

Original Research Article

Efficacy of Biopesticide Formula containing *Streptomyces* sp. and *Trichoderma* sp. Against Southern Green Stink Bug (*Nezara viridula*) on Soybean (*Glycine max* L.)

ABSTRACT

Streptomyces sp. and *Trichoderma* sp. are soil microorganisms isolated from shallot fields that can act as biological agents and increase crop production. *Nezara viridula*, the southern green stink bug, is the leading pest of soybean during the generative period, which can cause damage up to 80%. This study aimed to determine the efficacy of a liquid biopesticide formula using a mixture of coconut water containing *Streptomyces* sp. and *Trichoderma* sp. This study used a randomized block design. The first factor was the time of application and the second factor was the concentration level. There were 8 treatment combinations and 2 controls. Each treatment combination was repeated three times. Probit LC₅₀ and LT₅₀ were performed to determine the effectiveness of biopesticides. The calculation of probit analysis obtained results of 84,443 ppm or about 84% for LC₅₀, while the LT₅₀ analysis obtained results of 4.7 days.

Keyword : Lethal concentration, Biopesticide, *Streptomyces* sp., *Trichoderma* sp., *Nezara viridula*

1. INTRODUCTION

Soybean is a food that has a high carbohydrate content of 14 grams and is used by Asian people as an essential ingredient of daily food. The high population level accompanied by the availability of soybeans must be sufficient so that there is no gap, based on data from BPS (2017) [1] showing that there was a decline in production in 2015, reaching 344.998 tons, but in 2016 (274.317) and 2017 (200.916) there was a decrease of about 70.000 tons, one of the factors causing a decrease in soybean production is the attack of the Southern green stink bug (*Nezara viridula*).

Nezara viridula is a pest that attacks soybeans during the generative period when pods begin to form. *N. viridula* can cause 80% damage. This is due to the high level of mobility and the ability to produce many offspring, Radiyanto (2010) [2] reported that females are able to lay eggs up to 104 – 470, which are placed in groups during their lifetime, *N. viridula* is also able to act as a vector of plant diseases.

Trichoderma sp. and *Streptomyces* sp. is a soil microbe that is often found because the presence of this microbe they can provide a good impact on plants because *Trichoderma* sp. able to protect plants from pests and diseases while encouraging soybean production based on Hasibuan's research (2022) [3] *T. harzianum* was able to increase the number of pods, dry stover, and dry weight per plant, Saputri's research (2015) [4] showed that the administration of *T. harzianum*, *T. honingii*, and *T. viridae* were able to inhibit the growth of *S. rolfisii* disease, Ritongga's study (2022) [5] showed that administration of *T. harzianum* could give mortality as much as 83% within 24-26 hours after application, while Hidayah's study (2019) [6] where *Streptomyces* sp. can control larvae of *Lepidopta stigma* and *Streptomyces* sp. able to play a role as PGPR (Plant Growth Promoting Rhizobium) Vurukonda's research (2018) [7] reported that *Streptomyces* sp. capable of producing antibiotics and volatile organic compounds in soil and in planta. The presence of diverse microorganisms can encourage the resistance of these plants.

Microorganism such as *Streptomyces* sp. and *Trichoderma* sp. can work as BCA (Biological Control Agents) because it can produce enzyme or compounds that can damage the cells such as chitinase enzyme, this enzyme can degrade chitin on the shell or cuticle of an insect. Sidabutar's research (2022) [8] showed that by applying *Trichoderma viridae* 60 g/ 10 L was able to control the population of *Oryctes rhinoceros* larvae as much as 91.67 % with an LT₅₀ 15 days, Safri *et al* (2017) [9] showed that *Streptomyces* sp. able to hinder the fruit fly (*Bactrocera* sp.) pupation process with a spore density of 10⁻². Several research used a combination of *Streptomyces* sp. dan *Trichoderma* sp. as an entomopathogen such as Fitriana *et al* (2019) [10] that can cause the reduce feeding activity of *Spodoptera litura* also Avrianto *et al* (2022) [11] research showed that using a single microorganism *Streptomyces* sp. can give better result than using a combination of *Streptomyces* sp. and *Trichoderma* sp. to repel the soybeans pest such as *Aphid* spp, *Bemisia tabaci*, and *Nezara viridula*. Calculation of probit analysis LC₅₀ and LT₅₀ was performed to determine how effective these biopesticides were in controlling pests, the purpose of this efficacy test was to determine the

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effectiveness of biopesticides with active ingredients *Streptomyces* sp. and *Trichoderma* sp. in a liquid formula against *Nezara viridula* attack on soybean plants.

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2.2 MATERIAL AND METHOD

2. EXPERIMENTAL DETAILS

2.1 Research Methode

The study were conducted at January 2022 to February 2022 in Plant protection laboratorium at agriculture faculty at UPN "Veteran" east java. The statistical method used is a factorial randomized block design with two factors. The first factor was the application time before *N. viridula* infestation (S0) and after *N. viridula* (S1) infestation. The second factor is the level of concentration consist of 25% (K1), 50% (K2), 75% (K3), and 100% (K4), 0% (K0) as a control, 200 ml of biopesticide will be administered on each replication.

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2.2 *Streptomyces* sp. and *Trichoderma* sp. isolation

Exploration was carried out in Pare, Kediri on healthy shallot farming land, 500 grams of soil samples were taken at random and then 1 gram was taken as an isolating material. Isolation of *Streptomyces* sp. and *Trichoderma* sp. used soil plating method by Dhingra and Sinclair (2017) [12]. It was carried using 10^5 and 10^6 dilutions for *Streptomyces* sp. then inoculated on GNA (*Glucose Nutrient Agar*) media and incubated for 2 weeks. *Trichoderma* sp. diluted in 10^4 and 10^5 dilutions then inoculated on PDA media (*Potato Dextrose Agar*) and incubated for 3 days.

2.3 Provision of Biopesticide Concentration

The process of dissolving the concentration of biopesticides using sterile distilled water as a solvent. Concentration 25% consist of 125 ml biopesticide with 375 ml aquadest, concentration 50% consist of 250 ml biopesticide with 250 ml aquadest, concentration 75% consist of 375 ml biopesticide with 125 ml aquadest, concentration 100% consist of 500 ml biopesticide, while 0% concentration was control for this treatment.

2.4 *Nezara viridula* Rearing

Nezara viridula was placed in a rearing box measuring 50 cm x 40 cm X 60 CM, which was covered with. Long beans were used as feed for the test insects and replaced every 2 days. *N. viridula* Imago will be used as a test insect because it has the highest Feeding Activity (Bowling, 1980) [13]

2.5 Application and Infestation

The study used soybean that are in generative stage where its already produced pods, each polybag containing 3 soybean plants and there are 30 polibag. The infestation process used 300 imagos of *N. viridula* total, every treatment need 10 imagos *N. viridula*, biopesticide which will be applied for 200 ml and observed for around 10 days.

3. RESULT AND DISCUSSION

3.1 Symptom and Mortality Percentage of *Nezara viridula*

The symptom cause by *streptomyces* sp. is shown by the mophological changes in its cuticle thus resulting colour changing in the abdomen, thorax, and head (figure. 1) this is aligned with [Trisnawati's research \(2018\) \[14\]](#) that the colour change in the abdominal area is the result of chitin degradation thus changing the colour to dark black compared to the control.



Figure 1. Morphological deformation of *Nezara viridula*

Comment [e172]: Figure 1. Which is treated and which one is control?

Nezara viridula has a thick cuticle layer based on research by [Rawda \(2015\) \[15\]](#) showed that *N. viridula* has a chitin content of 2% in its cuticle so that it can affect the infection process of a microorganism. *Streptomyces* sp. is able to produce chitinase enzyme, but the amount of chitinase enzyme is influenced by the type of species, the age of the isolate, and the environment [Sowmya \(2012\) \[16\]](#) reported that *Streptomyces* sp. is able to produce as much as 0.4% (w/v) at the optimum state of pH 7 with a temperature of 30°C.

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Table 1. The mortality percentage of *Nezara viridula* on various concentration and application time for 10 days

Days	Before infestation (S0)					After infestation (S1)				
	25%	50%	75%	100%	0%	25%	50%	75%	100%	0%
1	0,00%	0,00%	0,00%	0,00%	0,00%	13,33%	0,00%	0,00%	23,33%	0,00%
2	0,00%	0,00%	0,00%	0,00%	0,00%	23,33%	23,33%	23,33%	43,33%	0,00%
3	0,00%	0,00%	10,00%	0,00%	10,00%	26,67%	26,67%	30,00%	50,00%	0,00%
4	0,00%	13,33%	10,00%	0,00%	10,00%	26,67%	26,67%	30,00%	50,00%	0,00%
5	0,00%	13,33%	26,67%	20,00%	16,67%	26,67%	26,67%	30,00%	50,00%	0,00%
6	16,67%	23,33%	30,00%	30,00%	16,67%	26,67%	26,67%	30,00%	50,00%	0,00%
7	26,67%	56,67%	30,00%	33,33%	16,67%	26,67%	26,67%	30,00%	50,00%	0,00%
8	33,33%	60,00%	30,00%	43,33%	16,67%	26,67%	26,67%	30,00%	50,00%	3,33%
9	36,67%	60,00%	30,00%	43,33%	16,67%	26,67%	26,67%	30,00%	50,00%	6,67%
10	36,67%	60,00%	30,00%	43,33%	16,67%	26,67%	26,67%	30,00%	50,00%	13,33%

Table 1. showed that the time of death occurred at different times this is because of the before and after infestation treatment, the treatment makes the biopesticide act as stomach poison and contact poison this is aligned with [Djojsumarto \(2008\) \[17\]](#) statement that contact poison works through the insect cuticle while the stomach poison works if the part affected by the biopesticide is being eaten, the research by [Fitriana et al \(2019\) \[18\]](#) showed that by giving a combination of *streptomyces* sp. and *trichoderma* sp. will act as stomach poison for *spodoptera litura* that resulted in increasing feeding activity according to [Lu et al \(1994\) \[19\]](#) insect require a lot of energy to neutralize the poison insect their abdomen.

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The highest mortality results were obtained by the 50% concentration treatment, which obtained total mortality of 60% from 30 test insects, while the lowest result was in the 75% treatment, on average the treatment before infestation (S0) obtained a mortality result of 37.33% while after infestation (S1) obtained 29.33%. The factor that affects the effectiveness of contact poison is the level of chitin in the cuticle because *N. viridula* has a chitin level of 5 percent, while *N. viridula* has a piercing-sucking mouth type, so the amount of fluid taken is not maximal.

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Before infestation (S0), mortality occurred in 4 DAA in 50% and 75% of treatment, respectively. It was recorded that 13% and 10% of mortality, the highest mortality rate occurred in 7 DAA in 75% treatment, which reached 50% normality. After infestation (S1), mortality occurred in 1 DAA in 25% and 100% treatment, respectively recorded at 13% and 23%. The highest mortality rate occurred in 3 DAA, reaching 50%. Based on the mortality results, it was concluded that the biopesticide with the active ingredient *Streptomyces* sp. and *Trichoderma* sp. It can be used as a stomach poison and a contact poison.

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3.2 LC₅₀ and LT₅₀ Analysis

3.2.1 Probit Analysis of LC₅₀

Probit analysis on the mortality of *N. viridula* after 10 days of application of the biopesticide formulation containing *Streptomyces* sp. and *Trichoderma* sp. is presented in figure 2. probit analysis is carried out to

determine the LC_{50} of the biopesticide the result showed a linier equation which is the correlation between the probit of mortality (Y) and the logarithm of concentration (X) as follows: $y = 0.2731x + 3.6547$ with a correlation value (R) 0.7605 while the regression (R^2) 0.5785, so it can be concluded that there is a correlation between the administration of biopesticide and the mortality of *N. viridula*. correlation value (R) is close to 1 therefore there's a strong connection that resulted in the percentage mortality of 52.35 % the results of probit analysis showed that the LC_{50} value with a time period of 10 days was 84,453 ppm or 84 %is the concentration required to control the population of *N. viridula*.

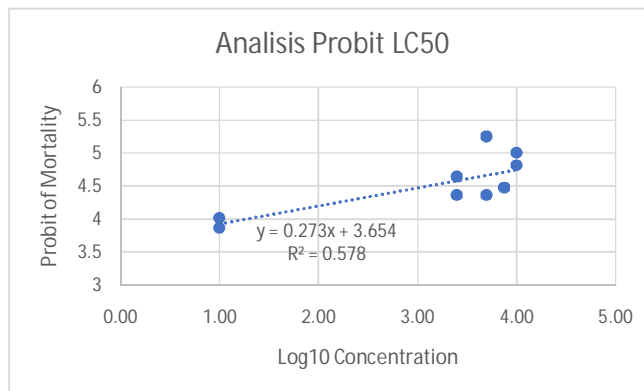


Figure2. Probit Analysis LC_{50} againsts *Nezara viridula* population

Figure 2 shows an increase of correlation between the concentration and probit of mortality, so it can be concluded that the higher the biopesticide concentration, the higher the mortality rate. This is in accordance with the results of [Handayani's research \(2019\) \[20\]](#) giving a concentration of 16 ppm of ethanol extract of *Tabernaemontana macrocarpa jackleaves* can control the population of *Artemia salina*, LC_{50} results showed 0.7440 g/ml, [Hasyim's research \(2019\) \[21\]](#) showed that the administration of botanical insecticides from *Cerbera manghas* leaf extract was able to control *S. exigua* as much as 85% with an LC_{50} of 1.002.67 Ppm and an LT_{50} of 46.98 hours.

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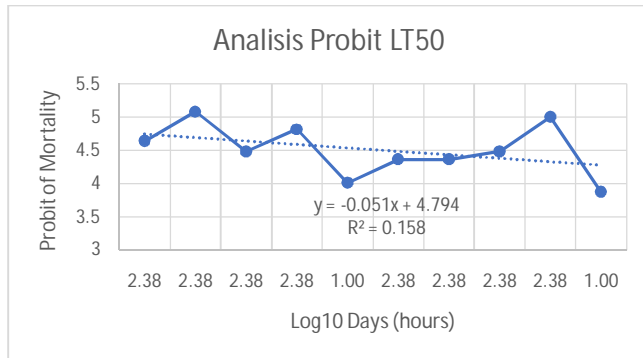
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3.2.2 Probit Analysis of LT_{50}

Probit LT_{50} analysis was used to determine on what days this biopesticide was able to control the pest population as much as 50%, Figure 3. shows a linear equation which is the correlation between probit mortality percentage (y) and the logarithm of days (hours) (x) as follows: $y = -0.0516 x + 4.794$ with a correlation value of (R) is 0.398. The calculation of probit analysis shows that the regression (R^2) obtained is 0.1589, and the correlation value (R) is 0.3986 with a value (R) close to 0 so it can be concluded that there is no a strong relationship this is due to the presence of unknown factors. The trendline shows that the decline is due to the weakening influence between variables, causing a decrease.

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Figure 3. Probit Analysis of LT_{50} againsts *Nezara viridula* population



The analysis of probit LT₅₀ with a period of 10 days was obtained in the form of 113 hours. If converted in days, there are 4.7 days needed to control the insect pest population *N. viridula*.

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Probit analysis LC₅₀ and LT₅₀ can be affected by the type and age of micro organism, and the target insect, [Nia's reasearch \(2016\) \[22\]](#) showed that by administrated *Metharizium* on *M. Anisopliae* on *Lepidiota stigma* 3rd instarit can give result of LT₅₀ 7.7 days while the LC₅₀ is 8.2 x 10⁸ conidia/ml this was due to shedding or molting which could inhibit the infection process, [Cahyani's research \(2022\) \[23\]](#) showed that by administrated *Aspergillus niger* to *Aedes aegypti* larvae it give a result of LC₅₀ 6.1 x 10⁷ and LT₅₀ 1.919 hours.

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4. CONCLUSION

Efficacy test of biopesticide with active ingredient *Streptomyces* sp. and *Trichoderma* sp. in controlling the insect pest *N. viridula* showed symptoms of death of *N. viridula* caused by the degradation of chitin in the abdomen and thorax, the LC₅₀ analysis showed a concentration of 84% while the LT₅₀ analysis showed of 4.7 days, this biopesticide can also be used as a stomach poison and contact poison.

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REFERENCES

1. Central Bureau of Statistics. (2017). Harvest Area, Productivity, and Production of Soybeans in East Java 2002 – 2017 at <https://jatim.bps.go.id/statictable/2018/10/31/1342/wide-panen-productivity-dan-hasil-kedelai-di-jawa-timur-2002-2017.html>. Accessed on July 3, 2021.

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2. Radiyanto, B., Sodiq, M., & Nurcahyani, N. M. (2010). Diversity of insect pests and natural enemies on soybean plantations in Balong-Ponorogo District. *Indonesian Journal of Entomology*, 7(2), 116-116.
3. Hasibuan, S. A., Zurni, T. C., & Syamsuddin, S. (2022). The Effectiveness of BioPriming *Trichoderma harzianum* and Compost Application of Oil Palm Empty Fruit Bunches (TKKS) on Growth and Production of Soybean (*Glycine max* L. Merr.) Plants. *Journal of Agrista*, 26(1), 9-16.
4. Saputri, E., Lisnawita, L., & Pinem, M. I. (2015). Encapsulation of Several Types of *Trichoderma* sp. on Soybean Seeds to Control Disease *Sclerotium rolfsii* Sacc. *Journal of Agroecotechnology*, University of North Sumatra, 3(3), 105478.
5. Ritonga, N. F., Nuraida, N., & Sari, A. (2022). Pathogenicity of *Trichoderma harzianum* to Larval Pests of Horn Beetle (*Oryctes rhinoceros*) in Oil Palm Plants (*Elaeis guineensis* Jacq.) in the Laboratory. *Journal of Agrofolium*, 2(2), 98-107.
6. Hidayah, A. R., Harijani, W. S., Widajati, W., & Ernawati, D. (2019). Potential entomo pathogenic fungi *Metarhizium anisopliae*, *Beauveria bassiana* and *Streptomyces* sp. on mortality of *Lepidiotia stigma* in sugarcane. *Plumula: Agrotechnology Scientific Periodic*, 7(2), 64-72.
7. Vurukonda, S. S. K. P., Giovanardi, D., & Stefani, E. (2018). Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. *International journal of molecular sciences*, 19(4), 952.
8. Sidabutar, M., Nuraida, N., & Sofian, A. (2022). Pathogenicity of *Trichoderma viride* Fungus against Horn Beetle Larvae Pests on Oil Palm Plants. *Journal of Agrofolium*, 2(2), 135-141.
9. Safri, M., Harijani, W. S., & Suryaminarsih, P. (2017). Pupa Viability Test of Fruit Flies (*Bactrocera* sp.) Become Imago by Giving *Streptomyces* sp. Biological Agent. *Agrotechnology-PLUMULA Scientific Periodic*, 5(1).
10. Fitriana, I. N., Suryaminarsih, P., & Mujoko, T. (2018). Potential of *Multientomopa Streptomyces* sp. and *Tripchoderma* sp. in Potato Extract Broth and Glucose Nitrate Broth Media on Pests (*Spodoptera litura*) Eating Behavior by in Vitro Test. *Nusantara Science and Technology Proceedings*, 270-276.
11. AVRIANTO, N. I. (2021). The Effect of Application of the Biological Agent Formula of *Streptomyces* sp. and *Trichoderma* sp. On the Presence of Insect Pests on Soybean Plants (*Glycines Max* L. Merrill) Vegetative Phase (Doctoral dissertation, "Veteran" National Development University, East Java).
12. Sinclair, J. B., & Dhingra, O. D. (2017). *Basic plant pathology methods*. CRC press.
13. Bowling, C. C. (1980). The stylet sheath as an indicator of feeding activity by the southern green stink bug on soybeans. *Journal of Economic Entomology*, 73(1), 1-3
14. Trisnawati, Didin Julia, Wiwik Sri Harijani, and Penta Suryaminarsih. 2018. "Concentration Test of *Streptomyces* sp. Biological Agent. against the Pupae of the *Bactrocera* Fruitfly." 6(1):41-48.
15. Rawda M. Badawy and Hadeer I. Mohamed. Chitin extration, Composition of Different Six Insect Species and Their Comparable Characteristics with That of the Shrimp. *J Am Sci* 2015;11(6):127-134].
16. Sowmya, B., Gomathi, D., Kalaiselvi, M., Ravikumar, G., Arulraj, C., & Uma, C. (2012). Production and Purification of Chitinase by *Streptomyces* sp. from Soil. *Journal of Advanced Scientific Research*, 3(3).
17. Djojsumarto, P. (2008). *Complete guide to pesticides & their application*. agromedia.
18. Fitriana, I. N., Suryaminarsih, P., & Mujoko, T. (2018). Potential of *Multientomopa Streptomyces* sp. and *Tripchoderma* sp. in Potato Extract Broth and Glucose Nitrate Broth Media on Pests (*Spodoptera litura*) Eating Behavior by in Vitro Test. *Nusantara Science and Technology Proceedings*, 270-276.
19. Lu, H., Rajamohan, F., & Dean, D. H. (1994). Identification of amino acid residues of *Bacillus thuringiensis* delta-endotoxin CryIAa associated with membrane binding and toxicity to *Bombyx mori*. *Journal of Bacteriology*, 176(17), 5554-5559.

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20. Handayani, F. F., Sentat, T., & Rahim, A. (2019). Acute Toxicity Test of Ethanol Extract of Selutui Puka Leaves (*Tabernaemontana macrocarpa* Jack.) on Larvae (*Artemia salina* Leach). *Journal of the World of Pharmacy*, 4(1), 1-7.
21. Hasyim, A., Lukman, L., & Marhaeni, L. S. (2019). Evaluation of lethal concentration and lethal time of botanical insecticides on onion caterpillar (*Spodoptera exigua*) in the laboratory. *Journal of Horticulture*, 29(1), 69-80.
22. Niã, L., Himawan, T., & Mudjiono, G. (2016). Pathogenicity test of entomopathogenic fungus *Metarhizium anisopliae* (Moniliales: Moniliaceae) against uret pest *Lepidiotia stigma* F. (Coleoptera: Scarabaeidae). *Journal of Plant Pests and Diseases*, 4(1), 24-31.
23. Cahyani, N. D. K. S., Wiadnya, I. B. R., Khusuma, A., & Getas, I. W. (2022). Analysis of Lethal Concentration and Lethal Time of *Aspergillus Niger* Fungus Isolate Against *Aedes Aeg* Mosquitoes. *Journal of Bioscience Medical Analysts (JAMBS)*, 9(2), 78-86.

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