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Application of Biopesticide with Active Ingredients Containing *Trichoderma* sp., *Streptomyces* sp., and Chitosan for Groundnut Aphid (*Aphis craccivora* Koch.)

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ABSTRACT

Indonesia is the second largest exporter of peanuts to Europe. One of the export requirements is that exported peanuts do not contain pests and high levels of pesticides. Aphis craccivora *Koch. is the main pest in peanuts and controlled generally using pesticides which cause high pesticide levels. The aim of this research is to determine the application technique for biopesticide containing enthomopathogen* (Streptomyces sp. *and* Trichoderma sp.) *and chitosan which were expected to control the population of the pest* Aphis craccivora *and the intensity of plant damage. The research was conducted and designed using factorial completely randomized design where the first factor was application time (before and after pest investment). The second factor was chitosan concentration of 0.6%, 0.9%, and 1.2% in potato-sugar extract solution. Each treatment combination was repeated 4 times. The results obtained that the pre-investment application technique with a chitosan concentration of 1.2% was more capable of incrasing the mortality of the pest* A. craccivora *by up to 70% and the application of biopesticide before investment with a concentration of 1.2% had a lower level of damage, namely 38.25%.*

1. INTRODUCTION

Peanuts (*Arachis hypogaea* L.) are a legume that is widely used by Indonesian people. Peanuts play an important role in meeting food needs which have quite high economic value. Peanut production in Indonesia tends to decline year by year. Based on data from the Center for Agricultural Data and Information System, Ministry of Agriculture (CADIS, 2024), peanut production in Indonesia had declined from 420,000 ton in 2019 to 350,000 ton in 2023. The rate of declining increased from –4.69%/year during 2021-2022 (CADIS, 2022) to –7,87 during 2022-2023 (CADIS, 2024). A decrease in peanut production can occur due to various factors. The decline in peanut production was mainly caused by the decrease in harvested area and attacks by plant pests (OPT) (Megasari *et al*., 2022).

Aphids (*Aphis craccivora* Koch.) are one of the pests that attack peanut plantations. Aphids love young shoots and petioles with symptoms such as stripes on the leaves in the form of dark green and light green, chlorosis, and can cause stunting. Aphids (*Aphis craccivora* Koch.) attack land plants in groups and suck the fluids of peanut plants because they are one of the pests with piercing-sucking mouthparts (Oka, 2015). Aphids act as vectors for the virus that causes peanut stripe disease and are carriers of various types of peanut diseases (Megasari *et al*., 2022).

The combination of *Streptomyces* sp. *Trichoderma* sp. can be used as an entomopathogen to control armyworm pests (*Spodoptera frugiperda*), fruit flies (*Bactrocera* sp.) with the chitinase enzyme which degrades the polysaccharide chitin during the insect's molting process (Suryaminarsih *et al*., 2019; Togola *et al*., 2020). This biopesticide used against the

pest N. viridula causes up to 60% death, symptoms of death due to chitin degradation in the stomach and chest (Suryaminarsih *et al*., 2022). Biocide with the active ingredient *Streptomyces* sp. *Trichoderma* sp. This can also increase plant growth and production, increase the number of peripheral roots, induce plant resistance with the ability to recover roots that experience necrosis (tissue death), increase the amount of alkaloids in tomato leaves/plants (Madhumitha & Sridhar, 2023). Several studies have found a combination of *Streptomyces* sp. *Trichoderma* sp. with production media reduces the population of insect pests on plants (M, Putri, 2023). The MOA of *Streptomyces* in producing chitinase can be induced by adding chitin (Soeka & Triana, 2016) and by providing an opponent in the form of *Trichoderma* sp. can increase chitinase production (Sowmya *et al*., 2012).

Biopesticide based on *Streptomyces* sp. and *Trichoderma* sp. is an organic pesticide that uses a concert between two microorganisms where each microorganism has the ability to control pest populations. If chitosan is added, its effectiveness can be increased. Biopesticide given to pests can act as stomach poisons and contact poisons, this depends on how the biopesticide are applied. Rajput *et al*. (2020) stated that contact poison works by degrading the chitin in the insect's cuticle so that it can make the insect paralyzed or have low mobility while stomach poison works by damaging the digestive system so that it makes the insect vomit and decreases appetite. The administration of biopesticide both directly and indirectly does not determine contact or stomach poison but rather the nature of the active ingredients contained in the biopesticide determines it. Fauzah (2021) shows that administering papaya leaf extract to *Aphis gossypii* directly can cause *A. gossypii* to decrease its appetite and make morphological changes. Research by Fauzana & Faradilla (2018) shows that giving “krinyuh” (*Eupatorium odoratum* L.) leaf extract to armyworm (*Spodoptera litura*) feed can cause its morphology to rot and its digestive system. Oka (2015) shows that administering a solution of papaya leaves (*Carica papaya* L.) directly to aphids (*A. craccivora*) can cause decreased mobility and undergo morphological deformation.

The aim of this research is to determine the application techniques and concentrations of biopesticide containing the active ingredients *Streptomyces* sp., *Trichoderma* sp. and chitosan which is needed for the mortality and intensity of damage to *Aphis craccivora* Koch. The benefit of this research is to provide another alternative in making biopesticide and using chitosan.

2. MATERIALS AND METHODS

This research was carried out in 2024. Research activities were carried out in the laboratory and greenhouse of the Agrotechnology Study Program, Faculty of Agriculture, National Development University "Veteran" East Java. The research was carried out using an experimental method arranged in a completely randomized design (CRD) with a factorial pattern consisting of two factors, namely application time (T) and concentration (K). The application time factor (T) consisted of 2 levels, namely, T1 (before vector infestation) and T2 (after vector infestation). The Concentration factor (K) consisted of 3 levels, namely, the biopesticide formula which contains the active ingredient *Streptomyces* sp. and *Trichoderma* sp., with chitosan concentrations of 0.6% (K1), 0.9% (K2) and 1.2% (K3) with 3 replications so there were 27 experimental units. In addition, a control without biopepticide application was observed.

The tools used in this research include an Olympus compound microscope model CX33RTFS2, micro pipette, Neubauer haemocytometer 0.100 m/0.0025 mm², autoclave, cork borer 0.5, Erlenmeyer, laboratory shaker. The materials used in carrying out this research included aphids (*Aphis craccivora* Koch.), Potato-Sugar Extract media, PDA media (Potato Dextrose Agar), GNA media (Glucose Nutrient Agar), biopesticide *Streptomyces* sp., *Trichoderma* sp. (3:1), chitosan, distilled water, and peanut seeds (TAKAR 2).

2.1. Biopesticide Preparation with *Streptomyces* sp. and *Trichoderma* sp.

Streptomyces sp. and *Trichoderma* sp. isolates were provided from the research collection of Dr. Ir. Penta Suryaminarsih, M.P. The growth medium used is ECG media (Potato Extract Glucose Agar, PEGA). The ECG media was made by cutting 250 g of potatoes, adding 1 L of water, adding 20 g of sugar and boiling it for 30 min then filtering and adding chitosan (0.6%, 0.9% and 1.2%) referring to research by Megasari *et al*., (2015). Finally, the ECG media was sterilized in an autoclave at a pressure of 1.5 atm and temperature 121°C for 30 min. The ECG media was inoculated with *Streptomyces* sp. and *Trichoderma* sp. and shaken in an orbital shaker for 10 days at a speed of 60/min.

2.2. Preparation for the Pest *Aphis craccivora* Koch. and Test Plants

Aphids (*Aphis craccivora* Koch.) were obtained from peanut fields. Maintenance and propagation of aphids were carried out by moving the aphids to the breeding site in a cage containing peanut plants as a propagation host. Aphids were reared until the aphid imago produces offspring. The aphid imago resulting from propagation was used in the treatment. Planting of test plants was carried out by planting peanut seeds in polybags measuring 10 × 15 cm. The planting medium used is a mixture of soil and compost in a ratio of 2:1. Two peanut seeds were planted in each polybag and the best plant was selected for treatment. Treatment was carried out when the peanut plants were 14 days after planting (DAP).

2.3. Biopesticide Application against *Aphis craccivora* Koch.

Treatment plants that were 14 HST old were sprayed with biopesticide containing *Trichoderma* sp., *Streptomyces* sp. and chitosan according to each treatment that has been given a research label. Two application time were investigated, namely before and after vector infestation. Application before infestation was carried out by spraying biopesticide *Trichoderma* sp., *Streptomyces* sp., and chitosan using a sprayer on peanut plants which were covered with a hood and covered with gauze. The aphid insects were then infested on the plants as many as 5 for 30 min. Application after infestation was carried out by infesting 5 aphids on the plants then spraying biopesticide *Trichoderma* sp., *Streptomyces* sp. and chitosan. Plants were covered and screened to prevent infestation by other insects. Spraying was done as much as 100 mL at a distance of about 30 cm so that the spray droplets formed are fine granules on the leaves. Each treatment consisted of 4 plants as replications. Observations of aphid mortality on treated plants were carried out at 24, 48, and 72 hours after treatment.

2.4. Observation Parameters

a. Mortality of Aphids *Aphis craccivora* Koch.

The mortality rate of aphids can be determined from the symptoms of death caused by chitin degradation. Trisnawati *et al*. (2019) said that changes in color and body odor of insects are caused by the chitinase enzyme which degrades insect morphology. Observations related to mortality symptoms and mortality rates (Z) were observed and calculated every 24 h for 3 days after application using the following formula:

$$Z = \frac{a}{b} \times 100\% \quad (1)$$

where *a* is number of dead aphids, and *b* is total number of aphids

b. Damage Intensity of Peanut Plants

The results of observations on plant damage caused by pests were used to calculate attack intensity (*I*) as follows:

$$I = \frac{\sum(n \times v)}{N \times V} \times 100\% \quad (2)$$

where n_i is the number of leaves showing the i score, v_i is score of the i^{th} leaf (i = 0 to 10), V is the highest score (10), and N is the number of leaves observed.

2.5. Data analysis

The research data was analyzed statistically using Analysis of Varience (ANOVA) to determine whether there was an effect of each treatment. If it was known that there is a significant effect from the treatment, a further test was carried out using the Honestly Significant Difference (HSD) test at the α = 5% level.

3. RESULTS AND DISCUSSION

3.1. Probit Analysis

Probit analysis showed that a dose LD_{50} of 6 ml per treatment was required to kill the *Aphis craccivora* Koch population in vitro. Probit analysis also showed that it takes 2.6 days to kill *A. craccivora* populations in vitro. Figure 1 shows that

the regression resulted in determination coefficient (R^2) of 0.9603 and *r* of 0.9799, concluding that there was a very strong relationship between probit mortality of *Aphis craccivora* Koch. and bipesticide dose treatment.

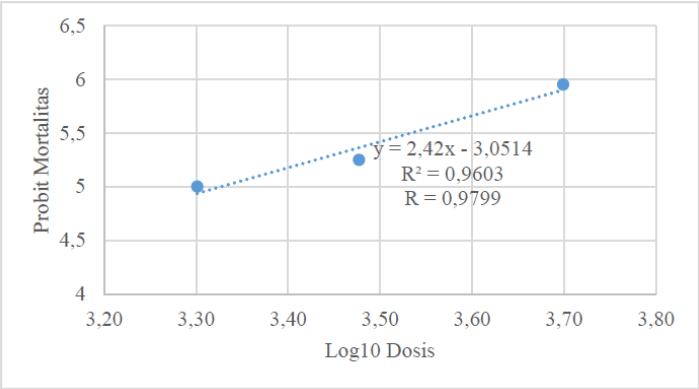

Figure 1. Graph of probit analysis of LD_{50} *A. craccivora* in vitro

The probit analysis can be influenced by several factors, such as the type of isolate, the test insects, and the growth medium, and the type of biopesticide used. As a comparison, Jayanti *et al.* (2019) showed that addition of red galangal (*Alpinia purpurata* K. Schum) rhizome pellets to adult cockroaches (*Periplaneta americana*) resulted in LD_{50} of 70 ml. Shofiyah (2018) showed that addition of soursop (*Annona muricata* L.) leaf and seed extract resulted in LD_{50} of 12 ml/plant for peach aphids (*Mycus persicae* Sulz). The use of gamal (*Gliricidia sepium*) leaves showed an LD_{50} of 20 ml/plant for chili aphids (*Aphis gossypii*) (Risningsih, 2023) and LD_{50} of 63 ml/plant for papaya mealybugs (*Paracoccus marginatus*) (Kanedi *et al*., 2018). Meanwhile, a study by Kastilong et al., (2021) showed that addition of *Beauveria bassiana* Bals. at 10^8 conides/ml to the brown plant hopper (*Leptocorisa acuta*) resulted in an LT_{50} of 6.8 days. A study by Ibrahim *et al.* (2024) showed that the use of tuba root (*Derris elliptica*) extract to the armyworm (*Spodoptera frugiperda*) resulted in an LT_{50} of 1.86 days.

3.1. Mortality of *Aphis craccivora* Koch.

Table 1 shows the effect of treatment on the mortality of *Aphis craccivora* Koch. Results of analysis of variance (ANOVA) on the mortality of *Aphis craccivora* Koch. showed that F-count value was 5.069 for application time factor (T) which is higher than F-table of 4.414 at 5% significant level. Meanwhile, F-count value for biopesticide formulation is 4.034, higher than F-table of 3.555 at 5%. These imply that both factors significantly influence the mortality of *Aphis craccivora* Koch. The interaction of both factors, however, is not significant. It shows that application time before infestation is better than that of after infestation. Application before infestation resulted in *Aphis* mortality of 63.3% (in average), higher than that of application after infestation which produce lower mortality of 51.7% in average. The table also shows that increasing chitosan resulted in the increase in *Aphis* mortality. Biopesticide consisting of 1.2% chitosan resulted in average 60% mortality, and this is significantly higher than those of lower chitosan. Overall, the application of biopesticide increased *Aphis* mortality as compared to the control (without biopesticide application) which showed *Aphis* mortality of only 20%. This imply that the addition of biopesticide containing *Streptomyces* sp. and *Trichoderma* sp. is able to act as an abdomen poison.

Table 1. Effect of treatment combination on the mortality of *Aphis craccivora* Koch

Treatment	K1	K2	K3	Average
T1 (Before)	55	65	70	63.3 a
T2 (After)	40	55	60	51.7 b
Average	47.5 c	60 b	65 a	

Note: Number followed by same letter are not significantly different based on LSD test at 5% significant level. Lowercases for biopesticide formulation factor, uppercases for application time factor.

The T1 treatment showed the highest mortality results, this was because there was stomach poison in the pesticide solution, most likely from the chitinase enzyme based on visual observations in the field. Haedar *et al*., (2017) stated that the chitinase enzyme is an enzyme that can degrade chitin into simple compounds so that it can be absorbed by the bacteria. Symptoms of mortality of *Aphis craccivora* Koch. caused by the administration of biopesticides based on *Streptomyces* sp., *Trichoderma* sp., and chitosan showed morphological changes in the form of abdominal shrinkage (Figure 2a). This is consistent with research by Oka (2015), which showed that *Aphis craccivora* Koch. treated with papaya leaf-based biopesticides caused decreased mobility and appetite. Research by Raharjo (2023) showed that the administration of biopesticides containing *Streptomyces* sp. and *Trichoderma* sp. to green ladybugs (*Nezara viridula*) resulted in abdominal shrinkage.

Biopesticides containing *Trichoderma* sp. and *Streptomyces* sp. produce the enzyme chitinase that is capable of degrading chitin found in the insect cuticle. Microbes in the form of *Streptomyces* sp. can produce chitinase enzymes (Fatmawati, 2015). *Trichoderma* sp. can also produce chitinase enzymes, but not as much as *Streptomyces* sp. (Saputra *et al*., 2017). Mumba and Rante (2020) stated that abdomen poison works when pests eat plant parts that have been treated, in this case, with biopesticide containing *Streptomyces* sp. and *Trichoderma* sp.

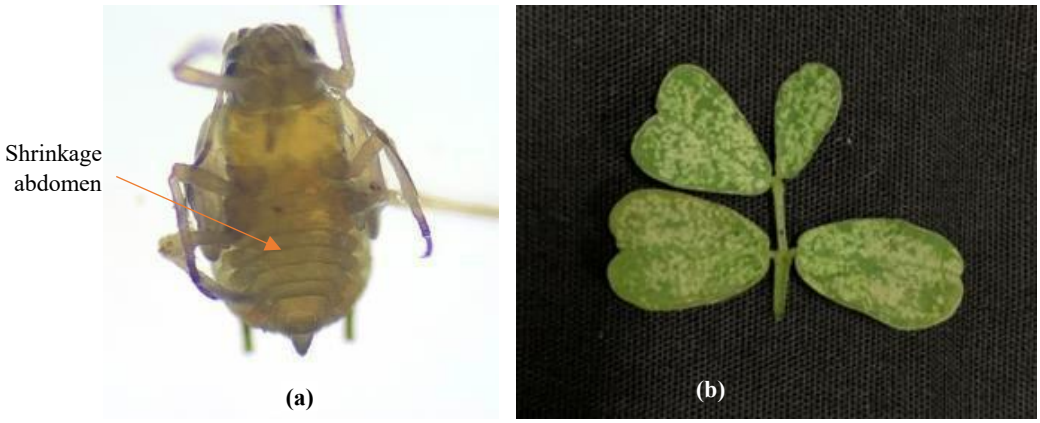

Figure 2. (a) *Aphis craccivora* Koch. under biopesticide treatment observed in this research, and (b) Symptoms of *Aphis craccivora* Koch. damage on the leaves of peanut plants

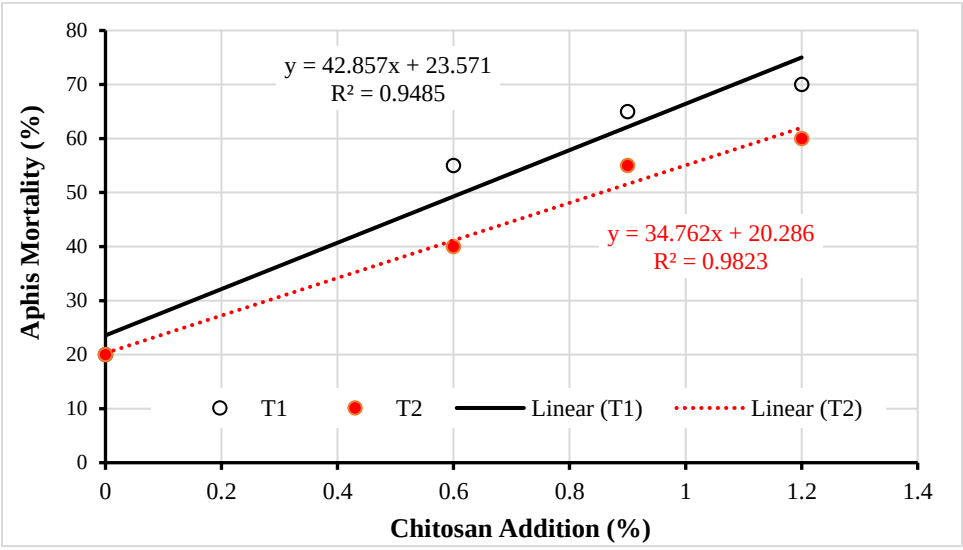

Figure 3. Regression value (R^2) on mortality of *Aphis craccivora* Koch. for 3 days

The regression value (R^2) found was 0.1849 (Figure 3). R^2 is also called the coefficient of determination which explains how far dependent data can be explained by independent data. R^2 has a value between 0 and 1 with the condition that the closer to one the better, so it can be concluded that there is a poor relationship between treatment and the observed parameter in the form of mortality of *Aphis craccivora* Koch. This is likely influenced by the surrounding environmental conditions. The results of colony counting contained in the biopesticide showed that *Streptomyces* sp. 2.4×10^3 CFU/ml and spore density of *Trichoderma* sp. 2×10^2 Conidia/ml in 1.2% chitosan, *Streptomyces* sp. 2.1×10^2 CFU/ml and *Trichoderma* sp. 3.2×10^4 conidia/ml in 0.9% chitosan, *Streptomyces* sp. 2.7×10^3 and *Trichoderma* sp. 2.8×10^2 conidia/ml in 0.6% chitosan, this is considered low. Herlinda *et al*., (2006) stated that biopesticide containing a type of microbe, both bacteria and fungi, must have a high density depending on the type used in order to obtain high mortality, so the right formulation needs to be made in order to increase the microbial population. The addition of chitosan might be encourage the production of the chitinase enzyme, this is because *Streptomyces* sp. and *Trichoderma* sp. able to make chitinase enzymes where this enzyme can degrade chitin as a nutritional source (Haedar *et al*., 2017).

3.2. Plant Damage Intensity

Aphis craccivora Koch. which attacks peanut plants makes the leaves wrinkle and have white spots (Figure 2b) and if left too long the leaves will wrinkle then turn yellow and eventually dry (Figure 4). Javindra *et al*., (2022) stated that *Aphis craccivora* Koch. attacks plants by piercing and sucking the transport tissue (Phloem and Xylemi) thereby making the plant lack elements and causing chlorosis on the leaves. The results of ANOVA (Table 2) on plant damage intensity showed significantly different (p-value < 0.05) for treatment interaction obtained after 3 days of observation. The highest damage intensity was in the treatment before infestation + 0.6% chitosan (T1K1) of 55.22%, while the lowest damage intensity was in the treatment before infestation + 1.2% chitosan (T1K3) of 38.25%. On average, the treatment before infestation (T1) had a lower damage intensity than those of after infestation (T2). All treatment combinations resulted in lower damage intensity as compared to that of plant without biopesticide application with damage intensity of 80%.

Figure 4. Development of symptoms of damage to peanut plants by the aphid *Aphis craccivora* Koch.

Table 2. Average intensity of plant damage due to biopesticide application

Treatment	T1K1	T1K2	TIK3	T2K1	T2K2	T2K3
	55.25 b	38.50 a	38.25 a	51.50 b	41.00 a	50.75 b
HSD 5%	5.03					

Note: Numbers accompanied by the same letter in the same column and with the same treatment are not significantly different in the 5% BNJ test.

4. CONCLUSION

Providing biopesticide with active ingredients of *Streptomyces* sp., *Trichoderma* sp., and chitosan is able to reduce the population growth of *Aphis craccivora* Koch. Time of biopesticide application is important where application before vector infestation resulted in aphid mortality of average 63.3%, significantly higher than that of application after vector infestation with mortality of 51.7%. The chitosan concentration in the biopesticide increase aphid mortality from 47.5% to 65% when chitosan concentration increase from 0.6% to 1.2%. Results also reveal that application of biopesticide can reduce the intensity of plant damage caused by *Aphis craccivora* Koch. with T1K3 treatment had the lowest intensity

of 38.25% with the notation a. Suggestions for further research include testing the chitinase enzyme contained in the pesticide and preparing a practical solution for use by local farmers.

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