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The Potency of *Streptomyces* spp. from Shallot Land as Entomopathogen of Onion Caterpillar Pest *Spodoptera exigua*

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***Abstract*—*Spodoptera exigua* is a major pest of shallots (*Allium ascalonicum* L.), capable of causing up to 100% yield loss if uncontrolled. *Streptomyces* sp. is a soil-dwelling Actinomycetes bacterium that can be used to control *S. exigua*. This study aimed to determine the potential of *Streptomyces* spp. isolates from shallot fields as an entomopathogen against *S. exigua*. The research employed a Factorial Complete Randomized Design (CRD). The first factor consisted of one *Streptomyces* spp. isolate obtained from the Pare-Kediri region (BMP) and three isolates from Sidera-Palu (BMS1, BMS2, and BMS3). The second factor was the concentration of the *Streptomyces* sp. suspension consist of 0%; 5%; 10%; and 15% with three replication per treatment (48 units total). Data on larval mortality and damage intensity were analyzed using RStudio via ANOVA and DMRT (α=5%). The BMP (15%) treatment demonstrated the highest efficacy, achieving 76.67% larval mortality while maintaining the lowest damage intensity of 0.20% on shallot plants. These results highlight BMP isolate at 15% concentration as the most promising biocontrol agent and support its further field evaluation as a sustainable alternative to chemical insecticides.**

Keywords: Allium ascalonicum, Entomopathogen, Shallot, Spodoptera exigua, Streptomyces sp.

I. Introduction

Shallot (*Allium ascalonicum* L.) is a high-value horticultural commodity, leading many farmers to cultivate it. According to shallot consumption data, the consumption in Indonesia reaches 2.76 kg/capita/year [1]. The demand for shallots continues to rise with increasing societal needs due to population growth. Low domestic shallot production often leads to insufficient supply, necessitating reliance on imported shallots.

Several factors hinder shallot production, one of which is the attack by the pest *Spodoptera exigua*, which can cause up to 100% yield loss if not controlled [2]. Symptoms of *S. exigua* larval attack include transparent spots on leaves due to the consumption of internal leaf tissues, leaving the outer epidermis intact. Severe infestations cause leaves to dry and fall prematurely, reducing both the quality and quantity of the crop. The control of *S. exigua* predominantly relies on chemical insecticides, which can lead to environmental damage and long-term health issues.

Streptomyces sp. is an Actinomycetes bacterium that thrives around plant roots. This bacterium can control insect pests by producing chitinase enzymes, which degrade insect cuticles. Recent research by Hidayah et al. demonstrated that the application of *Streptomyces* sp. effectively controlled *Lepidiota stigma* populations, while the combination of *Streptomyces* sp. and *Trichoderma* sp. could control the pest *Spodoptera litura* [3], [4]. Currently, research on the use of *Streptomyces* sp. on shallots plant to control *S. exigua* is still limited. This study aims to determine the effectiveness of *Streptomyces* spp. obtained from three different regions for controlling *S. exigua*.

II. Research Methods

A. Exploration and Isolation of Streptomyces spp.

Streptomyces spp. exploration was carried out by collecting soil samples from shallot farmer fields in Sidera Village, Sigi Biromaru District, Sigi Regency, Central Sulawesi Province. The isolation process was performed using serial dilution. The dilutions used were 10^{-5} and 10^{-6}, which were then plated onto Glucose Nutrient Agar (GNA) media and incubated for 14 days (two weeks). Observation and identification of the isolated *Streptomyces* spp. were conducted both microscopically and macroscopically, focusing on colony shape, colony margin, elevation, colony size, colony color, and bacterial Gram staining [5].

B. Rearing of Spodoptera exigua

Spodoptera exigua were obtained using a searching and collecting method from shallot fields infested with *S. exigua*. The *S. exigua* eggs that have been collected and placed in a gauze container along with shallot leaves as food for the hatching *S. exigua* larvae. For the experiment, third-instar *S. exigua* larvae were selected. Third instar larvae are characterized by a green coloration visible on the abdomen, a transverse black band, and a length of 6.2 mm – 8 mm.

C. *Application of Streptomyces* spp.

The Bacterial Control Agent (BCA) solution was applied to shallot plants at 45 days after planting (DAP) before pest infestation. The BCA solution was applied around the plants and left for approximately 7 days [4]. Pest infestation was carried out 7 days after the BCA solution was applied to the plants. The prepared APH formula was then applied directly to the larval bodies as a contact poison.

This study utilized a Factorial Completely Randomized Design (CRD) with two factors. The first factor was the BCA derived from *Streptomyces* spp. isolates, coded as BMP, BMS 1, BMS 2, and BMS 3. The second factor consisted of treatment concentrations for each BCA type: 0% (control), 5%, 10%, and 15%, coded as K0, K1, K2, and K3. Each treatment was replicated 3 times, resulting in 48 experimental units.

D. *Mortality of Spodoptera exigua*

The mortality of *S. exigua* was observed daily for 10 days. Symptoms caused by *Streptomyces* sp. infection included dried, shrunken, and rigid larval bodies, with slight moisture and visible bacterial colonies growing on the larval surface. The mortality of *S. exigua* was calculated using the following systemic formula [6]:

$$\text{Mortality (\%)} = \frac{a}{b}\ x\ 100\ \%$$

Where:
a: Number of dead larvae
b: Total number of larvae

E. *Damage intensity of Spodoptera exigua*

The damage intensity of *S. exigua* was observed daily for 10 days. Symptoms of *S. exigua* larval attack observed were transparent spots on the leaves due to the consumption of the inner leaf tissue, while the outer epidermis was left intact [7]. Severe attacks caused leaves to dry and fall prematurely, reducing both the quality and quantity of the crop yield. The calculation for *S. exigua* damage intensity was performed using a non-systemic calculation:

$$\text{Damage Intensity} = \frac{\sum(n\ x\ v)}{z\ x\ N}\ X\ 100\ \%$$

Where:
n: Number of leaves with the same score
N: Total number of leaves observed
V: Score value for each attack category
Z: Highest score value for the attack category

F. *Data Analysis*

The collected data were processed using Microsoft Excel. Data analysis was performed using ANOVA and the DMRT 5% post-hoc test in RStudio software.

III. Results and Discussion

A. *Exploration and Isolation of Streptomyces* spp.

Based on the exploration, four isolates with characteristics consistent with *Streptomyces* sp. were obtained. These characteristics included colony shape, colony margin, elevation, colony size, colony color, and Gram staining. The isolates obtained were: BMP (*Streptomyces* sp. Pare), BMS1 (*Streptomyces* sp. Sidera 1), BMS2 (*Streptomyces* sp. Sidera 2), and BMS3 (*Streptomyces* sp. Sidera 3) (Table 1).

Macroscopic observations revealed that all four colonies exhibited a fibrous shape with thread-like margins. In terms of elevation, the colonies found tended to be convex, pulvinate (flat-raised), or umbonate (convex with a pointed center). The observed colony sizes varied from punctiform (pinpoint), small, to moderate, with colony colors ranging from white, opaque white, brownish-white, to reddish-white (Table 2).

TABLE 1. MACROSCOPIC OBSERVATION RESULT OF STREPTOMYCES SPP.

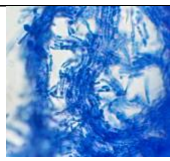
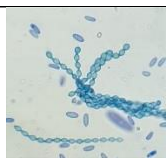
No	Isolate	Colony Shape	Colony Margin	Elevation	Colony Size	Color
1	BMP	Filamentous	Thread-like	Umbonate	Moderate	Reddish-white
2	BMS1	Filamentous	Thread-like	Pulvinate	Moderate	Brownish-white
3	BMS2	Filamentous	Thread-like	Convex	Small	Milky white
4	BMS3	Filamentous	Thread-like	Convex	Punctiform	White

The isolated *Streptomyces* spp. displayed diverse characteristics. Observations of the four *Streptomyces* isolates showed similarities in colony shape and margin, both being fibrous with thread-like edges. This aligns with recent research findings that *Streptomyces* bacteria are fibrous and spore-forming [8]. Similarities were also found in their Gram staining characteristics; all isolated strains were Gram-positive bacteria. This is consistent with Indrawan et al., who stated that *Streptomyces* belongs to the Gram-positive Actinomycetes class [9].

The results of the characteristic observations also revealed varied colony colors, including reddish-white, brownish-white, milky white, and white. Similar research about Streptomyces also found that *Streptomyces* can have diverse mycelial colors such as white, reddish-white, brownish-white, brown, and gray [10], [11]. Differences were also noted in colony elevation and size. When examining colony size, the isolated *Streptomyces* had sizes ranging from small to moderate (2-3 mm). Generally, *Streptomyces* typically ranges from small to moderate in size (1-10 mm) [12]. These variations can be attributed to differences in the species or strain of *Streptomyces* identified.

Furthermore, morphological identification also focused on colony elevation and size. The elevation of the four isolated colonies varied, including pulvinate (flat with a raised center), convex, and umbonate (convex only in the center). In terms of size, the colonies of the four *Streptomyces* isolates ranged from punctiform (dots), small, to moderate. These morphological differences can be attributed to variations in *Streptomyces* species or strains. Different *Streptomyces* species can have distinct colony morphological characteristics [11].

TABLE 2. MICROSCOPIC OBSERVATION RESULT OF STREPTOMYCES SPP.

No.	Isolate	Spore Chain	Gram Test
1.	 Streptomyces from Pare (BMP)	*Biverticillate*	(+) Positive
2.	 Streptomyces from Sidera 1 (BMS-1)	*Open Loop*	(+) Positive
3.	 Streptomyces from Sidera 2 (BMS-2)	*Flexous*	(+) Positive
4.	 Streptomyces from Sidera 3 (BMS-3)	*Flexous*	(+) Positive

Microscopic observations of the four isolates confirmed that the isolates were *Streptomyces* sp. through the identification of visible characteristics, namely spore chain morphology and Gram staining (Table 2). In detail, the spore chain morphology of the four identified isolates ranged from biverticillate, open loop, to flexuous. According to another research, *Streptomyces* possesses structures ranging from biverticillate to flexuous [13], [14]. Based on the Gram staining results, all four isolates showed fungal body structures ranging from dark blue to light blue . The staining results indicated that all four *Streptomyces* isolates were Gram-positive due to their thick cell walls composed of peptidoglycan, which retains crystal violet as a test indicator. Indrawan et al. and Sipriyadi et al. stated that *Streptomyces* are Gram-positive actinobacteria [11], [15].

B. Mortality of Spodoptera exigua

The results of the *S. exigua* mortality at 10 days after Streptomyces treatment are presented in Table 3. It was found that, on average, the highest concentration treatment (15%) resulted in high mortality, with the following details: BMP caused 76.67% mortality, BMS-1 resulted in 80.00% mortality, BMS-2 caused 40.00% mortality, and BMS-3 resulted in 26.67%. According to Hidayah et al., a higher concentration implies a faster infection process by microorganisms due to a higher spore density [4]. The varied mortality rates caused by the treatments are attributed to the diversity of species or strains found.

TABLE 3. *EFFECT OF ISOLATE TYPE AND CONCENTRATION LEVEL ON SPODOPTERA EXIGUA MORTALITY*

No	Isolate and Concentration Treatment	Mortality Rate (%)
1	BMP 0%	16.67 a
2	BMP 5%	50.00 f
3	BMP 10%	53.33 f
4	BMP 15%	76.67 h
5	BMS-1 0%	13.33 a
6	BMS-1 5%	26.67 ab
7	BMS-1 10%	60.00 g
8	BMS-1 15%	80.00 i
9	BMS-2 0%	16.67 a
10	BMS-2 5%	30.00 abc
11	BMS-2 10%	33.33 cd
12	BMS-2 15%	40.00 e
13	BMS-3 0%	13.33 a
14	BMS-3 5%	30.00 abc
15	BMS-3 10%	36.67 de
16	BMS-3 15%	26.67% ab

Notes: Numbers within the same column followed by the same letter indicate no significant difference based on the DMRT test at a=5% confidence level.

Each species or strain possesses a specific level of virulence towards certain insect species [16]. Based on the test results, isolate BMS-1 at 15% concentration level produced the highest mortality, at 80.00% rate mortality. *Streptomyces* can produce insecticidal secondary metabolites such as avermectin, prasinons, doramectin, milbemycin, nanchangmycin, dianemycin, and spinosad, which cause insect mortality [17], [18], [19]. The compatibility factor of entomopathogens, which varies by strain/species, also significantly influences their effectiveness in controlling insect pests, as each species and strain has a different level of efficacy in pest control [20], [21].

C. Damage intensity of Spodoptera exigua

Table 4 shows the systemic damage intensity based on the number of leaves affected. According to the Table 4, the BMP 15% treatment had a low damage intensity (0.20%). On average, treatments with higher concentrations resulted in lower systemic damage intensity: BMS-1 15% (0.30%), BMS-2 15% (0.25%), and BMS-3 15% (0.30%).

TABLE 4. EFFECT OF ISOLATE TYPE AND CONCENTRATION ON THE DAMAGE INTENSITY OF *S. EXIGUA*

No	Isolate and Concentration Treatment	Damage Intensity (%)
1	BMP 0%	0.80 h
2	BMP 5%	0.45 e
3	BMP 10%	0.55 f
4	BMP 15%	0.20 a
5	BMS-1 0%	0.75 h
6	BMS-1 5%	0.55 f
7	BMS-1 10%	0.40 d
8	BMS-1 15%	0.30 c
9	BMS-2 0%	0.65 g
10	BMS-2 5%	0.40 d
11	BMS-2 10%	0.45 e
12	BMS-2 15%	0.25 b
13	BMS-3 0%	0.45 e
14	BMS-3 5%	0.40 d
15	BMS-3 10%	0.30 c
16	BMS-3 15%	0.30 c

Notes: Numbers within the same column followed by the same letter indicate no significant difference based on the DMRT test at a=5% confidence level.

This is because *Streptomyces* produces specific compounds that lead to a decrease in appetite, thereby reducing the damage intensity from *Spodoptera* attacks. *Streptomyces* produces polyketide-group metabolites such as Avermectins and Milbemycins, which act as antifeedants and larvicides [18]. Another antifeedant compound, Antimycin, also contributes to reduced insect feeding. Ingested metabolites damage the digestive system, thus inhibiting feeding activity [22]. Administering compounds at higher concentrations also increases the probability of more compounds being ingested by the insects. Consequently, a greater intake of toxins that disrupt digestion leads to a decrease in insect metabolism [23].

IV. Conclusion

The isolates obtained from Pare, Kediri, and Sidera, Palu, were identified as *Streptomyces* spp. based on microscopic observations. The *Streptomyces* spp. isolate from the Pare, Kediri region showed the highest mortality rate of 76.67% while maintaining a low *Spodoptera exigua* damage intensity on shallot plants as low as 0.20%. These findings indicate that selected *Streptomyces* spp. isolates from shallot-growing areas have strong potential as entomopathogenic agents for the biological control of *S. exigua* in shallot cultivation and merit further development under field conditions.

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