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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 and potato extract 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 LC_{50} and LT_{50} were performed to determine the effectiveness of biopesticides. The calculation of probit analysis obtained results of 84,443 ppm or about 84% for LC_{50}, while the LT_{50} analysis obtained results of 4.7 days.

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Keywords: Lethal concentration; lethal time; time of application; Nezara viridula.

1. INTRODUCTION

Soybean is a ingredient that has a high carbohydrate content of 14 grams and is used by Asian people as an essential ingredient for daily meal. The high population level accompanied by the availability of soybeans must be sufficient so that there is no gap, based on data from BPS [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) [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 [3] *T. harzianum* was able to increase the number of pods, dry stover, and dry weight per plant, Saputri's research [4] showed that the administration of *T. harzianum*, *T. honingii*, and *T. viridae* were able to inhibit the growth of *S. rolfsii* 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 *Lepidiota 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.

Microorganisme 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 applicating *Trichoderma viridae* 60 g/ 10 L was able to control the population of *Orycetes rhinoceros* larvae as much as 91.67 % with an LT_{50} 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 a entomopathogens such as [10] that can cause the reduce feeding activity of *Spodoptera litura* also [11] research showed that using a single microorganisme *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.*

Streptomyces sp. and *Trichoderma* sp. both can be grown on potato extract liquid media but by adding another suplement such as coconut water it can boost its nutritional value [12] stated that coconut water have 4 % carbohidrate, 0.1 % fat, 0.02 calcium, 0.01 % phospor, 0.5 % iron, and 9 g/l of protein that can support the growth of *Pseudomonas flourescens* colony, both of the BCA have an antagonistic charasteristic that can rival each other but according to [13] *Streptomyces* sp. and *Trichoderma* sp. can be compatible this was done by inoculate them in a PDA media pH 6 and 7. Combining 2 BCA types can give an astonishing result because by using two it can cover each other flaw, *Trichoderma* sp. have a rapid growth rate but have a low production of chitinase enzym while *Streptomyces* sp. have a slow growth rate but have a high production of chitinase enzym, by combining these two BCA it can be achieve a high level of chitinase enzym.

Calculation of probit analysis LC_{50} and LT_{50} was performed to determine how effective these biopesticides were in controlling pests, the main objective of this efficacy test was to determine the effectiveness of biopesticides with active ingredients *Streptomyces* sp. and *Trichoderma* sp. in a liquid formula against *Nezara viridula* attack on soybean plants.

2. MATERIALS AND METHODS

2.1 Experimental Details

2.1.1 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 study used a factorial randomized block design with 2 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. Mortality rate of *nezara viridula* will the main focus for this research.

2.1.2 *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 platting method by Dhingra and Sinclair (2017) [14]. 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.

2.2 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.

2.3 *Nezara viridula* Rearing

Nezara viridula was placed in a rearing box measuring 50 cm x 40 cm X 60 CM, which was covered by a net. 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 [15].

Chart 1. Layout of ecperiment design

$S_0K_{1\,(1)}$	$S_1K_{2\,(1)}$	$S_0K_{2\,(1)}$	$S_1K_{3\,(1)}$	$S_0K_{3\,(1)}$	$S_1K_{4\,(1)}$	$S_0K_{4\,(1)}$	$S_1K_{0\,(1)}$	$S_0K_{0\,(1)}$	$S_1K_{1\,(3)}$
$S_1K_{1\,(1)}$	$S_0K_{1\,(2)}$	$S_1K_{2\,(2)}$	$S_0K_{2\,(2)}$	$S_1K_{3\,(2)}$	$S_0K_{3\,(2)}$	$S_1K_{4\,(2)}$	$S_0K_{4\,(2)}$	$S_1K_{0\,(2)}$	$S_0K_{\mathbf{0}\,(2)}$
$S_0K_{0\,(3)}$	$S_1K_{2\,(2)}$	$S_0K_{1\,(3)}$	$S_1K_{2\,(3)}$	$S_0K_{2\,(3)}$	$S_1K_{3\,(3)}$	$S_0K_{3\,(3)}$	$S_1K_{4\,(3)}$	$S_0K_{4\,(3)}$	$S_1K_{0\,(3)}$

Chart 2. Treatments used in the experiments

Codes	Treatments	Concentration level
S_0K_1	Before infestastion application	25 %
S_0K_2	Before infestastion application	50 %
S_0K_3	Before infestastion application	75 %
S_0K_4	Before infestastion application	100 &
S_0K_0	control	0 %
S_1K_1	After infestasion application	25 %
S_1K_2	After infestasion application	50 %
S_1K_3	After infestasion application	75 %
S_1K_4	After infestasion application	100 &
S_1K_0	control	0 %

Note : 200 ml of biopesticide containing Streptomyces sp. and Trichoderma sp. will be givin once each replicant

2.4 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 applicated for 200 ml once and observed for around 10 days.

2.5 Statistical Analysis

Data analysis will be using ANOVA (Analysis of Variance) and DMRT (Duncan Multiple Range Test) with a probability level of 5 % while probit analysis of LC_{50} and LT_{50} will be conducted by using regression linear, data were submitted to Microsoft excel 2019.

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 (Fig. 1) this is aligned with [16] 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.

Nezara viridula has a thick cuticle layer based on research by [17] 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 [18] 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^{o}C.

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 [19] 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 [20] 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 [21] insect require a lot of energy to neutralize the poison insect their abdomen.

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.

Fig. 1. Morphological deformation of *Nezara viridula*

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

Days	Before infestation (S_0)					After infestation (S_1)				
	25% (K_1)	50% (K_2)	75% (K_3)	100% (K_4)	0% (K_0)	25% (K_1)	50% (K_2)	75% (K_3)	100% (K_4)	0% (K_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 2. Time of infestation and concentration level effect on *Nezara viridula* mortality

No	Treatment (time of infestation, concentration)	Mortality rate	Notasi
1	Before infestation. concentration 25 %	0,36	bcd
2	Before infestation. concentration 50 %	0,6	e
3	Before infestation. concentration 75 %	0,3	abcd
4	Before infestation. concentration 100 %	0,43	cd
5	Before infestation. concentration 0 % (control)	0,16	ab
6	After infestation. concentration 25 %	0,26	abc
7	After infestation. concentration 50 %	0,26	abc
8	After infestation. concentration 75 %	0,3	abcd
9	After infestation. concentration 100 %	0,5	d
10	After infestation. concentration 0 % (control)	0,13	a

Means within column for each treatment followed by the same letter are not significantly different at $P \leq 0.05$

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%. Mortality rate shown that the S_0K_2 have the highest result of 60 % rather than the S_0K_4 (43,33%) this was cause due the enviroment temperature (33^{o}C) that can cause the evaporation of the biopesticide, some will evaporize while some of it absorbed by the seed pods this can be act as a stomach poison because the liquid that containg the BCA's have an enzyme that can degrade the chitin on insect, seed pods tend to hold the moisture around it to keep the seed inside moist and cool. S_1K_4 treatment that have a concentration level of 100% gives an instant result, in under 12 hours it already killed nearly 23,33% after 48 hours it was already at 50% then it became stagnant, the reason it became stagnant varies from evaporation, *N. viridula* might have a thicker cuticle, or it needs another dose of biopesticed in order to keep the mortality rate rising. 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.

3.2 LC_{50} and LT_{50} Analysis

3.2.1 Probit analysis of LC_{50}

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 Fig. 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*.

Fig. 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 [22] giving a concentration of 16 ppm of ethanol extract of *Tabernaemontana macrocarpa jack* leaves can control the population of *Artemia salina*, LC_{50} results showed 0.7440 g/ml, [23] 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.

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%, Fig. 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. 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*.

Probit analysis LC_{50} and LT_{50} can be affected by the type and age of micro organisme, and the target insect, [24] showed that by administrated *Metharizium* on *M. Anisopliae* on *Lepidiota stigma* 3rd instar it can give result of LT_{50} 7.7 days while the LC_{50} is 8.2 x 108 conidia/ml this was due to shedding or molting which could inhibit the infection process, [25] showed that by administrated *Aspergillus niger* to *Aedes aegypti* larvae it give a result of LC_{50} 6.1 x 10^{-7} and LT_{50} 1.919 hours.

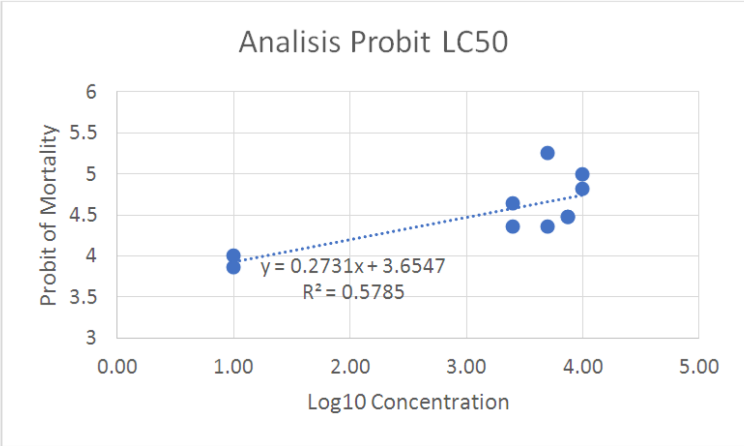

Fig. 2. Probit Analysis LC_{50} againts *Nezara viridula* population

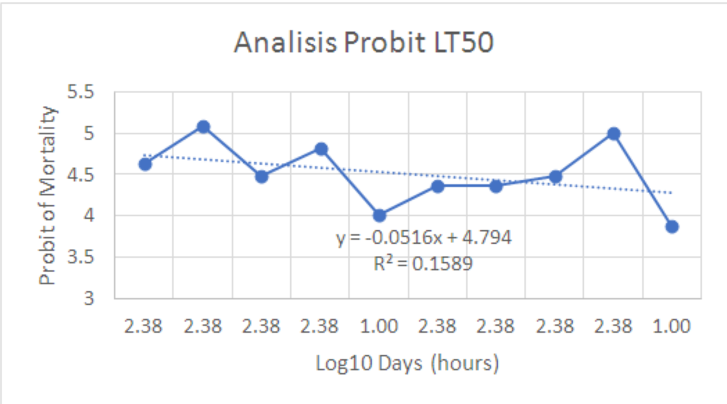

Fig. 3. Probit Analysis of LT_{50} againts *Nezara viridula* population

4. CONCLUSSION

Efficacy test of biopesticide containing *Streptomyces* sp. and *Trichoderma* sp. in controlling the insect pest *N. viridula* showed symptoms of death caused by the degradation of chitin in the abdomen and thorax, mortality rate showed that S0K2 (60.00 %) treatment have the highest mortality rate while the lowest is from S1K1 and S1K2 (26.67 %). LC_{50} analysis showed a concentration of 84% while the LT_{50} analysis showed of 4.7 days, this biopesticide can also be used as a stomach poison and contact poison. Therefore this biopesticide is effective but not efficient.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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