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Potential Secondary Metabolites of *Streptomyces* sp. and *Trichoderma* sp. in Suppressing the Percentage of *Spodoptera litura* Attacks on Corn Plants

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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

Spodoptera litura F. (Lepidoptera:Noctuidae) is one of the main pests on corn which is polyphagous and can cause crop failure due to damage to the leaves of the plant. Secondary metabolite compounds produced by microorganisms have many roles and functions, namely as compounds to protect plants from pest attacks. *Streptomyces* sp. and *Trichoderma* sp. are biological control agent

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which have the potential to produce chitinase enzymes capable of degrading the cell walls of larval and pupal stages. This study aims to determine the potential of secondary metabolites of *Streptomyces* sp. and *Trichoderma* sp. on various production media on the percentage of *S. litura* larval attack on corn plants. The study was conducted using a completely randomized factorial design with the first factor being the types and combination of biological control agents used, namely *Streptomyces* sp. and *Trichoderma* sp. While the second factor is the type of propagation media used *i.e* glucose nitrate (GN) and potato dextrose agar (PDA). The results showed that the combination of *Streptomyces* sp. and *Trichoderma* sp. on PDA production media with a concentration ratio of 5:1 can increase the potential of secondary metabolites in suppressing the percentage of *S. litura* larval attack on corn plants by up to 10%.

Keywords: Production media; mortality; larval.

1. INTRODUCTION

Spodoptera litura F. (Lepidoptera: Noctuidae) is one of the main pests of maize, a polyphagous pest, and can cause crop failure due to damage to the leaves and fruit of the plant [1]. Symptoms of *S. litura* attack are similar to the symptoms of grasshopper attack. *S. litura* begins to eat the edges of the leaves to the bones of the leaves [2]. Attacks on young plants can inhibit plant growth and even kill plants. Larval appear on the plants after 11-30 days after planting. If pest control efforts are not carried out properly, farmers will experience considerable losses. *S. litura* attack caused 12.5% and more than 20% damage to plants aged more than 20 days after planting (Trizelia, et al. 2011).

Control of using chemical pesticides in excess which is often used by farmers carries several risks. The active ingredients contained in pesticides can lead to the possibility of water and soil contamination [3]. Currently, biological control is being developed using secondary metabolites from biological agents. Secondary metabolites have beneficial and appropriate properties when applied in various ways. The properties of secondary metabolites according to [4] include; being easily soluble in water, so that it can blend with water and does not require a grader or adhesive, not leave residues in plant tissues, so that agricultural products are safe against residual hazards, are not volatile, make biological control agent secondary metabolites durable in nature, a large amount of secondary metabolites it takes only a small amount, but provides great benefits and is easy to apply in a variety of ways and conditions.

Streptomyces spp. is one of the important microbes that can provide potential new bioactive compounds to be used as biological agents to control insect pests [5]. Several active extracts from metabolites of the Streptomyces genus, such as avermectin, emamectin, polynactins, milbemycin, and spinosad, have been established as potential protective agents against various insect pests [6].

The fungus *Trichoderma* sp. has enzymes contained in secondary metabolites including protease, cellulase, cellobiase, chitinase, and 1,3-β-glucanase [4]. *Streptomyces* sp. and *Trichoderma* sp. also produce the enzyme chitinase which can degrade the cell wall of pests in the larval and pupal stages. *Streptomyces* sp. exhibited 78% and 81% larval mortality against *H. armigera* and *S. litura*, respectively, were at par with insecticides such as Emamectin benzoate 5G and Spinosad 45SC [7] In addition to antibiotics, other bioactive metabolites are produced by Streptomyces sp., including volatile organic compounds (VOCs) and enzymes that degrade cell walls, such as chitinases [8].

The media has an important role in the production of secondary metabolites, the right composition of the media can increase the amount of enzymes and compounds in secondary metabolites [9-11]. Based on research Arasu, et al. 2013 the production of secondary metabolites of *Streptomyces* spp. ERI04 was at its maximum level when grown on media with high amount of glucose as the sole carbon source. The combination of *Streptomyces* sp. and *Trichoderma* sp on potato dextrose agar (PDA) production media and a concentration ratio of 5:1 can increase the cytotoxicity of secondary metabolites against *S. litura* larval *In vitro* [12]. This study aims to determine the potential of secondary metabolites of *Streptomyces* sp. and *Trichoderma* sp. growing on PDA and GN media on the percentage of *S. litura* larval attack on corn plants and types of secondary metabolites that can affect the mortality of *S. litura* larval.

2. MATERIALS AND METHODS

This research was carried out in 2019 at the Plant Health Laboratory, and the experimental garden screen house of the Faculty of Agriculture, UPN "Veteran" East Java.

2.1 Preparation of Corn Plants

The corn seed used was Bonanza F_1 variety of corn. The seeds were planted in polybags measuring 15 kg with a media ratio of soil: compost (3:1), then polybags were arranged with a distance between treatments of 35 cm.

2.2 Preparation of Bioagent Isolate

In this study, two isolates were used namely, *Streptomyces* sp and *Trichoderma* sp and belonged to Dr. Ir. Penta Suryaminarsih, MP. Replace to whose effectiveness is known against larval pests of *S. litura.* *Streptomyces* sp. isolates rejuvenated on glucose sodium agar media (glucose 1gr/L, KH2PO4 1.75 gr/L, NaNO3 0.85 gr/L, KCl 0.75 gr/L, MgSO4 7H2O 2.5 gr/L, agar, and distilled water; 30^{o}C [13] was incubated for 14 days and *Trichoderma* sp. rejuvenated on Potato Dextrose Agar medium (PDA 39 g/L; 28^{o} C) [14] incubated for 7 days.

2.3 Propagation in Liquid Medium

Two isolates *Streptomyces* sp and *Trichoderma* sp were grown individually in 150 ml of liquid media with a combination of sugar potato extract and glucose sodium. The treatment series used were the 1st Erlenmeyer was inoculated with 6 pieces of agar containing a single isolate of *Streptomyces* sp. (±8mm in diameter), the 2nd Erlenmeyer was inoculated with 6 pieces of agar containing a single isolate of *Trichoderma* sp. (±8mm in diameter), and the 3rd, 4th and 5th erlenmeyer were inoculated with a combination (co-culture) of a single isolate of *Streptomyces* sp. and *Trichoderma* sp. in a ratio of 3 : 3, 4 : 2 and 5 : 1. All cultures were incubated for 7 days using an orbital shaker (25^{o}C – 28^{o}C ; 160 rpm).

2.4 Extraction the Secondary Metabolites from *Streptomyces* sp and *Trichoderma* sp.

Secondary metabolite was extraction from the media containing *Trichoderma sp* and *Streptomyces sp* at a speed of 4,000 rpm for 15 minutes [14]. Then filtered was done three times using whatman paper No.1 using 0.2 µm syringe to get the supernatant. Then filtered using whatman paper number 1. to get more sterile results.

2.5 Secondary Metabolite Chitinolytic Index

The chitinase test was carried out using the disc diffusion method. Whatman filter paper No. 1 (±8 mm diameter) which had been previously dipped with secondary metabolites of each treatment and then incubated for 48-72 hours. The Chitinolytic index is the ratio between The clear zone and colony diameter to obtain potential isolates. The chitinolytic index formula is as follows [15].

CI = Clear Zone D./Colony D.

Where:

CI : Chitinolytic index
Clear Zone D. : Clear Zone Diameter
Colony D. : Colony Diameter

2.6 Symptoms of *S. litura* Mortality

We observed the symptoms of mortality and counting the number of *S. litura* mortality for approximately 3 x 24 hours. The population in this study was *S. litura* from BALITAS Karang Ploso Malang. The sample of this study was 300 *S. litura* in instar III.

2.7 Percentage of *Spodoptera litura* Larval Attack on Corn Plants

The secondary metabolite test was carried out *in vivo* on corn plants in the experimental field of the Faculty of Agriculture, UPN "Veteran" East Java. Corn plants were prepared until the age of 50 days after planting (DAP), then 3nd instar larval of *S. litura* were inoculated on corn plants as many as 5 individuals per plant. Secondary metabolite spraying was carried out 2 days after the plant (his) on the leaves of the corn plant according to the treatment. The symptoms resulting from *S. litura* were observed and the damage caused on corn plants was known after 85 DAP. The formula for calculating the percentage of pest attacks is as follows:

$$P = \frac{A}{B} X\ 100\%$$

P : Percentage of pest attack
A : Number of affected plants
B : Number of observed plants (Pratama, et al. 2015).

3. DATA ANALYSIS

Potential secondary metabolites of *Trichoderma* sp. and *Streptomyces* sp. which were grown on different production media with different combinations were analyzed based on the content of secondary metabolites, larval mortality symptoms and the percentage of *S. litura* larval attack on corn plants descriptively and using analysis of variance test.

4. RESULTS

4.1 Secondary Metabolite Chitinolytic Index

The results of the chitinolytic test on chitin agar media showed the difference between the 5:1 *Sterptomyces* sp. and *Trichoderma* sp in Glucose Potato Extract (GPEST) formula which had the highest chitinolytic index value was 3, while the 5:1 *Sterptomyces* sp. and *Trichoderma* sp in Glucose Nitrate (GNST) formula with the more low chitinolytic index value was 0.33 . The Formula of *Sterptomyces* sp. and *Trichoderma* sp. with a concentration ratio of 3:3 in GPE had the lower chitinolytic index value of 1.77 than the Formula of *Sterptomyces* sp. and *Trichoderma* sp. with a concentration ratio of 3:3 in GN value of 2.33 while the concentration ratio of 4:2 in GPE than had the lowest chitinolytic index value of of 0 (Table 1).

4.2 Symtomps of *S. litura* larval Mortality

The dead *S. litura* larval in the 5:1 EKGST formula showed a characteristic shrinking body with a size of ± 8 mm, blackened, and released fluid. S litura larval without secondary metabolites have a body length of ± 10 mm with a proportional shape, body color and pattern are clearly visible, and the larval are actively moving (Fig. 1).

4.3 Percentage of *S. litura* Attacks on Corn Plants

The application of secondary metabolites of *Streptomyces* sp. and *Trichoderma* sp. with various combinations of treatments on *Spodoptera litura* of maize pests has not shown a significant effect between treatments on the percentage of pest attacks. The combination formula of *Sterptomyces* sp. and *Trichoderma* sp. isolates on PDA media with a concentration ratio of 5:1 was able to control the most effective pest attack with an attack percentage of 10% on the 7th day compared to the combination formula of *Trichoderma* sp. isolates. on GN media where the attack percentage was 68% (Fig. 2).

5. DISCUSSION

5.1 Secondary Metabolite Chitinolytic Index

The chitinolytic index value which is included in the high category has a value of more than two (> 2). If it has a value of less than two (<2) then it is classified as a low category (Fitriana et al. 2016). This indicates that the activity of the chitinase enzyme in the 5:1 EKGST formula is high. Meanwhile, the lowest index value was found in the 4:2 EKGST formula. The formation of a clear zone in the media according to Wibowo et al. [16] is caused by the homogeneous cleavage of *β1, 4 N-acetylglucosamine homopolymer* in the chitin contained in the media into *N-acetylglucosamine monomer* so that the iodine solution cannot bind *β1, 4 N-acetylglucosamine homopolymer* and then zone clear formed. Chitinolytic index indicates the activity of the chitinase enzyme produced by the test microorganisms.

According to [17], secondary metabolites not only contain antibiotics, but can also be in the form of extracellular enzymes, namely chitinase and cellulase. Based on research (Arasu et al. 2009 the production of secondary metabolites was at its maximum level when cultured on media with a high amount of glucose as the sole carbon source. In the combination formula of *Sterptomyces* sp. and *Trichoderma* sp. isolates on PDA media with a concentration ratio of 5:1 the carbon source was obtained from potato extract and glucose in larger amounts when compared to GN media where the carbon source only came from glucose.

5.2 Symtomps of *S. litura* larval Mortality

Symptoms of larval mortality are characterized by a shrinking body, blackening, and releasing fluid. This is because the chitin of *S. litura* larval is degraded by the chitinase enzyme during contact application and enters the digestive system through feed that has been sprayed with formula which can cause the death of *S. litura* larval. Chitinase enzyme causes the body of *S. litura* larval to become soft, shrink, and blacken [18]. According to Singh et al. [19] the chitinase enzyme causes tissue damage such as the

digestive tract, body muscles, nervous system, and respiration and causes the death of larval. The chitinase enzyme enters the larval body and degrades the peritrophic membrane, this causes damage to the epithelial cells so that the toxin compounds attached to the insect digestive system results in cytoplasmic leakage and death of the larval [20].

5.3 Percentage of *Spodoptera litura* Larval Attack on Corn Plants

The application of spraying secondary metabolites produced by the combination of *Streptomyces* sp. and *Trichoderma* sp. can be used as an insecticidal agent which is stomach poison to control the population of *S. litura* larval. Research by Kaur et al. [5] showed that administration of secondary metabolites *Streptomyces hydrogenans* with a high concentration of 1,600 µg/ml was significantly effective on larval mortality of 70%. Secondary metabolites of *Streotomyces* sp. AP-123 showed high larvicidal activity against *Helicoverpa armigera* (63.11%) and *S. litura* (58.22%) [21].

Insect control can be effective by optimizing the administration of chitinase enzymes from either single isolates or combinations [22]. In addition to chitinase, the combination of *Streptoomyces* sp. and *Trichoderma* sp. isolates was able to produce toxins such as emamectin. Emamectin is very effective in killing *H. armigera* and *S. litura* in a short time [23]. *Streptomyces* sp. produces secondary metabolites of chitinase which can control fruit flies and pupae up to 100% in vitro [24]. This indicates that the secondary metabolites produced by *Streptomyces* sp. and *Trichoderma* sp. can be used to increase resistance. Arasu et al. 2013 also found that *Streptomyces* sp AP-123 showed significant antifeedant, larvicidal activity against polyphagous pests.

Table 1. The mean chinolytic index of secondary metabolites of the Microorganism *Streptomyces* sp. and *Trichoderma* sp.

Media treatment	APH microorganisms	Average	Notation
Glucose Potato Extract (GPE)	*Streptomyces* sp, *Trichoderma* sp 4:2	0.00	a
Glocose Nitrat (GN)	*Streptomyces* sp,	0.00	a
GN	*Streptomyces* sp, *Trichoderma* sp 5:1	0.33	ab
GN	*Trichoderma* sp, *Trichoderma* sp 4: 2	1.00	abc
GPE	*Streptomyces* sp,	1.17	abc
GPE	*Trichoderma* sp	1.17	abc
GN	*Trichoderma* sp	1.17	abc
GPE	*Streptomyces* sp, *Trichoderma* sp 3: 3	1.77	bcd
GN	*Streptomyces* sp, *Trichoderma* sp 3: 3	2.33	cd
GPE	*Streptomyces* sp, *Trichoderma* sp 5 : 1	3.00	d
BNT 5%		**1.64**	

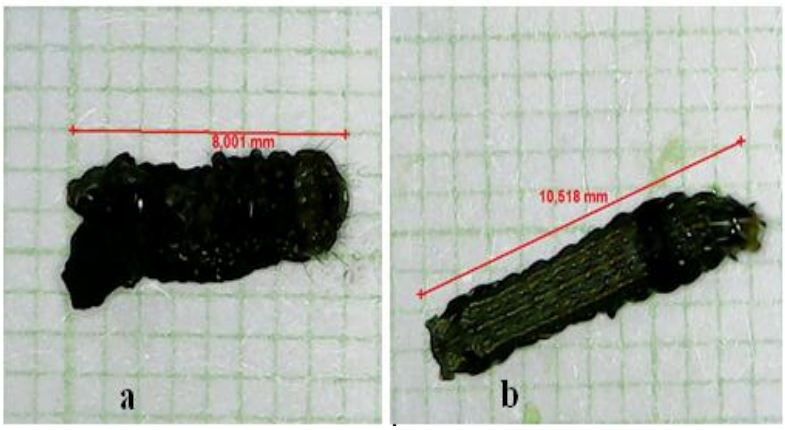

Fig. 1. Larval of *Spodoptera litura* instar VI
a. EKGST formula treatment 5 : 1
b. Control

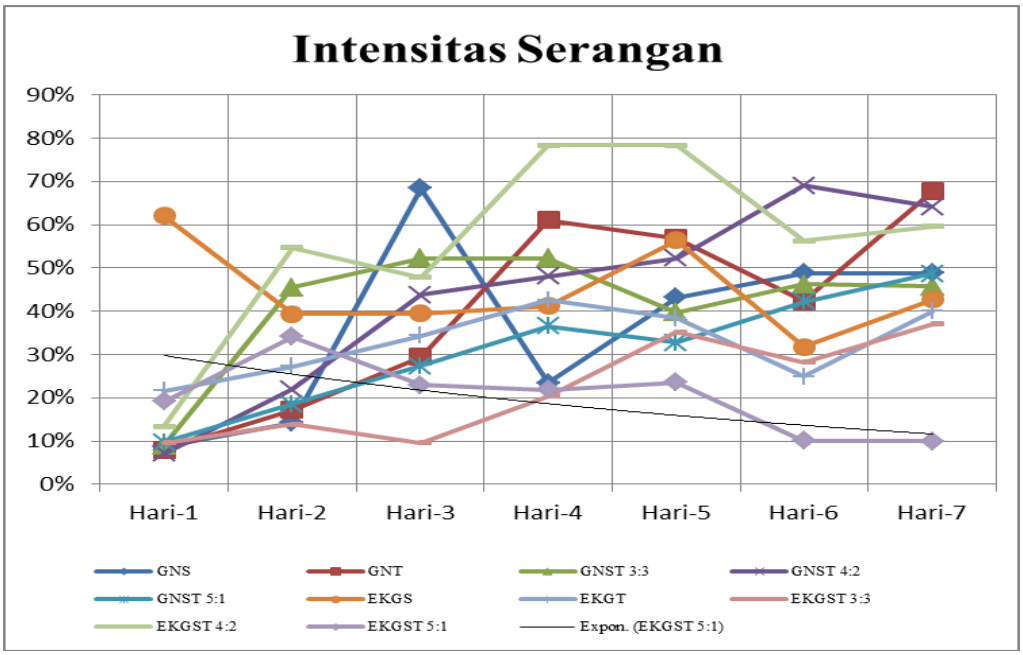

Fig. 2. *Spodoptera litura* larva attack intensity chart

Chemical analysis revealed that *T. atroviride* produces secondary metabolites of volatile antifeedants 1-octen-3-ol and 6-pentyl-2H-pyran-2-one, which can reduce leaf tissue consumption and change the diet of *S. frugiperda* [25].

6. CONCLUSION

Secondary metabolites of the combination *Streptomyces* sp. and *Trichoderma* sp. produced on Glucose Potato Extract media containing high chinolytic enzyme 2, can cause the death of *Spodoptera litura* larval with symptoms of a shrinking body, blackening and releasing fluid. The secondary metabolite combination of *Streptomyces* sp. and *Trichoderma* sp. potential as larvacida and antifeedant that can reduce the percentage of *S. litura* larval attack up to 10% on corn plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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