

Interactive effects of Rhizobacteria for Managing *Spodoptera exigua* on Shallot Plants (*Allium cepa* Linnaeus) under Field Conditions

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Received: 14 February 2024

1st Revised: 12 March 2024

Accepted: 27 June 2024

Published: 30 June 2024

Abstract

Spodoptera exigua is the primary pest attacking shallot plants from the vegetative to the generative phase. Yield loss caused by *S. exigua* attack can reach 57%. Rhizobacteria have been known to be used as biocontrol agents for controlling insect pests. The research objectives were to determine and compare the effectiveness of Yuyaos and BTPH rhizobacteria formula (*Bacillus* sp.) in suppressing the attack of *S. exigua* on shallot-growing areas. The study used a completely randomized design (CRD) of 3 treatments and five replications. The treatment consisted of shallot seedlings treated with two types of rhizobacteria (Yuyaos and BTPH) and water (without rhizobacteria) as control before planting. Beneficial effects of the rhizobacteria on shallot plants were observed in the following variables, including the development of the *S. exigua* population, damage level of *S. exigua*, and shallot growth. The results showed that introducing Yuyaos rhizobacteria (RZ2.1AG1, *Bacillus thuringiensis*), which originated from the rhizosphere of the chili plant, was the best way to protect shallot plants from *S. exigua*. Effectively reduced the population of *S. exigua* (78.90%), leaf damage (62.12%), and damage intensity (38.88%). On the other hand, Yuyaos was also influential in increasing tuber growth (11.11%), plant height (43.29%), number of leaves (58.33%), and number of tillers (22.22%).

Keywords: *Bacillus* sp., BTPH, effectiveness, resistance induction, Yuyaos

Introduction

Shallot (*Allium cepa* Linnaeus) is one of Indonesia's most essential horticultural commodities, and it is widely cultivated. National shallots productivity is relatively stable from 2015 to 2019 respectively by 10.06 tons/ha, 9.67 tons/ha, 9.31 tons/ha, 9.59 tons/ha, and 9.93 tons/ha (Badan Pusat

Statistik, 2019). This productivity is still relatively low compared to the optimal productivity of shallots, as reported by Sumarni and Hidayat (2005), which can reach 20 tons/ha. One of the main constraints causing low productivity in shallot plants is pest attacks, such as *Spodoptera exigua* Hubner (leaf caterpillar) (Lepidoptera: Noctuidae).

Yield loss caused by *S. exigua* attack can reach 57% from the initial growth to the tuber maturation stages of shallot plants. The effect of attack can even lead to crop failure, especially in the dry growing season, if no early pest control is carried out (Prasetyo, 2016). It is reported that severe yield losses in shallot plants due to pest attacks range from 24 to 100% (Rosariastuti and Hartati, 2022).

Currently, farmers still rely on synthetic insecticides to control *S. Exigua*. However, this approach is commonly known to cause adverse environmental impacts such as killing natural enemies, causing pest resistance and resurgence, leaving residues on agricultural products, and being harmful to humans (Kardinan, 2001; Amin, 2021; Dao, 2021). Actually, controlling pest can be done using a light trap, which can suppress the attack of *S. exigua* by up to 15.69% (Sari *et al.*, 2017). But this method is costly and less effective and efficient when applied by farmers.

Control of pathogens by endophytic bacteria can occur through the production of antifungals or antibacterials, siderophore secretion, competition for nutrients, and induction of plant resistance (Habazar *et al.*, 2016; Resti *et al.*, 2016). The biocontrol agent, Plant Growth Promoting Rhizobacteria (PGPR), is an alternative way to control *S. exigua*, which can suppress pest populations by inducing plant resistance (Soesanto, 2008).

Previous studies reported that rhizobacteria showed their effectiveness in suppressing some pests' attacks on plants. It is reported that applying *Bacillus turingensis* (30 gr/l water) effectively suppressed *Spodoptera litura* (Tampubolon *et al.*, 2013) Furthermore, *Crocicidolomia pavonana* (Khaeruni *et al.*, 2012; Muliani *et al.*, 2021) accounted for 100% mortality. *Serratia sp.* could suppress the development of *S. litura* by up to 88% (Sari, 2016). Yuyaos, formula of rhizobacteria, was effective in reducing the population of

thrips by up to 22.88% in chili plants, while the BPTPH formula of rhizobacteria was effective in controlling thrips by up to 15.89% (Susanti, 2020). However, the effectiveness of the two rhizobacteria formula in suppressing the population of *S. exigua* has yet to be determined. This research aimed to determine and compare the effectiveness of Yuyaos and BPTPH formula of rhizobacteria in suppressing the attack of *S. exigua* and increasing shallot plant growth.

Methods

The research was conducted in the farmers' fields in Nagari Situjuh Gadang (altitude: 651 m above sea level, latitude: 0°17'28"S, 100°38'4"E), Lima Puluh Kota District, West Sumatra Province, Indonesia, from January to March 2021.

Method

The experiment was arranged in a completely randomized design (CRD) consisting of three treatments and five replications. Treatments were: A = Control (Without Rhizobacteria), B = Rhizobacteria Yuyaos (RZ2.1AG1, *Bacillus thuringiensis* originated from the rhizosphere of the chili plant), C = Rhizobacteria of BPTPH formula (*Bacillus sp.*) (Yanti *et al.*, 2018).

The experimental unit consisted of beds with a 2.5 x 1.5 m² size. There were 15 beds in this experiment, each of which contained 90 plants. A total of 5 plants for each bed were taken and observed randomly.

Land Preparation and Bed-making

The land was first cleaned of the grass and plant residues. Then, the land was processed and loosened using a hoe at a depth of ±20 cm. Then, 12 beds were made with a size of 2.5 x 1.5 m² each. The distance between beds was 50 cm. The soil was mixed with manure at 4 tons/ha (1.5 kg/bed). After mixing, the beds were incubated for 1 week, followed by the second soil processing, which

was carried out by loosening and leveling the soil. The beds were further covered using plastic mulch given holes according to the planting distance (20x15 cm²). Shallot seedlings were planted one week after the second processing.

Rhizobacteria Formula

Yuyaos rhizobacteria were obtained from Yanti's collection (2015) and stored at the Microbiology Laboratory of the Faculty of Agriculture, Universitas Andalas, with a population size of 10-8/cell/ml. The rhizobacteria isolates were rejuvenated: 1 ose of bacteria was transferred to NA medium in a petri dish using the scratch method and incubated at room temperature for 2x24 hours.

The propagation of rhizobacteria consists of 2 stages, including (1) *pre-culture* one colony of rhizobacteria taken from pure culture was transferred to 10 ml of NB medium in a balm bottle and incubated on a rotary shaker at 150 rpm for 24 hours at room temperature, (2) *Main culture*, 1 ml suspension of *pre-culture* was transferred into 25 ml of sterile coconut water in a bottle of balm and incubated in the same way for 3x24 hours (Bonaterra *et al.*, 2022). Rhizobacteria population density in suspension from central culture was determined based on the bandi only with an eight-scale McFarland solution.

Observation

Population of larvae (individual)

The number of larvae per plant was counted weekly, from two weeks after planting until harvesting.

The effectiveness of the treatment was compared with control and calculated using the following formula:

$$E \frac{K-P}{K} \times 100 \% \quad \dots\dots\dots (1)$$

(E = Effectiveness, P = Treatment, K = Control)

Leaves affected (%)

The Percentage of affected leaves was observed weekly, starting 2 WAP. The Percentage of leaves attacked by pests was calculated using the formula:

$$P = \frac{A}{B} \times 100 \% \quad \dots\dots\dots (2)$$

Notes: P = Percentage of leaves attacked by pests, A = Number of leaves affected by pests, B = Number of leaves observed.

Treatment effectiveness was compared to control and calculated using the formula 1.

Damage intensity (%)

Damage intensity was observed weekly from 1 WAP until one week before harvesting. Damage intensity was calculated using the formula by Costa (2019):

$$I = \frac{\sum(n \times v)}{N \times Z} \times 100 \% \quad \dots\dots\dots (3)$$

Notes: I = Damage intensity (%), n = Number of leaves from each damage category, v = Value of score damage category (Table 1, Figure 1), N = Number of observed plant leaves, Z = Highest damage category value.

The effectiveness of the treatment was compared with the control and calculated using the formula 1.

Table 1. Indicators and criteria for pest damage scale of *Spodoptera exigua* (Haryanto *et al.*, 2006) in Hartono *et al.* (2012)

Scale	Indicator	Criteria
0	0%	No symptom
I	1-25%	Low
II	26-50%	Medium
III	51-75%	High
IV	>75%	Very high

Growing shallot tubers (%)

Determination of the percentage of shallot tubers growing was aimed at measuring the quality of shallot tubers, observed from 7-21 DAP. The percentage was calculated by the following formula:

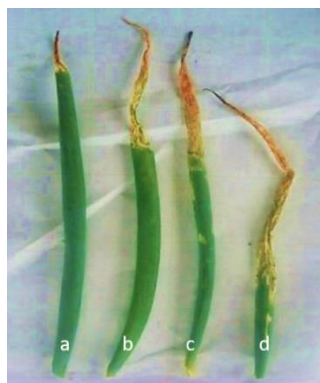


Figure 1. The scale of pest damage on shallot plants: (a) Scale I, (b) Scale II, (c) Scale III and (d) Scale IV.

$$P = \frac{b}{B} \times 100\% \quad \text{..... (4)}$$

Notes: P = Percentage of tubers growing, b = Number of tubers growing, B = Number of tubers planted.

The effectiveness of the treatments was compared with the control and calculated using the Sivan and Chet (1986) formula:

$$E = \frac{P-K}{K} \times 100\% \quad \text{..... (5)}$$

Notes: E= Effectiveness, P = Treatment, K = Control

Plant height

Plant height was measured every week starting the 1 WAP until the plant growth was stable (no longer increasing). Plant height was measured from the clump's base to the highest leaf. The effectiveness of the treatment was compared with the control and calculated using formula 5.

Number of leaves

The number of leaves was counted weekly, and the plant height was observed. The effectiveness of the treatment was compared with the control and calculated using formula 5.

Number of tillers

The number of tillers was counted together with the observation of plant height. The effectiveness of the treatment was

compared with the control and calculated using formula 5.

Data Analysis

Data were analyzed by variance analysis and continued with the Least Significant Difference (LSD) at a 5% level.

Results

Population of Larvae (Individual)

Introducing rhizobacteria in shallots could reduce the population of *S. exigua*. The Yuyaos formula was significantly different from the control, with an effectiveness of 78.90%. Yuyaos's treatment showed no significant difference from BTPH, and BTPH showed no significant difference from the control (Table 2).

Table 2. Population of *S. exigua* (individual/plant) larvae on shallot plants introduced by difference rhizobacteria

Treatment	<i>S. exigua</i> larva	Effectiveness (%)
Yuyaos	0.28 a	78.90
BTPH	0.66 ab	49.17
Control	1.30 b	0.00

Leaves Affected

The introduction of rhizobacteria using the Yuyaos formula in shallot plants showed a decreased percentage of leaves damaged by *S. exigua*, with an effectiveness value of 62.12%. The percentage of leaves damaged by the Yuyaos treatment significantly differed from that of the control and BTPH formula (Table 3).

Damage Intensity (%)

Introducing rhizobacteria in shallot reduced damage intensity by *S. exigua* (Table 4). The Yuyaos formula was more effective than the control and BTPH, with an effectiveness of 38.88%.

Table 3. Shallot leaves affected by *S. exigua* after applying of rhizobacteria formulas at 56 days after planting (DAP)

Treatment	Leaves damaged (%)	Effectiveness (%)
Yuyaos	30.04 a	62.12
BTPPH	72.25 b	8.91
Control	79.32 b	0.00

Table 4. Damage intensity of *S. exigua* on shallot plants after applied by difference rhizobacteria at 56 days after planting (DAP)

Treatment	Damage Intensity (%)	Effectiveness (%)
Yuyaos	40.48 a	38.88
BTPPH	64.91 ab	2.00
Control	66.24 b	0.00

Growing Shallot Tubers (%)

Introducing rhizobacteria in shallot plants increased the percentage of growing tubers (Table 5). The Yuyaos formula was more effective than the control and BTPPH, with an effectiveness of 11.11%.

Table 5. Percentage of shallot tubers growing after treatment with difference rhizobacteria

Treatment	Tuber growing (%)	Effectiveness (%)
Yuyaos	100.00 a	11.11
BTPPH	90.00 b	0.00
Control	90.00 b	0.00

Plant Height

Introducing rhizobacteria in shallots can increase their height. The Yuyaos formula was more effective than the control, with an effectiveness of 43.29%. However, there was no significant difference between the Yuyaos and BTPPH (Table 6).

Number of Leaves

Introducing rhizobacteria in shallots can increase the number of leaves (Table 7, Figure 2). The Yuyaos formula showed the highest number of leaves, with effectiveness of 58.33%, and was significantly different from both BTPPH and the control.

Table 6. Plant height of shallots introduced by two rhizobacterial formulas (56 DAP)

Treatment	Plant Height (cm)	Effectiveness (%)
Yuyaos	35.00 a	43.29
BTPPH	25.23 b	3.27
Control	24.43 b	0.00

Table 7. The number of leaves of shallots introduced by the two formulas rhizobacteria (56 DAP)

Treatment	Number of leaves (strands)	Effectiveness (%)
Yuyaos	35.15 a	58.33
BTPPH Formula	25.25 b	13.73
Control	22.20 b	0.00

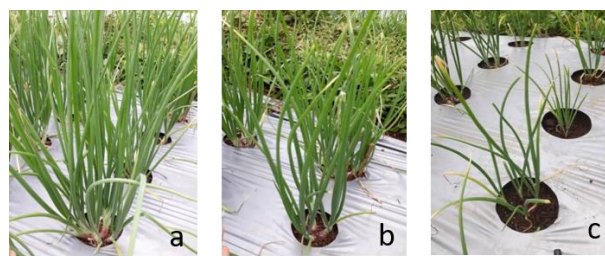


Figure 2. Shallot plants at 35 DAP after introducing difference rhizobacteria: (a) Yuyaos, (b) BTPPH, (c) control.

Number of Tillers

Introducing rhizobacteria in shallot can increase the number of tillers. The number of tillers in plants treated with Yuyaos formula was not significantly different from BTPPH but was significantly higher than the control, with an effectiveness of 22.22% (Table 8).

Table 8. Number of tillers of shallot plants after the introduction of two rhizobacteria formulas (56 DAP)

Treatment	Number of tillers	Effectiveness (%)
Yuyaos	5.50 a	22.22
BTPPH	4.85 ab	7.77
Control	4.50 b	0.00

Discussion

Introducing the difference rhizobacteria, Yuyaos and BTPPH formulas,

demonstrated that across all observed parameters - growth and *Spodoptera exigua* - the treatment with the Yuyaos formula generally showed superior compared to BTPH. On the other hand, the BTPH formula showed outcomes that were typically similar to control.

The introduction of both rhizobacteria formulas on shallots reduced the larval population of *S. exigua* (Table 2), a decreased percentage of leaves affected (Table 3), and a lower intensity of damage (Table 4). Although both treatments did not show significant differences in population parameters and damage intensity, there was a tendency for Yuyaos to perform better than BTPH. The effectiveness of reducing the *S. exigua* larval population with the Yuyaos formula was 78.90%, whereas, with BTPH, it was 49.17%. This high effectiveness in reducing *S. exigua* larval population was also associated with a lower percentage of affected leaves and damage intensity.

The lowest population of *S. exigua* was observed during the Yuyaos treatment, which is assumed to be due to the presence of chemical compounds produced by plants through resistance induction. Rhizobacteria induce plant resistance by stimulating plants to produce secondary metabolites such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), which are essential compounds in plant defense against pests (Van Oosten *et al.*, 2008). The induction of resistance to insect pests in plants differed from that of pathogens. According to Sami *et al.* (2017), rhizobacteria do not kill insects but cause some physiological changes in the host plant that prevent insects from eating, such as reducing the population of cucumber beetles (*Dibrotica undecimputata* and *Acalymma Vitattum*) known to transmit disease. Bacteria that are pathogenic to insects, such as *Bacillus thuringiensis*, are among the most prominent

and potential biological agents. *B. thuringiensis* can form parasporal inclusion bodies during sporulation. In these parasporal inclusion bodies, endotoxin accumulates. When ingested by sensitive insect larvae, endotoxin in the form of this protoxin in the digestive tract of insects in an alkaline environment is converted into an active toxin. The digestive tract of insect larvae also contains proteases that play a role in converting toxins into active toxins. In addition, proteases change the binding capacity of receptors in the digestive tract so that the toxin can bind to the receptor to initiate its toxicity (Adang *et al.*, 2014).

As conducted in the present study, introducing rhizobacteria in shallot plants reduced the percentage of affected leaves (Table 3) and the damaged intensity of *S. exigua* (Table 4). Yuyaos formula was the best treatment in suppressing damaged leaves by *S. exigua*, with an effectiveness of 62.12%. Meanwhile, for the damage intensity of *S. exigua*, applying the Yuyaos formula showed a better effect than that of BTPH and control, with 38.88% effectiveness. This is presumably due to the chemical compounds produced by plants so that plants can reduce the intensity of the affected leaves of *S. exigua*. According to Ramadhan *et al.* (2016), the PGPR could secrete compounds beneficial for plant growth, produce antibiotics, compete in food, and induce plant resistance to disease and pest pathogens. It is reported that Plant Growth Promoting Rhizobacteria (PGPR) can reduce the damage of cotyledons and cucumber stems caused by cucumber beetles (Zehnder *et al.*, 2001).

The introduction of rhizobacteria in shallots showed an increase in the percentage of growing shallot tubers (Table 5). Compared to the BTPH formula and control, the Yuyaos formula was the best at increasing the seedling growth rate. This result is similar to

Farida (2023), who states that introducing rhizobacteria to shallot tubers can increase plant growth. In addition to an increase in the percentage of growing seedlings, shallot plants introduced by the Yuyaos formula also showed an increase in plant height (Table 6), number of leaves (Table 7), and number of tillers. This is presumably because the rhizobacteria produce hormones that can stimulate plant growth.

Conclusion

Introducing Yuyaos rhizobacteria (RZ2. 1AG1, *Bacillus thuringiensis*), which originated from the rhizosphere of the chili plant, was the best way to protect shallot plants from *S.exigua*. Effectively reduced the population of *S.exigua* (78.90%), leaf damage (62.12%), and damage intensity (38.88%). On the other hand, Yuyaos was also influential in increasing tuber growth (11.11%), plant height (43.29%), number of leaves (58.33%), and number of tillers (22.22%).

Acknowledgement

This research was carried out in collaboration with the Carano farmer group located in Situjuh Gadang, Lima Puluh Kota District, West Sumatra, Indonesia. Therefore, the authors thank the Carano farmer group and all parties who have helped complete this research.

Declaration

Author contribution

Hidayani is the main contributor for this paper. Zahlul Ikhsan is corresponding author. Nurbailis and Fitri Yeni are co-author. All authors read and approved the final paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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How to cite: Hidayani, Nurbailis, F Yeni, and Z Ikhsan. 2024. Application of Rhizobacteria for Managing *Spodoptera exigua* Hübner on Shallot Plants (*Allium cepa* Linnaeus) in the Field. JPT: Jurnal Proteksi Tanaman (Journal of Plant Protection) 8(1): 12 – 20.