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Bioinsecticide Toxicity of Active Ingredients *Serratia sp*. with Bentonite Carrier Material on *Spodoptera Litura* and its Effect on Survival

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ABSTRACT

Spodoptera litura is a polyphagus pest with a wide host range. In Indonesia, this pest is spread in almost all areas of soybean and horticultural crops. The attack level of *S. litura* on soybean plants varies greatly between, 23-45%. The case of *S. litura* resistance to synthetic insecticides encourages environmentally friendly control efforts using bioinsecticides with active ingredients from microorganisms. *Serratia marcescens* isolated from the soil is capable of producing prodigiosin, a chitinase enzyme, effectively killing pest insect and has the potential to be a biocontrol agent. This study aims to determined the toxicity of bioinsecticides with active ingedient bacteria of which were suspected to be *Serratia* sp. with bentonite carrier material on *S. litura* and its impact on survival. Started with isolating *Serratia* sp bacteria from the rhizosphere taken from Bangkalan Regency with the D-E Shmit Ferguson climate category using media selective of CHROMagar™-Serratia. The isolates obtained were selected based on their ability to cause the highest mortality of level in the larvae of the beetle *Tenebrio molitor.* The isolate was cultured on nutrien agar medium added with 1.5% threhalose, then formulated in powder form with bentonite carrier. Toxicity as stomach poison to *S. litura* was determined using probit analysis for $LC_{50\text{-}90}$ and $LT_{50\text{-}90}$ values. The results showed that at low concentration levels (0.01% and 0.1%) and short observation times (24 hours and 48 hours) the bioinsecticides were non-toxic, the test insects could survive to pupae but none of the pupae survived until adulthood.

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1. INTRODUCTION

Armyworm or *Spodoptera litura* is a polyphagous pest with a wide range of hosts (tobacco, peanuts, chilies, onions, castor oil, cabbage, fiber crops, vegetables and ornamental plants) [1]. This polyphagous nature allows the pest to maintain its life cycle in nature throughout the year by obtaining food from other host plants when the main host plant is not available. The spread of this pest in subtropical and tropical areas [2], includes Asia, Africa, North America (USA), Europe (France, Portugal and Russia) and Oceania. The biggest attack of *S. litura* occurs at the beginning of the dry season until towards the end of the dry season or until the beginning of the rainy season [3]. In Indonesia, this pest is spread in almost all areas of soybean and horticultural crops. The attack level of *S. litura* on soybean plants varies greatly. In Banswara India in 2015 and 2016 the range of attacks due to was 22-55%, while in Indonesia it was 23-45% [4]. Efforts to control this insect of pests still

use synthetic insecticides, in fact 71% of farmers use insecticides that are not recommended [5]. Excessive and unwise use of insecticides not only results in resistance to insect pests, but also residues in the soil thereby reducing the population of organisms macro and micro soil [3]. Babu and Singh [6] and Ramadhan *et al* [7] explain that pest insect resistance is indicated by the inability of certain synthetic insecticides to control populations of insect pest species in an area where the population was previously susceptible to this type of insecticide. The emergence of various cases of resistance encourages efforts to control insect pests that are more environmentally friendly by using bioinsecticides, namely insecticides with active ingredients from microorganisms such as bacteria [8] or metabolite seconder of plant extracts [9], [10]. Sanjaya *et al*. [5] explained that bacteria isolated from the soil (rhizosphere) have great potential as active bioinsecticides that are toxic, including against *S. litura.*

S. marcescens bacteria isolated from soil can produce prodigiosin which is antifungal, antibacterial, aglycidal, antiprotozoal, antimalarial activity, immunosuppressive and anticancer activity [11], [12]. In addition, *S. marcescens* also produces biosurfactants have emerged as a promising source of antimicrobial, antifouling and antitumour compounds that possess emulsification and surface activity [13]. *S. marcescens* is one of the bacteria that can produce chitinase enzymes and is one of the most effective bacteria to degrade chitin. *S. marcescens* bacteria can be used as a biopesticide to control plant pests [14], [15]. Using of biological agents from *S. marcescens* bacteria has the potential to suppress the growth of pest attacks and to be of developed futher as a biocontrol agent [16], [17]. Whereas Lestari *et al*. [18], showed that the extract *S. marcecsens* strain MBC1 was toxic to third instar Aedes aegypti larvae, the probit analysis obtained showed the LC_{50} and LC_{90} values of the *S. marcescens* extract were 66,426.02 and 749,001.41 ppm within 18 days after treatment of the MBC1 strain *S. marcescens* extract, and showed an effect on the length of the life cycle and the death of *A. aegypti* larvae before reaching the adult stage. Additionally described by Jensen *et al*. [19] that toxicity to insects is relative, to find out the exact mechanism of larval toxicity can be done by comparing the *S. marcescens* genome, this is because the level of toxicity of *S. marcescen* to insects does not correlate with pigmentation, colony morphology, motility, surfactant production, antibiotic resistance, or exoenzyme production: protease, DNase, lipase, or phospholipase. The differences in toxicity were more determined in the production of chitinase, a homoserine quorum lactone sensing molecule, but neither of these differences correlated with virulence in *Manduca sexta* larval strains.Then, apart from the toxicity of bioinsecticides, it is affected by the type (bacteria) of the active ingredient, but also the type of carrier material.

These carriers material will have an effect on maintaining cell density in the formulation thereby determining viability which in turn leads to bioinsecticide killing power [20], [21]. Based on this background this study aims to determine the toxicity level of bioinsecticides with active bacterial ingredients *Serratia* sp. with bentonite carrier material to *S. litura* and its effect on survival.

2. METHODS

This research was conducted at the Protection and Environment of plant Laboratory of the Agroecotechnology Study Program, Faculty of Agriculture, University of Trunojoyo Madura on February 9 - March 16, 2023. The tools and materials used were: autoclave, volume pipette, Erlenmeyer, oven, mortar stamper, petri dish, digital balance, brush, label paper, media selective of CHROMagar™-Serratia, nutrient broth, agar media, bentonite, sterile water, larvae *T. molitor* larvae third instar larvae *S. litura* and their feed.

2.1. Isolation

Isolation was carried out from soil/rhizosphere sample taken from Bangkalan Regency with climate category D-E Shmit Ferguson (Kamal, Labang, Kwanyar, Modung Districts). Furthermore, the soil samples were stored in the refrigerator. Bacterial isolation was carried out within 72 hours. The soil samples were composited and homogenized, then 100 g was taken and suspended with 900 ml of physiological solution. Then the suspension was diluted to 10-8, then pour plates were carried out on CHROMagar™-Serratia selective agar media, to detect *S. marcescens* [22]. Then incubated at room temperature for 48 hours. Separate colonies, colored blue (indicated as *S. marcescens*) growing on the surface of the media, were isolated and cultured on slant agar and then stored in the refrigerator used as a pure culture.

2.2. Isolate selection

The isolates obtained were then selected based on their ability to cause death in the test insects, using the larvae of the beetle *Tenebrio molitor* [23]. Twenty *T. molitor* beetle larvae with uniform body sizes were placed in sterile plastic petri dishes, starved for 12 hours. *S. marcescen* suspension was prepared aseptically by streaking isolat of *S. marcescens* on slanted agar and then incubated for 24 hours. After incubation, 2 ml of physiological solution was added and homogenized with a vortex until all bacterial colonies were suspended.

Then the suspension was added to the physiological solution to obtain 50 ml of the suspension which was used to soak rice as feed inoculated with *S. marcescens*. After starvation, they were then given 20 grains of wind-dried rice which had previously been soaked in a suspension of *S. marcescens* bacteria isolate for 5 hours. Then the mortality of *T. molitor* larvae was observed in each *S. marcescens* isolate for up to 98 hours. Isolate *S. marcescens* which caused the highest mortality was used as an active ingredien of bioinsecticide.

2.3. Formulation

Based on the results of isolate selection, *S. marcescens* sm14. isolate was chosen, this isolate produced the highest mortality rate. Furthermore, to increase the stability of the viability of the isolates against drought, the isolates were treated with threhalose as an osmoprotectant in the following way, namely the isolates were cultured on agar slants and incubated at room temperature for 24 hours. After the incubation period, one loop of the isolate was cultured in 2 ml of nutrient solution, which was added to 3 ml of threhalose with a concentration of 1.5% and 0.7 M NaCl, then incubated at room temperature for 24 hours. Then after the incubation period or obtaining a minimum cell density of 10^9 cfu/ml, 50 ml of sterile water and 30 g or 60% w/v of bentonite powder are added as a carrier. The suspension is mixed until homogeneous, then dried by placing it in a certain area in an incubator with a temperature of 37^{o}C for 36 hours. After drying, grinding was carried out with a particle size of less than 100 µm by grinding it in a mortal.

2.4. Determination Toxicity

Toxicity was determined based on the LC_{50-90} and LT_{50-90} values using probit analysis (mortality) by the SPSS version 22 application. The formulated bioinsecticides were suspended at several concentration levels, namely 0.01%, 0.1%, 1%, and 10%. The application of bioinsecticide residue was carried out by feeding the third instar larvae of *S. litura*, cut into three cm^2 sizes, immersed in each concentration of bioinsecticide suspension for ± 10 minutes, then drained to air dry. Then 20 third instar *S. litura* larvae were placed in sterile plastic petri dishes and starved for 6 hours. After starvation, they are fed feed that has been treated with bioinsecticide residues. Then observed the death rate every 24 hours. After the feed given bioinsecticide residues runs out, it is given feed without being given bioinsecticide residues. Furthermore, the provision and replacement of feed is carried out every day without taking into account the depletion of all the feed.

3. RESULTS AND DISCUSSION

Based on observations (Table 1), it is generally seen that the higher the concentration level, and the longer the insects are exposed to *S. marcescens* through feed containing bioinsecticide residues will the higher mortality its.

Table 1. Number of deaths and mortality level at various concentration test

Concentration (%)	Number of deaths and % mortality							
	24 hours		48 hours		72 hours		96 hours	
10	2	10 %	6	30%	6	30%	15	75%
1	2	10%	2	10%	4	20%	9	45%
0.1	0	0%	2	10%	2	10%	4	20%
0.01	0	0%	1	5%	1	5%	2	10%

Meanwhile, on exposed the bioinsecticides by low concentration, namely 0.01% and 0.1%. and short observation time (1-2 days) obtained the number of deaths and low rate of mortality, this condition indicates that at low concentration levels and short observation time bioinsecticides were non-toxic, but based on observations of their survival (Table 2) it appears that none of the test insects survived to maturity, even though they were able to survive to become pupae, none of the pupae managed to live to adulthood. In line with it Devi et al. [24], they found that the mortality rate of *S. litura* second instar larvae increased from 32 to 58% due to *S. marcescens* at concentrations ranging from 2.6×10^8 to 5.2×10^9 cfu/ml.

Table 2. Survival results at various bioinsecticide concentrations

Concentration (%)	Larval period (days)	Percentage until pupation	Pupal period (days)	Pupa state
0.01	20	1	9	dead
0,1	15	1	9	dead
1	16	1	0	dead
10	0	0	0	dead

This difference can occur due to different strains of *S. litura* or different feed given so that the nutrients received will be different which will ultimately affect differences in their resistance to exposure to *S. marcescens*. In addition, the differences in formulation processes also affect it. Furthermore, based on the results of probit analysis, it can be seen that up to 72 hours of observation the LC_{50-90} values were quite high, namely at concentration values of 16.8% and 34% respectively, and at LT_{50-90} values at a concentrations of 0.1% it was achieved after 145.5 hours and 233.5 hours, and at a concentration of 0.01% it was achieved after 186.10 hours and 278.15 hours (Table 3). This condition indicates that the toxicity of bioinsecticides to third instar larvae of *S. litura* is low.

Table 3. Probit analysis results

Lethal Concentration	Hours			
	24	48	72	96
LC_{50}	28.97%	15.79%	16.80%	5.10%
LC_{90}	48.58%	30.05%	34.22%	13.73%
Lethal Time	Bioinsecticide concentration (%)			
	10	1	0,1	0.01
LT_{50}	77.34 hours	110.30 hours	145.40 hours	186.10 hours
LT_{90}	129.83 hours	183.27 hours	233.49 hours	278.15 hours

Described by several authors [25], [17], [26], that the infection process occurs through the activity of the chitinase enzyme produced by *S. marcescens* which damages the peritropic membrane which in turn causes bacteria to migrate to the hemolymph. The body becomes soft, the skin on the body breaks easily and emits a red-black liquid (Fig. 1).

Fig. 1. Symptoms of *S. marcescens* infection in third instar *S. litura* larvae

On the other author [27] explained that insect mortality occurs due to the presence of prodigiosin (a red pigment compound) produced by *S. marcescens*, a compound that plays a role in its proteolytic and hemolytic activities, but virulence and mortality in insects with older instars or in adult insects other than the presence of prodigiosin also requires other metabolites. Other than that explained that non-virulence *S. marcescens* strains induced *S. litura* larvae of a strong immune response, whereas a weak response was shown by virulent *S. marcescens* strains, this is due to the higher activity of lysozyme and phenol oxidase in larval hemolymph, which are enzymes related to the defense system [28], and toxicity to insects is relative, not correlated with pigmentation, colony morphology, motility, surfactant production, antibiotic resistance, or exoenzyme production: protease, DNase, lipase, or phospholipase. The difference in the toxicity of *S. marcescens* is more determined by the production of chitinase, a type of homoserine lactone quorum sensing molecule, and and virulence does not correlate with the target insect strain [19]. In addition to being influenced by the type (bacteria) of the active ingredients, the toxicity of bioinsecticides is also determined by the type of carrier material. These carriers will have an effect on maintaining cell density in the formulation thereby determining viability which in turn leads to insecticide killing power [20]. Related to the carrier material described that characteristic of bentonoite is plastic with a fine particle size, thus presenting strong colloidal properties can bind metabolites/nutrients as well as store microbes [29] [30]. The results of observations on the survival of *S. litura* due to exposure to *S. marcescens* (Table 2), none of the tested insects managed to survive exposure to *S. marcescens*. This shows that at low concentration levels and short observation times, the toxic compounds produced by *S. marcescens* are not yet at a sufficient concentration to kill *S. litura* larvae, this condition can occur because the immune system of *S. litura* cannot recognize the presence of *S. marcescens* [17], so that with

increasing time the bacteria continue to reproduce until the density level is sufficient to produce toxic compounds at a concentration level that can kill *S. litura*. However, other authors explained that the larvae died after 48 hours [31]. According to several previous studies, *S. marcescens* are pathogenic bacteria for insects and larvae because they can produce several hydrolytic enzymes, such as proteases [32] chitinase [33], and lipase which is a poison. These different conditions can be caused by different Serratia species or strains.

4. CONCLUSION

At low concentration levels (0.01% and 0.1%) and short observation times (24 hours and 48 hours) the bioinsecticides were non-toxic, the test insects were able to survive to become pupae but none of the pupae managed to live to maturity.

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Conflict of Interest

Declare conflicts of interest or state "The authors declare no conflict of interest."

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