VOLUME 13 NOMOR 1 JUNE 2026

ISSN 2548 – 611X

JURNAL

BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejournal.brin.go.id/JBBI/index

EFFECTIVENESS OF *Bacillus spp.* IN CONTROLLING *Fusarium sp.* AND ITS EFFECT ON GROWTH AND PHYTOCHEMICAL OF SHALLOT (*Allium ascalonicum L.)*

Efektivitas *Bacillus spp.* dalam Mengendalikan *Fusarium sp.* dan Pengaruhnya Terhadap Pertumbuhan Serta Senyawa Fitokimia Bawang Merah (*Allium ascalonicum L.*)

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ABSTRACT

Shallot (*Allium ascalonicum* L.) is a key horticultural crop in Indonesia, but its productivity is constrained by moler disease caused by *Fusarium* sp. Chemical fungicides have limited effectiveness and raise environmental concerns, necessitating eco-friendly alternatives such as *Bacillus* spp. This study aimed to evaluate the potential of *Bacillus* spp. in suppressing moler disease and promoting shallot growth and resistance. A factorial Completely Randomized Design (CRD) was employed under *in vitro* and *in vivo* conditions, with two factors: *Fusarium* sp. inoculation (with and without) and *Bacillus* spp. treatments (control, Bcz 14, and Bcz 20). Results demonstrated that *Bacillus* spp., particularly isolate Bcz 20, significantly reduced disease intensity by up to 73.91% and delayed symptom development. Moreover, *Bacillus* spp. enhanced plant growth and induced the accumulation of flavonoids and saponins. These findings indicate that *Bacillus* spp. function as effective biological control agents and plant growth promoting rhizobacteria (PGPR), offering a sustainable strategy to improve shallot productivity while reducing reliance on chemical fungicides.

Keywords: *Bacillus* spp.*, Biological control agents, Fusarium* sp.*, Plant growth promoting rhizobacteria, Shallot*

ABSTRAK

Bawang merah (*Allium ascalonicum* L.) merupakan komoditas hortikultura penting di Indonesia, namun produksinya terhambat oleh penyakit moler akibat *Fusarium* sp. Pengendalian berbasis fungisida memiliki keterbatasan sehingga diperlukan alternatif ramah lingkungan, salah satunya melalui pemanfaatan *Bacillus* spp. Penelitian ini bertujuan mengkaji efektivitas *Bacillus* spp. dalam mengendalikan penyakit moler serta menginduksi ketahanan dan pertumbuhan bawang merah. Penelitian dilaksanakan menggunakan Rancangan Acak Lengkap (RAL) faktorial secara in vitro dan in vivo dengan dua faktor, yaitu inokulasi *Fusarium* sp. (F0 = tanpa, F1 = dengan) dan jenis *Bacillus* spp. (B0 = tanpa, B1 = Bcz 14, B2 = Bcz 20). Parameter pengamatan meliputi daya hambat, masa inkubasi, intensitas penyakit, tinggi tanaman, berat umbi dan senyawa fitokimia (flavonoid, tanin, saponin, alkaloid). Hasil penelitian menunjukkan bahwa aplikasi *Bacillus* spp., khususnya isolat Bcz 20, mampu menurunkan intensitas penyakit moler hingga 73,91% dibandingkan kontrol positif dan memperpanjang masa inkubasi penyakit. Aplikasi *Bacillus* spp. juga menginduksi pembentukan senyawa flavonoid dan saponin. *Bacillus* spp.juga berfungsi sebagai *plant growth promoting rhizobacteria* (PGPR) yang meningkatkan tinggi tanaman dan berat umbi. Dengan demikian, *Bacillus* spp., terutama isolat Bcz 20, berpotensi sebagai agens hayati untuk pengendalian penyakit moler sekaligus peningkatan pertumbuhan dan hasil bawang merah secara berkelanjutan serta mengurangi ketergantungan pada fungisida kimia.

***Received**: 27 March 2026* ***Accepted**: 30 March 2026* ***Published**: 12 June 2026*

Kata kunci: *Bawang merah, Bacillus* spp.*, Fusarium* sp.*, Pertumbuhan tanaman, Ketahanan terinduksi, Agensia haya*ti

INTRODUCTION

Shallot (*Allium ascalonicum* L.) is one of the leading vegetable commodities in Indonesia, cultivated intensively and playing an important role in household consumption. In 2022, shallot consumption reached 83.140 tons, an increase of 5.12% compared to the previous year (BPS, 2023). Despite the rising demand, shallot production decreased by 1.51%, from 2.00 million tons in 2021 to 1.98 million tons in 2022. This decline is influenced by various constraints in cultivation, one of which is the attack of Plant Pests and Diseases, particularly moler disease caused by *Fusarium* sp. Infection by this fungus results in symptoms such as yellowing leaves, root rot, and disrupted bulb development, which can reduce yield by up to 50% and even lead to crop failure (Udiarto *et al.,* 2005; Wiyatiningsih, 2003).

Conventional control methods, including the use of chemical fungicides, resistant cultivars, and crop rotation, have several limitations, such as chemical residues, health risks, and pathogen resistance (Soesanto, 2008; Degani & Kalman, 2021). Therefore, the development of environmentally friendly disease control strategies is essential. One promising approach is the use of biological control agents, particularly *Bacillus* spp., which are capable of suppressing pathogen growth through mechanisms such as nutrient competition, antibiosis, and induction of plant resistance (Radhakrishnan *et al.,* 2017; Pieterse *et al.,* 2014). This study aims to determine the effectiveness of *Bacillus* spp. in controlling moler disease in shallots, to investigate the mechanisms by which these bacteria induce plant resistance, and to analyze the metabolite compounds produced by shallots.

MATERIALS AND METHODS

Place and Time

The research was conducted at Laboratory of UPT Proteksi Tanaman Pangan dan Hortikultura Madiun from August to November 2024.

MATERIALS

Aquades sterile, *Bacillus* spp. isolate Bcz 14 dan Bcz 20 collected by Prof. Dr. Ir. Yenny Wuryandari, MP. Isolate *Fusarium* sp. collected by Wilker UPT PTPH Jember, alcohol 70%, alcohol 96%, methylated spirit, *Potato Dextrose Agar* (PDA), Plastic wrap, aluminum foil, Bayclin (bleach or sodium hypochlorite solution), tissue, filter paper, label paper, cotton or cotton wool, sterile distilled water, streptomycin (antibacterial), potassium permanganate powder (PK), chloroform, ammonia, concentrated sulfuric acid (H_2SO_4), Dragendorff's reagent, Meyer's reagent, Wagner's reagent, methanol, sulfuric acid (H_2SO_4), 30% ethanol, ether (diethyl ether), acetic anhydride, ethyl acetate, magnesium, concentrated hydrochloric acid, 1% ferric chloride solution ($FeCl_3$), 5% formalin solution, shallot seeds, polybags, soil, manure, and rice husk charcoal.

Methods

The sterilization of Erlenmeyer flasks, measuring cylinders, inoculating loops, pipettes, Petri dishes, test tubes, object glass, cover glass, forceps, and microtube pipettes was carried out by placing them in heat-resistant plastic and autoclaving at a temperature of 121°C and a pressure of 1.5 atm for 60 minutes (Wulandari *et al.,* 2021). The preparation of Potato Dextrose Agar (PDA) medium was done by dissolving 39 grams of PDA powder in 1 L of sterile distilled water and heating it until boiling. The solution was then poured into an Erlenmeyer flask, covered with sterile cotton and aluminum foil, and sterilized for approximately 15 minutes (Azzahra *et al.,* 2020).

Fusarium sp. isolate was taken using a single inoculating loop and cultured on slanted PDA medium, then incubated for 5–7 days (Wulandari *et al*., 2014). Pure culture of *Fusarium* sp. was propagated on Potato Extract Glucose (PEG) medium aseptically using a fermentor for 7 days (Hardianti *et al*., 2014). The conidial density of the fungus used was 10^6 conidia/mL (Tovar *et al*., 2013). The conidial density was calculated using the following formula (Rustama, 2008).

$$C = \frac{t}{(n \times 0.25)} 10^6$$

Where:

C : Spore density per mL of solution

T : Total number of spores observed in the sample chamber

n : Number of sample chambers (5 large squares × 16 small squares)

0.25 : Correction factor for the use of small-scale sample chambers in a hemocytometer

Bacteria *Bacillus* Bcz 14 and Bcz 20 were taken using a single loop and streaked on NA medium, then incubated for 48 hours and multiplied using PEG medium (Noor & Dewi, 2022). The population density used in this study was 10^8 CFU/mL. Soil sterilization was carried out by mixing soil, manure, and rice husk in a ratio of 2:1:1. A total of 75 mL of formalin was poured into a polybag containing 3 kg of the soil mixture, stirred evenly, then wrapped for 7 days. After that, the polybag was left open for another 7 days (Sianipar *et al*., 2016). The planting media was then ready for use.

The polybags were filled with planting media, and the shallot bulbs were cut at the top by approximately one-quarter of the bulb (Fatmawaty et al., 2015). A suspension of *Bacillus* spp. (20 mL) was inoculated by pouring it according to the treatment at the time of planting (Istiqomah et al., 2018). Inoculation of *Fusarium* sp. was carried out 7 days after planting by applying a 10 mL suspension per planting hole according to the treatment (Poromarto et al., 2020).

Research Design

The research was conducted using both *in vitro* and *in vivo* methods. The *in vitro* study used a Completely Randomized Design (CRD) consisting of 3 treatments and 5 replications, resulting in a total of 15 experimental units. The treatments applied were: F0 = (*Fusarium* sp.), F1 = (*Fusarium* sp. + *Bacillus* isolate Bcz 14), and F2 = (*Fusarium* sp. + *Bacillus* isolate Bcz 20). The inhibitory activity test of *Bacillus* spp. against *Fusarium* sp. was conducted in Petri dishes containing sterile Potato Dextrose Agar (PDA), following Flori *et al.,* (2020). The following is the schematic layout of the inoculum placement in the antagonistic assay.

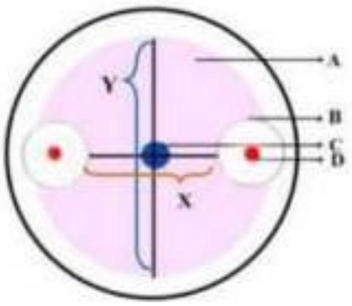

Figure 1. Schematic Layout of the Antagonistic Assay

Figure 1 shows the placement of *Bacillus* sp*p*. bacterial isolates against *Fusarium* fungal isolates in a Petri dish. In the figure, A represents the *Fusarium* fungal colony, while D indicates the *Bacillus* sp*p*. bacterial colony. The interaction between the two is shown by the formation of an inhibition zone, labeled as B, where the growth of the fungus is suppressed by the bacteria. C indicates the center point of the fungal colony. The diameter of the inhibited fungal growth is represented by X, whereas Y represents the diameter of the normal fungal colony. (Suryanto et al., 2016)).

The growth of the fungus *Fusarium* sp. is measured by calculating the increase in the colony diameter each day over a period of 7 days. The percentage of inhibition of *Fusarium* sp. colony growth is calculated using the formula (Hanif, 2016):

$$Inhibition\ (\%) = \frac{Y - X}{Y} \times 100\%$$

Where:

Y = The diameter of the normal colony of *Fusarium* sp.

X = The diameter of the fungal colony with inhibited growth

The observation parameters include inhibition ability and the mechanism of antagonism.

The in vivo study used a Completely Randomized Factorial Design (RAL Faktorial) with two treatment factors. Factor 1 was *Fusarium* sp. inoculation, consisting of 2 levels, namely F0: without *Fusarium* sp. and F1: with *Fusarium* sp. inoculation. Factor 2 was the type of *Bacillus* spp. isolate, consisting of 3 levels, namely B0: without *Bacillus* spp., B1: *Bacillus* isolate Bcz 14, and B2: *Bacillus* isolate Bcz 20. The combination of treatments resulted in 6 treatment combinations with 3 replications. Each experimental unit consisted of 9 plants, so the total number of plants was 162. The observation parameters included incubation period, disease intensity, plant height, and tuber weight. The category used to determine Disease Intensity (DI) was based on leaf symptoms and calculated using the formula (Ambar *et al*., 2010).

$$Disease\ intensity\ (\%) = \frac{\sum(ni \times vi)}{Z \times N}$$

Where:

ni = the number of plants in each severity category,
vi = the scale value of each severity category,
Z = the scale value of the highest severity category, and
N = the total number of plants observed.

The score used to calculate the severity of moler disease in shallots follows a 0–4 scale based on Ambar et al. (2010), as follows:

0 = healthy plants (no wilting)
1 = 0–25% of leaves wilted (some leaves wilted)
2 = 26–50% of leaves wilted (most leaves wilted)
3 = 51–75% of leaves wilted (all leaves wilted, but the stem remains fresh)
4 = 76–100% of leaves wilted or the plant is dead

Phytochemical Analysis

1. Flavonoid Analysis

0.5 g sample is taken and added to 10 mL of distilled water. The mixture is heated until boiling and then filtered while still hot. From the filtrate, 5 mL is taken and added with 0.1 g of magnesium powder, 1 mL of concentrated hydrochloric acid, and 2 mL of amyl alcohol. The mixture is shaken and allowed to separate. A positive result for flavonoids is indicated by the appearance of a yellow-orange to red color in the amyl alcohol layer (Hasibuan *et al*., 2020; Mustikasari & Ariyani, 2010).

2. Tannin Analysis

The sample is dissolved in 10 mL of distilled water, heated, and filtered. From the filtrate, 2 mL is taken and added with 2 drops of 1% $FeCl_3$ reagent. The formation of a blue or dark green color indicates the presence of tannins (Harborne, 1987; Prabowo & Noor, 2020).

3. Saponin Analysis

The sample is dissolved in 10 mL of distilled water, heated, filtered, and cooled. A 2 mL portion is taken and shaken vigorously for 10 seconds. The formation of stable foam (1–10 cm high) lasting at least 10 minutes indicates the presence of saponins. Then, 1 drop of 1% hydrochloric acid is added; if the foam remains, the test is considered positive (Prabowo *et al*., 2020).

4. Alkaloid Analysis

The sample is mixed with 1 mL of 2N hydrochloric acid and 9 mL of distilled water. The mixture is heated for 2 minutes, then cooled and filtered. The filtrate is used for alkaloid testing. Three test tubes are prepared, each tube filled with 0.5 mL of the filtrate. To the first tube, 2 drops of Mayer's reagent are added; to the second tube, 2 drops of Wagner's reagent; and to the third tube, 2 drops of Dragendorff's reagent (Ditjen POM, 1995). The formation of a precipitate in any of these tests indicates the presence of alkaloids. A positive result with Mayer's reagent is indicated by a white precipitate, Wagner's reagent by a yellow to orange precipitate, and Dragendorff's reagent by a light brown to yellow precipitate

Data analysis

The data were analyzed using an F-test. If significant differences were found, the analysis was followed by Duncan's Multiple Range Test (DMRT) at a 5% confidence level using R Studio software.

Results and Discussion

Antagonism of *Bacillus* spp. and *Fusarium* sp.

Based on the inhibition assay of *Bacillus* spp. against *Fusarium* sp. over a 7-day observation period, the results showed differences as presented in Table 1.

Table 1. Inhibitory Effect of Bacillus spp. Against Fusarium sp.

Treatment	*Inhibitory Effect* (%)						
	1 HSI	2 HSI	3 HSI	4 HSI	5 HSI	6 HSI	7 HSI
F1	14.90	40.70	54.28 a	60.70	63.65 a	64.68 a	65.34 a
F2	16.70	42.30	62.56 b	70.40	72.39 b	76.23 b	77.60 b
DMRT 5%	tn	tn	7.34	tn	7.48	10.03	10.17

Note: Values followed by the same letter in the same column are not significantly different according to the DMRT test at the 5% level. F1 = (*Fusarium* sp. + *Bacillus* isolate Bcz 14) and F2 = (*Fusarium* sp. + *Bacillus* isolate Bcz 20).

The inhibition observation results showed that all treatments of *Bacillus* isolates Bcz 14 and Bcz 20 exhibited antagonistic properties against *Fusarium* sp. Both *Bacillus* isolates Bcz 14 and Bcz 20 had demonstrated inhibitory effects on *Fusarium* sp. from the early stages of growth up to day 7. The treatment with *Bacillus* isolate Bcz 20 significantly inhibited the growth of *Fusarium* sp. colonies (F2) by 77.60%, which was higher than the treatment with *Bacillus* isolate Bcz 14 (F1), which only reached 65.34%. This is presumably because *Bacillus* isolate Bcz 20 is more capable of competing for nutrients and producing stronger inhibitory compounds than *Bacillus* isolate Bcz 14. Research by Wuryandari (2022) showed that *Bacillus* isolates Bcz 14 and Bcz 20 were able to suppress *Fusarium* sp. in chili plants by 64% and 65.33%, respectively. The colonies of *Fusarium* sp. inhibited by *Bacillus* isolates Bcz 14 and Bcz 20 in the inhibition test are shown in Figure 2.

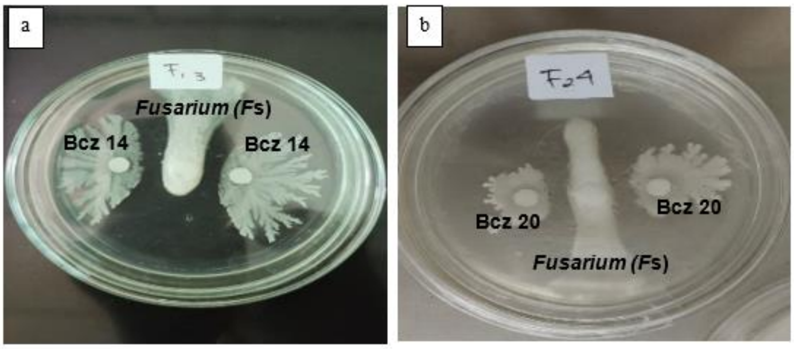

Figure 2. Inhibition of *Fusarium* sp. by *Bacillus* spp. at 7 days after inoculation (DAI). *Fusarium* sp. vs. *Bacillus* isolate Bcz 14 (a); *Fusarium* sp. vs. *Bacillus* isolate Bcz 20 (b).

Changes in *Fusarium* sp. Hyphae by *Bacillus* spp.

Based on microscopic observations, the treatment with *Bacillus* isolates Bcz 14 and Bcz 20 caused abnormalities in the hyphae of *Fusarium* sp. The antifungal compounds produced by *Bacillus* isolates Bcz 14 and Bcz 20 were able to suppress *Fusarium* sp. In the treatment of *Fusarium* sp. with *Bacillus* spp., the hyphal morphology showed signs of lysis.

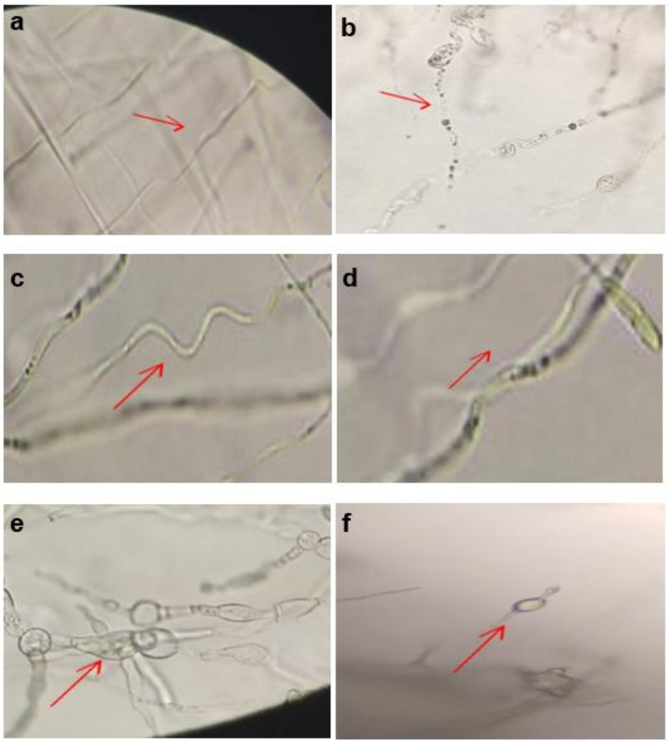

Figure 3. Hyphal morphology of *Fusarium* sp. in the antagonism test with *Bacillus* spp. (400×). Normal hyphae (a), lysis (b), curling (c), coiling (d), swelling (e), and chlamydospore formation (f).

The lysis of hyphae may be caused by compounds produced by *Bacillus* isolate Bcz 14. *Bacillus* sp. is capable of producing the enzyme chitinase, which can degrade chitin as a component of the cell wall of *Fusarium* sp. (Abidin *et al*., 2015). Novina & Suryanto (2013) stated that *Bacillus* spp. synthesizes antifungal secondary metabolites that cause abnormal hyphal morphology, such as lysis, swelling, bending, wrinkling, and breakage. In addition to hyphal malformation, *Fusarium* sp. also forms chlamydospores, which are spores produced as an adaptive mechanism to survive unfavorable environmental conditions.

Phytochemical of Shallot

Based on the phytochemical analysis results (Table 2), shallot leaves at 14 days after planting (14 DAP) showed the presence of secondary metabolite compounds, including alkaloids, flavonoids, tannins, and saponins. The test was conducted on six treatment combinations, which consisted of treatments without *Fusarium* sp. (F0) and with *Fusarium* sp. (F1), as well as two *Bacillus* spp. isolates (B1 and B2).

Table 2. Phytochemical Compounds Identified in Shallot Leaves

Compound	Test results					
	F0B0	F0B1	F0B2	F1B0	F1B1	F1B2
Flavonoid	-	+	+	+	+	+
Tanin	-	+	-	+	+	-
Saponin	-	+	+	-	+	+
Alkaloid	+	+	+	+	+	+
Mayer Reactant	+	+	+	+	+	+
Wagner Reactant	+	+	+	+	+	+
Dragendorff Reactant	+	+	+	+	+	+

Notes: − = absent, + = positive for the presence of secondary metabolite compounds. F0 = without *Fusarium* sp., F1 = application of *Fusarium* sp., B0 = without *Bacillus* spp., B1 = *Bacillus* isolate Bcz 14, and B2 = *Bacillus* isolate Bcz 20

Alkaloid compounds were detected in all treatments. This indicates that shallot plants naturally contain alkaloids, both in the absence of *Fusarium* infection and without the application of *Bacillus*. Alkaloids have several functions, including defense against pathogens, regulation of plant growth, and serving as a reserve of nitrogen in plants.

Flavonoid compounds were not detected in the negative control (F0B0), but were present in all other treatments, whether with *Bacillus*, *Fusarium*, or both. This suggests that flavonoids are likely induced as a response to biotic stress, either from the pathogen *Fusarium* sp. or the biocontrol agent *Bacillus* spp.

Tannin compounds appeared inconsistently, being detected only in F0B1, F1B0, and F1B1 treatments. Tannins are known for their antimicrobial properties and their ability to inhibit enzymes involved in fungal cell wall degradation; however, their role appears to be more selective or influenced by specific treatments. In this study, *Bacillus* isolate Bcz 14 contributed to tannin formation.

Meanwhile, saponin compounds were absent in the control treatments (F0B0 and F1B0), but were present in treatments with *Bacillus* isolates Bcz 14 and Bcz 20 (B1 and B2), with or without *Fusarium* sp. This indicates that saponins are likely induced by the presence of *Bacillus* spp., which act as inducers of systemic resistance (Induced Systemic Resistance/ISR) and have the ability to disrupt pathogen cell membranes.

Incubation Period of *Fusarium* sp.

Observation of the incubation period of *Fusarium* sp. was conducted to determine the time required for the pathogen, from inoculation until the appearance of disease symptoms in shallot plants. Asrul *et al.* (2021) stated that symptoms of Fusarium wilt include drying or necrosis of leaves starting from the tip of the leaf blade and eventually leading to plant death. Plants infected by this fungus are easily uprooted when pulled because the growth of roots or the basal stem is disturbed, and may even rot.

Based on the analysis of variance, the incubation period data showed no significant effect among treatments with the application of *Bacillus* isolates Bcz 14 and Bcz 20 on the incubation period of moler disease caused by *Fusarium* sp., as presented in Table 3.

Table 3. Incubation Period of *Fusarium* sp.

Treatment	Incubation Period (day)
F1B0	22.80
F1B1	22.90
F1B2	26.30
DMRT 5%	tn

Note: Values followed by the same letter in the same column indicate no significant difference based on the 5% DMRT test. F1B0 represents *Fusarium* sp. without *Bacillus* spp. (control), F1B1 represents *Fusarium* sp. with *Bacillus* isolate Bcz 14, and F1B2 represents *Fusarium* sp. with *Bacillus* isolate Bcz 20.

The application of *Bacillus* isolate Bcz 20 against *Fusarium* sp. (F1B2) resulted in the longest incubation period compared to other treatments, with a delay of 4 days, or symptom appearance occurring 26 days after inoculation. This extended incubation period may be caused by competition between *Fusarium* sp. and *Bacillus* isolate Bcz 20. *Bacillus* isolate Bcz 20 is also antagonistic to *Fusarium* sp. This is supported by the in vitro inhibition test, which showed that *Bacillus* isolate Bcz 20 had a higher inhibitory effect than *Bacillus* isolate Bcz 14 (F1B1).

Shallot plants treated with *Bacillus* isolate Bcz 20 produced saponin compounds. These saponins are thought to play a role in inhibiting the growth of *Fusarium* sp., thereby delaying the incubation period of *Fusarium* sp. during infection in shallot plants.

Disease Intensity

Based on the observation results, the application of the fungus *Fusarium* sp. significantly affected the level of damage in shallot plants, as indicated by the disease intensity presented in Table 4.

Table 4. Disease Intensity of *Fusarium* sp. on Shallot Plants

Treatment	Disease Intensity (%) Days After Planting (DAP)					
	21	28	35	42	49	56
F0B0	0.00	0.00 b	0.00 b	0.00 c	0.00 c	0.00 c
F0B1	0.00	0.00 b	0.00 b	0.00	0.00 c	0.00 c
F0B2	0.00	0.00 b	0.00 b	0.00 c	0.00 c	0.00 c
F1B0	2.78	6.94 a	16.67 a	25.00 a	26.39 a	31.94 a
F1B1	1.39	5.56 a	8.33 b	12.50 b	12.50 b	13.89 b
F1B2	1.39	1.39 b	5.56 b	5.56 bc	5.56 bc	8.33 bc
DMRT 5%	tn	3.03	8.19	11.05	9.41	11.98

Note: Values followed by the same letter in the same column indicate no significant difference according to the 5% DMRT test. F0B0 is the negative control (without *Fusarium* sp. and *Bacillus* spp.), F0B1 is *Bacillus* isolate Bcz 14 without *Fusarium sp.*, F0B2 is *Bacillus* isolate Bcz 20 without *Fusarium sp.*, F1B0 is the positive control (*Fusarium sp.* without *Bacillus spp.*), F1B1 is *Fusarium sp.* combined with *Bacillus* isolate Bcz 14, and F1B2 is *Fusarium sp.* combined with *Bacillus* isolate Bcz 20.

In shallot plants that were not treated with *Fusarium* sp., no disease symptoms were observed. In the single-factor treatments, the application of *Bacillus* isolates Bcz 14 and Bcz 20 also did not significantly affect the intensity of moler disease. The treatment of plants with *Fusarium* sp. without *Bacillus* spp. (positive control, F1B0) showed a significant increase in moler disease intensity over time, starting from 2.78% and rising to 31.94%.

In the treatment with *Fusarium* sp. combined with *Bacillus* isolate Bcz 14, the disease intensity was lower than the positive control. The highest disease intensity was 13.89% at 56 days after planting (DAP), which reduced the disease intensity by 56.51% compared to the positive control. Meanwhile, the treatment with *Fusarium* sp. combined with *Bacillus* isolate Bcz 20 (F1B2) showed that this isolate was able to suppress disease intensity down to 8.33%, reducing disease intensity by 73.91% compared to the positive control. This is consistent with the statement by Poromarto *et al.* (2020) that the application of *Bacillus* sp. can control moler disease in shallots in vivo by up to 24.76%

Effect on Plant Growth

Plant Height

The analysis of variance results showed that there was an interaction between the treatments of *Fusarium* sp. and *Bacillus* spp. on the height of shallot plants at 7, 14, and 56 days after planting (DAP). Based on Table 5, shallot plants at 14 DAP under the negative control treatment were taller than all other treatments, reaching 20.57 cm. This is likely due to *Bacillus* spp. not yet having adapted and optimally colonized the plant growth environment. Meanwhile, the positive control treatment showed the lowest plant height, at 11.57 cm. In this treatment, the fungus *Fusarium* sp. was able to inhibit plant growth. During the optimal vegetative phase at 42 DAP, the positive control treatment showed a significant slowdown in plant height growth compared to the negative control, with values of 18.13 cm and 30.68 cm, respectively.

Table 5. Effect of the application of *Fusarium* sp. and *Bacillus* spp. on the height of shallot plants

Treatment	Plant Height (cm) at… *Days After Planting (DAP)*							
	7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP	49 DAP	56 DAP
F0B0	12.49 a	20.57 a	24.02	26.07	27.56	30.68	34.08	36.90 ab
F0B1	10.19 ab	19.08 a	22.88	25.41	29.01	32.74	35.90	38.64 a
F0B2	7.82 b	18.33 ab	23.45	25.98	28.41	30.60	32.56	34.74 abc
F1B0	2.73 b	11.57 ab	15.57	16.27	17.33	18.13	20.03	22.00 bc
F1B1	9.21 b	16.17 b	20.48	23.39	24.87	26.22	27.96	29.27 c
F1B2	9.70 b	18.56 ab	22.71	26.91	29.79	32.39	34.31	35.74 abc
DMRT 5%	2.30	2.42	tn	tn	tn	tn	tn	6.17

Note: Values followed by the same letter within the same column are not significantly different according to the 5% DMRT (Duncan's Multiple Range Test).

The treatment with *Bacillus* isolate Bcz 14 without *Fusarium* sp. and *Bacillus* isolate Bcz 20 with *Fusarium* sp. resulted in the production of secondary metabolite compounds that play a role in increasing plant height. Flavonoid compounds can help transport the hormone auxin, which is responsible for stem elongation. They also act as antioxidants that help protect cells from stress caused by pathogens. Saponin compounds indirectly assist in nutrient uptake, thereby stimulating increased plant height. Tannin and alkaloid compounds do not directly affect plant height but are toxic to microbes. Indirectly, tannins and alkaloids inhibit the growth of *Fusarium* sp., allowing plants to grow optimally. Secondary metabolite compounds such as flavonoids, saponins, tannins, and alkaloids are produced by plants as part of physiological processes and play a role in plant defense and adaptation to biotic and abiotic stress.

The plant height under the treatment with *Bacillus* isolate Bcz 14 without *Fusarium* sp. showed the highest result compared to other treatments, possibly due to the formation of all phenolic compounds, namely flavonoids, tannins, saponins, and alkaloids, as well as the absence of stress from *Fusarium* sp. Flavonoids and saponins help shallot plants continue to grow even under *Fusarium* infection and act as growth promoters.

The application of bacteria can increase phenolic compounds (tannins, saponins, and glycosides) in plant tissues, which can reduce the intensity of fusarium wilt disease, suppress the infection rate, and increase the plant height of tomatoes (Soesanto et al., 2010). The treatment with *Bacillus* isolates Bcz 14 and Bcz 20 with *Fusarium* sp. at 42 days after planting (DAP) was able to increase plant height by approximately 44.65% and 78.67% compared to the positive control. According to Pieterse et al. (2014), *Bacillus* sp. interacts indirectly with pathogens by inducing plant resistance and is capable of producing secondary metabolic compounds.

Bulb weight

Based on the analysis of variance, there were significant differences in the weight of shallot tubers due to the treatment of *Fusarium* sp. and *Bacillus* spp. (Table 6).

Table 6. Effect of the application of Fusarium sp. and Bacillus spp. on the bulb weight of shallot plants

Treatment	Bulb weight (g)
F0B0	20.29 ab
F0B1	23.48 a
F0B2	20.15 ab
F1B0	12.14 bc
F1B1	10.22 c
F1B2	19.52 abc
DMRT 5%	8.96

Notes: Values followed by the same letter in the same column are not significantly different based on the DMRT test at the 5% significance level.

The positive control treatment resulted in a lower tuber weight compared to the negative control or without *Fusarium* sp. Fusarium sp.. *Bacillus* isolate Bcz 14 (F0B1) produced heavier tubers compared to *Bacillus* isolate Bcz 20 (F0B2) and the negative control (F0B0). Under *Fusarium* sp. infection, *Bacillus* isolate Bcz 20 (F1B2) produced a higher tuber weight than *Bacillus* isolate Bcz 14 (F1B1), namely 19.52 grams. The treatment with the best average tuber weight was F0B1, which was 23.48 grams. *Bacillus* isolate Bcz 14 was able to increase tuber weight, although the tuber weight was less optimal under *Fusarium* sp. infection (F1B1), which was 10.22 grams.

Fusarium sp. infection in shallot plants was able to reduce tuber weight in all treatments. The reduction in tuber weight reached 40.17% due to *Fusarium* sp. infection. The application of *Bacillus* isolate Bcz 14 was able to increase tuber weight by 15.7% compared to the negative control. Adiyoga *et al*. (2000) stated that *Fusarium* sp. infection in shallots can reduce tuber weight. This disease can reduce yield by up to 27–75%.

This indicates that *Fusarium* sp. exerts significant biotic stress on shallot yield. The tuber weight of shallot plants infected with *Fusarium* sp. and treated with *Bacillus* isolate Bcz 20 (F1B2) reflects optimal plant growth, where the plant produces flavonoids, saponins, and alkaloids that can induce plant resistance to *Fusarium* infection.

Phytochemical compounds play an important role in plant defense systems; however, their effect on tuber weight is not always linear. The presence of *Fusarium* sp. as a pathogen is the main determining factor, so the presence of *Bacillus* spp. as a biocontrol agent plays an important role in stimulating plant resistance through increased production of flavonoids and saponins, while also supporting tuber weight.

CONCLUSION

Bacillus isolate Bcz 20 showed higher inhibitory activity, namely 77.60%, compared to *Bacillus* isolate Bcz 14, which was only 65.34%. Both *Bacillus* isolates Bcz 14 and Bcz 20 act as Plant Growth Promoting Rhizobacteria (PGPR) that can stimulate the growth of shallot plants. *Bacillus* isolate Bcz 20 was the most effective in reducing disease intensity with suppression up to 73.91%, while *Bacillus* isolate Bcz 14 achieved only 56.51%. The application of *Bacillus* isolate Bcz 20 induced the production of flavonoids, saponins, and alkaloids, whereas *Bacillus* isolate Bcz 14 also produced tannins.

ACKNOWLEDGEMENTS

The author would like to thank the UPT Proteksi Tanaman Pangan dan Hortikultura Jawa Timur Wilayah Kerja Madiun and Universitas Pembangunan Nasional “Veteran” East Java for their support and collaboration.

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