100% of pests, so the effective treatment as a bioinsecticide is the combined extract treatment at a concentration of 30% because it has been able to cause larval mortality of 83.33% and close to K+ (permethrin).

Based on statistic test, there was an effect on the type and concentration of extracts, with the best result being the combination of extracts with a concentration of 30%. Interaction was found between the type and concentration of bitter melon leaf and bitter melon fruit extracts, and the combination of both has an effect on the mortality of *P. xylostella.*

DISCUSSION

The results of research that has been conducted on the effectiveness test of bitter melon leaf extracts, bitter melon fruit, and their combination on the anti-feeding activity and mortality of *P. xylostella* show that the most effective type of extract is the combination extract with a concentration of 30% which can produce a percentage of anti-feeding activity of 37.67% and mortality of 83.33%.

The increase in the percentage of anti-feeding activity and mortality of *P. xylostella* is influenced by the type of extract and the concentration of extract used. The higher the extract concentration, the greater the amount of active ingredients contained, so the higher the larval mortality (Yudiawati, 2019). Metabolite compounds accumulated in the extract can inhibit larval development through several compounds that are toxic or antifeedant (Sonia *et al.*, 2017).

The active compounds contained in bitter melon leaves and fruits are the same and different. Differences in the types and levels of active compounds in each organ in the same plant species are caused by differences in the biosynthesis process of metabolites in each different plant organ (Maslakhah, 2018). Bitter melon leaves contain active compounds of alkaloids, momordicin, flavonoids, saponins, triterpenoids, phenolic acids, and caratenoids (Rama, 2018). In comparison, the bitter melon fruit contains secondary metabolite compounds in the form of alkaloids, flavonoids, steroidal saponins, tannins, essential oils, triterpenoids (cucurbitacin), momordica acid (momordicin, momordin), charantin, polyphenols and lipids that can function as natural insecticides (Kumar *et al.*, 2010). These compounds act as antifeedants and can cause mortality in larvae. The function and mechanism of these active compounds vary in affecting larval survival (Maula & Adi, 2021).

Antifeedant compounds include alkaloid compounds, saponins, tannins, and momordicin. Alkaloids, as stomach poisons, can reduce appetite in larvae so that larvae starve and die slowly (Octaviana *et al.*, 2020). Saponins act as antifeedants and can irritate the stomach of insects (Desiyanti *et al.*, 2016). Tannins can inhibit the work of protease enzymes, causing disruption of the digestive system in larvae and then making larvae experience mortality (Sari & Isworo, 2020). Momordicin is the main compound in bitter melon leaves and bitter melon fruit. Momordicin compounds can inhibit the ability to eat larvae (Hasnah *et al.*, 2013).

Other toxic compounds that can cause mortality in larvae are flavonoids, triterpenoids, and essential oils. Flavonoids act as contact and respiratory toxins (fumigants). Flavonoids penetrate larval nerve cells and reduce the work of the respiration system so that O2 levels in the larval respiratory tract decrease. This finding is supported by Ervina et al. (2014), who state that flavonoid compounds are respiratory poisons that, if inhaled or ingested by larvae, can cause larvae to have difficulty breathing and eventually die. Triterpenoid compounds with cucurbitacin core are repellent to insects, so that these compounds can be utilized as natural insecticides (Budianto & Tukiran, 2012). Following research on biopesticides of tapak liman extract conducted by Yuliani (2022), the results showed that plants containing metabolite compounds such as alkaloids, tannins, saponins, and flavonoids can affect the mortality rate of *P. xylostella* by 96.65%. Essential oils found in bitter melon fruit play a role in inhibiting the work of the nervous system through contact poisons, stomach poisons, and respiratory poisons (fumigants) so that they can cause larvae to die (Wuragil *et al.,* 2019).

The single extract treatment of bitter melon leaves and bitter melon fruit (*M. charantia* L.) produced a percentage of anti-meal activity and mortality that was not significantly different from the combined extract treatment. Based on the study's results, the most effective type of extract is the combined extract with a concentration of 30%. This result is because the performance of the combined extract is enhanced by each compound contained in bitter melon leaves and fruits, such as alkaloid compounds, saponins, tannins, flavonoids, momordicin, triterpenoids (cucurbitacin), each of which is toxic, antifeedant and repellant. The combination of two compounds synergize, increasing feeding resistance, growth inhibition, and mortality of larvae (Lumowa & Nurbayah, 2017).

Based on the observation of anti-feeding activity and mortality, in the negative control treatment (distilled water), all the feed leaves were consumed by *P. xylostella,* and no larvae died until the end of observation. This finding follows the statement of Ningsih *et al.* (2016) that the negative control treatment (distilled water) does not contain active substances that inhibit eating activity so that pests can eat feed leaves as usual without any obstacles. Whereas in the positive control treatment (permethrin), the leaves were not eaten at all by the larvae, all larvae experienced mortality due to the content of synthetic insecticide compounds made from the active ingredient permethrin, which is very toxic so that the larvae do not eat the feed leaves at all and cause the larvae to experience rapid mortality. This result is due to permethrin, which can damage the insect nervous system and cause muscle spasms, paralysis, and death for insects (Toynton *et al.*, 2009).

Morphological characteristics when larvae experience inhibition of feeding activity after being treated with extracts are that the larvae move slowly or do not move, the body color becomes blackish brown when given a touch stimulus, and the larvae will bend their bodies (Cania & Setyaningrum, 2013). In Figure 4 (c), it can be seen that the morphology of *P. xylostella,* which experienced mortality after being treated with the extract, showed an unpleasant-smelling liquid coming out of its body. The body turned blackish brown and flabby, shrunk, and dried up at the end of the observation. This result follows research by Hidayati *et al.* (2013) that *P. xylostella* larvae that experience mortality show the characteristics of their bodies drying out; the body becomes black, and the body size shrinks.

The percentage of the ability of bitter melon leaf extract and bitter melon fruit as a vegetable insecticide is still below the percentage of the positive control treatment (synthetic insecticide). However, bitter melon leaf extract and bitter melon fruit are relatively safe to be used as bioinsecticides because excessive use of synthetic insecticides can damage the environment, pollute the environment, and affect human health (Muhidin *et al.*, 2020). The 30% concentration combination extract is the most effective because it can cause mortality of *P. xylostella* by 83.33%. This result has met the requirements of a vegetable insecticide that is said to be effective. Following the statement of

(Supriadi, 2013). an extract can be categorized as an effective bioinsecticide if it can cause the death of test insects with a mortality percentage of 80-90%.

The use of plant-based insecticides in the field is an application of the concept of Integrated Pest Management (IPM) because the use of plant-based insecticides prioritizes controlling or reducing pest populations and preventing plant damage rather than directly killing 100% of pests (Diaz, 2011). This study aims to preserve the natural enemies of pests. Natural enemies or predators are important in controlling (biocontrol) pest populations (Amrullah, 2019). Using vegetable insecticides is expected to realize healthy and environmentally friendly agriculture.

Based on the results of the Duncan test, it is known that the combined extract treatment with a concentration of 25% and 30% is not significantly different. So, the most effective treatment among other treatments is the combined extract of 25% and 30% concentration. However, the most recommended type and concentration for implementing bioinsecticides is the combined extract with a concentration of 30% because it can control *P. xylostella* by 83.33%.

CONCLUSION

Based on the research, it was found that bitter melon leaf extract, bitter melon fruit, and their combination can inhibit feeding activity and cause mortality in *P. xylostella* larvae. So, it can be concluded that extract type, extract concentration, and the interaction of the two affect the mortality of *P. xylostella* larvae. The combination extract with a concentration of 30% is the most effective treatment to be used as a bioinsecticide to control *P. xylostella* because it produces a percentage of anti-feeding activity of 37.67% and mortality of *P. xylostella* of 83.33%.

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CONFLICT OF INTEREST

There is no conflict of interest.

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