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Research Article

Effect of Biocontrol Agent (*Bacillus* and Mycorrhizal Fungi) Application against Twisted Disease (*Fusarium* spp.) in Off-Season Shallot Production

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ABSTRACT

The demand for shallots has increased along with the Indonesian population. To achieve shallot production goals, farmers began to plant shallots outside usual planting season. However, unfavourable environmental conditions and pest attacks are obstacles faced by farmers. The use of Biological Control Agents (BCA) is a way to improve plant growth and protect plants against plant pathogens or even abiotic stresses. The purpose of this study was to evaluate the response of shallot plants after BCA (*Bacillus* and Mycorrhizal Fungi) application, especially against twisted disease during off-planting season. This research was conducted by preparing BCA isolates, applying BCA, measuring disease incidence and intensity, observing plant growth development, analysing phenol content, and analysing phosphate content. Results showed BCA *Bacillus* and mycorrhizal fungi did not inhibit *Fusarium* spp. infection. Agronomic measurements showed no significant difference in crown weight and root weight, but isolate B8 significantly increase the number of leaves while isolate M significantly decreased plant height. Isolates B6, B7, and M significantly reduced the total phenol content in shallot plants. Phosphate analysis on isolate M did not have significant effects on shallot plants, while BCA *Bacillus* and Mycorrhizal fungi application could not suppress twisted disease. Nevertheless, B8 treatment has the potential to increase shallot growth; therefore, further research must be conducted.

Keywords: *Bacillus*; *Fusarium* spp.; indole-3-acetic-acid; phenol; shallot

INTRODUCTION

Shallots (*Allium cepa* L.) has become a basic need among many vegetable commodities due to Indonesian household consumers use in cooking. To this date, the demand for shallots in Indonesia is directly proportional to the increasing number of populations, thus shallot production must equal or even exceed this demand. To achieve production levels that exceeds consumers' demand, it is necessary to use culturing practices that increase shallot production.

In 2021, shallot farmers in Indonesia, especially in Bantul Regency, have the benefit of two planting seasons. The first planting season is between March and May 2021, while the second season is June to August 2021. Months outside these seasons (off season) have been reported to have environmental conditions that support pathogens growth and cause yield loss. During the first season in 2021, shallot plants can produce 12–14 tons/hectare, while during the second planting season they can produce 20–22 tons/hectare. Shallot farmers in Bantul Regency often plant shallots off-season due to economic factors. However, there are various challenges to obtain the possible benefits from increased shallot production.

Twisted disease caused by several Fusarium species, *Fusarium oxysporum* f.sp *cepae* (FOCe), is a constraint to shallot production. This pathogen has the potential to cause yields loss during vegetative and generative stages up to 20–100% (Adiyoga *et al*., 2004). Twisted disease is followed by favourable weather pattern which hampers the production process for farmers. The intensity of twisted disease during planting seasons are similar to off-seasons. During off season, shallot prices increases in respective to

their demand. High maintenance combined with high rainfall intensity makes production during off-seasons difficult. Regardless, this research was conducted to evaluate the effect of BCA during off-seasons.

Disease management can be done in several ways, especially the use of Biological Control Agents (BCA). BCA in shallot cultivation does not only control various pests, but can also to induce plants to bind various nutrients required by shallot plants to increase plants' defence system against stressors (Vacheron *et al*., 2013). Various research results have shown interactions between BCA and shallot to compounds produced to stimulate the defence metabolism of shallots. Research of Suwarno and Masnilah (2020) showed that application of *Bacillus* reduced colony *Fusarium* spp. by 30%. Mycorrhizal fungi reduced twisted disease intensity up to 60%. de Assis *et al.* (2020) stated that mycorrhizal fungi application has the ability to increased absorption of mineral inside in soil, thus increase the weight of shallot. The application of mycorrhizal fungi on shallot plant caused concentration of minerals (N, P, K, Zn, and Cu) to increase thus increasing infection tolerance (Fitriani *et al*., 2019). Compounds produced by the shallot include phenolic compounds, flavonoids, and sulphur (Alasalvar *et al*., 2001). This study aims to evaluate response of shallot plants after the application of BCA *Bacillus* and mycorrhizal fungi against twisted disease cultivated during off-season.

MATERIALS AND METHODS

Study Area

The study was carried out at the Laboratory of Plant Pathology, Faculty of Agriculture, Universitas Gadjah Mada and shallot field located in Sorobayan Village, Sanden District, Bantul Regency, Special Region of Yogyakarta, Indonesia between August and October, 2021. The eight bacterial strains (TL-9; BRB TI; EP-3; RC-76; A-11; KP-B51; A-9; and B-27 [Table 1]) used in this study were obtained from Laboratory of Plant Pathology, Faculty of Agriculture, Universitas Gadjah Mada.

Seed Treatment using Eight *Bacillus* and Mycorrhizal Fungi Isolates

Eight isolates of *Bacillus* were grown on Yeast Peptone Agar (YPA) medium (0.5% yeast extract, 1% polypeptone, 1.5% agar) and incubated for 48 h while Mycorrhizal fungi was obtained from Laboratory of Plant Pathology, Faculty of Agriculture, Universitas Gadjah Mada. *Bacillus* propagation was acquired by harvesting its grown colony. Afterwards, it was suspended in 300 mL sterile water and density of 10^8 CFU mL^{-1}. The application of *Bacillus* on shallot was done by immersing shallot for 45 minutes before planting as well as watering the plant once a week using *Bacillus* suspensions. The application of mycorrhizal fungi was done by placing 20 g in the planting holes (Saputri *et al*., 2020).

Table 1. *Bacillus* spp. isolates used in this study

Isolate	Species	Source
TL-9	-	
BRB TI	-	
EP-3	-	
RC-76	*B. cereus*	Jayanti *et al.* (2022)
A-11	-	
KP-B51	*B. subtilis*	Jayanti and Joko (2020)
A-9	-	
B-27	*B. velezensis*	Rahma *et al.* (2020)

Field Preparation

Field preparation consisted of ploughing and bund. Bund were 2×1.5×1.2 m and 0.5 m distance between bunds. Every bund was an experimental plot with 200 shallots. Fertilization used organic and inorganic fertilizer. Fertilizers used consist of organic fertilizer used was 100 kg/50 m^2, Phonska of 25 kg/50 m^2, and NPK 18 kg/50 m^2. Shallot bulbs (cv. Bima Brebes) treated using *Bacillus* suspension and mycorrhizal fungi were planted with distances of 15×20 cm. Routine watering was done twice a day during vegetative stage and once a day in generative stage. This study used a Completely Randomized Design (CRD) with 3 blocks for replications. A 10-unit plant samples was considered a replication.

Disease and Agronomic Parameter Observation in the Field

Observation was done on intensity and incidence of twisted disease and agronomic parameters such as, plant height, number of leaves, tuber weight, and root weight. Observations were conducted every week starting from 1–8 weeks of the plant age. Meanwhile tuber weight was measured on 7^{th} week after planting. Twisted disease symptoms were indicated from chlorosis and necrosis on the shallot's

leaves. Scores were based on chlorosis and necrosis percentages and disease intensity using the following categories: 0 = healthy plant, 1 = chlorosis on leaf tips, 2 = chlorosis up to 25%, 3 = necrosis less than 50%, 4 = necrosis up to 80%, 5 = plants were dead (Sintayehu *et al.*, 2011). Disease intensity (DI) were calculated using the following equation (Solekha *et al.*, 2020):

$$DI = \frac{\Sigma(m \times v)}{n \times z} \times 100\%$$

m = number of plants with the same damage category;v = score of each damage category; n = total number of plants observed; z = score for the most severe damage. Meanwhile, the Area Under of the Disease Progress Curve (AUDPC) was calculated based on the following equation (Ahmed *et al.*, 1997):

$$AUDPC = \frac{\sum_{i=1}^{n} \left[\frac{X_{i+1} + X_i}{2}\right] \times \left[t_{i+1} - t_i\right]}{N-1}$$

X_{i+1} = observation data i+1; X_i = observation data I; t_{i+1} = observation time i+1; t_i = observation time I; N = number of observations.

Phenolic Analysis (Septiani *et al.*, 2018)

The phenolic analysis of gallic acid was done by creating a gallic acid standard curve with the following concentrations: 200 ppm, 180 ppm, 120 ppm, 80 ppm, 40 ppm, and 0 ppm. As much as 0.2 mL of solution was taken from each test tube then 1 mL agent reagent Folin-Ciocalteau, 0.8 mL of 7.5% Na_2CO_3 solution and 3 mL of distilled water. Solutions were homogenized and incubated at room temperature for 30 minutes and calibrated with an absorbance of 750 nm. Furthermore, the coefficients a and b were calculated from each stock solution of gallic acid. Obtained coefficients were then used in a linear model: y = ax - b

Phenolic concentrations of samples were calculated using the following equation based on the previously obtained linear model:

$$\text{Total Phenolic} = \frac{(y = ax\text{-}b)}{g}$$

y = absorbance value; a = coefficient a value; x = dilution value; b = coefficient b value; g = sample weight (gram).

Phosphorus Analysis

Phosphorus content analysis was conducted by homogenized samples and adding 10 mL HNO_3: $HClO_4$ (1:1). Solutions were heated on a hot plate until boiling and distilled water was added until solutions reached 50 mL. Standard curve of phosphorus was created using stock solutions of the following concentrations: 0 ppm, 1 ppm, 4 ppm, 8 ppm, 16 ppm, and 32 ppm. As much as 1 mL of AMV solution was added and distilled water was added until solution reached 10 mL. Solutions were then homogenized and another 1 mL of AMV solution was added. Results were read using UV-Vis Spectrophotometer with a wavelength of 430 nm. Calculation of phosphate content used the following formula:

$$\text{PO4 Content} = \frac{\text{PO4 UV – Sis result} \times \text{Final volume} \times \text{Dillution factor}}{\text{Sample weight (g)}}$$

Statistical Analysis

Data on disease occurrence in the field was analysed using Analysis of Variance (ANOVA) with 95% confidence level. If significant differences were detected, post-hoc tests were done using Duncan's Multiple Range Test (DMRT) with 95% confidence level. In addition, the data were analysed descriptively and shown in graphics and figures.

RESULTS AND DISCUSSION

Disease Intensity and AUDPC

Twisted disease incidence and intensity after application of BCA *Bacillus* and mycorrhizal fungi on shallots were not significantly different from the control (Table 2 and 3). This shows that the application of BCA *Bacillus* and mycorrhizal fungi were not able to inhibit twisted disease on shallot plants. In off-season conditions, environment of shallot plantations often support growth of pathogens. According to rainfall data monthly, the rainfall from August to September 2021 showed high rainfall levels and followed by high humidity (Table 4). Agrios (2005) stated that high levels of rainfall will impact high humidity and increase *Fusarium* spp. growth to temperatures of 28°C. According to Francl (2001) the success of a pathogen to infect host plants is influenced by 3 factors, such as supportive environment, pathogen virulence, and host plant susceptibility.

Table 2. Twisted disease intensity development on shallots after treated using *Bacillus* and mycorrhizal fungi

Bacillus isolate	Disease Intensity (%)							
	Weeks After Planting							
	1	2	3	4	5	6	7	8
TL-9	0	18.43a	18.43a	26.57a	33.21a	50.00a	56.79a	79.53a
BRB TI	0	21.39a	26.57a	33.21a	40.00a	60.00a	63.43a	79.53a
EP-3	0	21.39a	24.12a	31.11a	37.29a	46.70a	56.79a	75.00a
RC-76	0	21.39a	26.57a	33.21a	40.00a	60.00a	58.89a	79.53a
A-11	0	18.43a	18.43a	26.57a	33.21a	60.00a	61.14a	79.53a
KP-B51	0	24.12a	26.57a	31.11a	37.29a	60.00a	65.88a	79.53a
A-9	0	18.43a	18.43a	26.57a	33.21a	56.70a	58.89a	75.00a
B-27	0	21.39a	26.57a	33.21a	46.70a	70.00a	68.61a	79.53a
Mycorrhizal fungi	0	18.43a	26.57a	33.21a	46.70a	66.70a	65.88a	79.53a
Control	0	21.39a	24.12a	31.11a	43.30a	63.30a	63.43a	79.53a
Sig.(*p*)	0	0.593	0.590	0.590	0.830	0.830	0.830	0.620

Notes: Numbers followed by different notations in the same column group show significant differences according to Duncan's Multiple Range Test (DMRT) at a 95% confidence level with arcsine Transformation Data $=\sqrt{x+0.5}$

Table 3. Area Under of the Disease Progress Curve (AUDPC) of twisted disease in shallots treated using *Bacillus* and mycorrhizal fungi after 6th week after planting

Bacillus isolate and mycorrhizal fungi	AUDPC
TL-9	287.28a
BRB TI	309.16a
EP-3	304.26a
RC-76	242.66a
A-11	204.16a
KP-B51	242.08a
A-9	285.83a
B-27	230.41a
Mycorrhizal fungi	274.16a
Control	230.41a
Sig. (*p*)	0.61

Notes: Numbers followed by different letters showed significant differences according to Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

A study conducted by Supyani *et al.* (2021) showed that the intensity of twisted disease in shallots increased to 52.14% in the rainy season.

Agronomic Parameter in the Field

Treatment B8 showed significant effects on the number of leaves and treatment M had significant effects on plant height (Table 5). For the number of leaves, treatment B8 had the highest value of 35.66, while treatment M had the tallest plant height of 14.60 cm. Meanwhile, Figure 1 showed that there were significant differences between treatment B8 and M compared to the control. The BCA *Bacillus* did not only provide stimulant to ISR but can also provide nutrients needed by plants according to research conducted by Rahma *et al.* (2020) that *Bacillus* sp. increased the growth of shallots, especially in isolate B-27 due to *Bacillus* sp. known role as Plant Growth Promoting Rhizobacteria (PGPR) through several mechanisms. One of these mechanisms is Indole-3-Acetic-Acid (IAA) that can synthesize auxin that affect plant growth rates and plant roots to increase acquisition of nutrients by plants (Kundan *et al.,* 2015). Inoculation of *Bacillus* spp. have also showed increase in the growth parameters of shallot, such as dry weight and leaf surface area (Hafri *et al.,* 2020).

Table 4. Monthly rainfall during 2021 in Bantul Regency

Month	Rainfall Monthly (mm)
January	369
February	447
March	208
April	122
May	1
June	261
July	8
August	33
September	48
October	151
November	551
December	339

However, other parameters such as plant height, root weight, and tuber weight were not significantly affected due to lack of doses, application frequency, and intensity of BCA *Bacillus* and mycorrhizal fungi treatment.

Analysis of Phenolic Content

Analysis of total phenol content aimed to determine the role of phenol content as a secondary metabolite in inhibiting the occurrence of twisted disease in shallots. Based on Table 6, treatments B6, B7, and M had significantly decreased phenol content in shallot. The cause of this decline has not yet been found. Onion plants basically have a high level of phenol (Yang *et al.,* 2004) because phenolic compounds are most commonly found in plants as a defence mechanism against plant pathogen.

Table 5. Shallots growth after treated with *Bacillus* and mycorrhizal fungi

Bacillus Isolates and Mycorrhizal Fungi	Root Weight (g)	Tuber Weight (g)	Number of Leaves	Plant Height (cm)
TL-9	14.28ab	92.05a	23.00a	15.50ab
BRB TI	8.38ab	101.94a	23.33ab	15.26ab
EP-3	7.38ab	126.21a	31.66bc	16.83bc
RC-76	9.09ab	161.68a	25.33ab	15.76ab
A-11	3.92a	95.18a	22.67a	15.93abc
KP-B51	8.39ab	67.41a	25.33ab	17.00bc
A-9	9.76ab	153.01a	20.33a	16.76bc
B-27	19.20b	112.64a	35.66c	17.70b
Mycorrhizal fungi	13.53ab	83.86a	26.33ab	14.60a
Control	15.78ab	99.54a	24.00ab	16.83bc
Sig. (*p*)	0.64	0.282	0.282	0.134

Notes: Numbers followed by different notations in the same column group showed significant differences according to Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

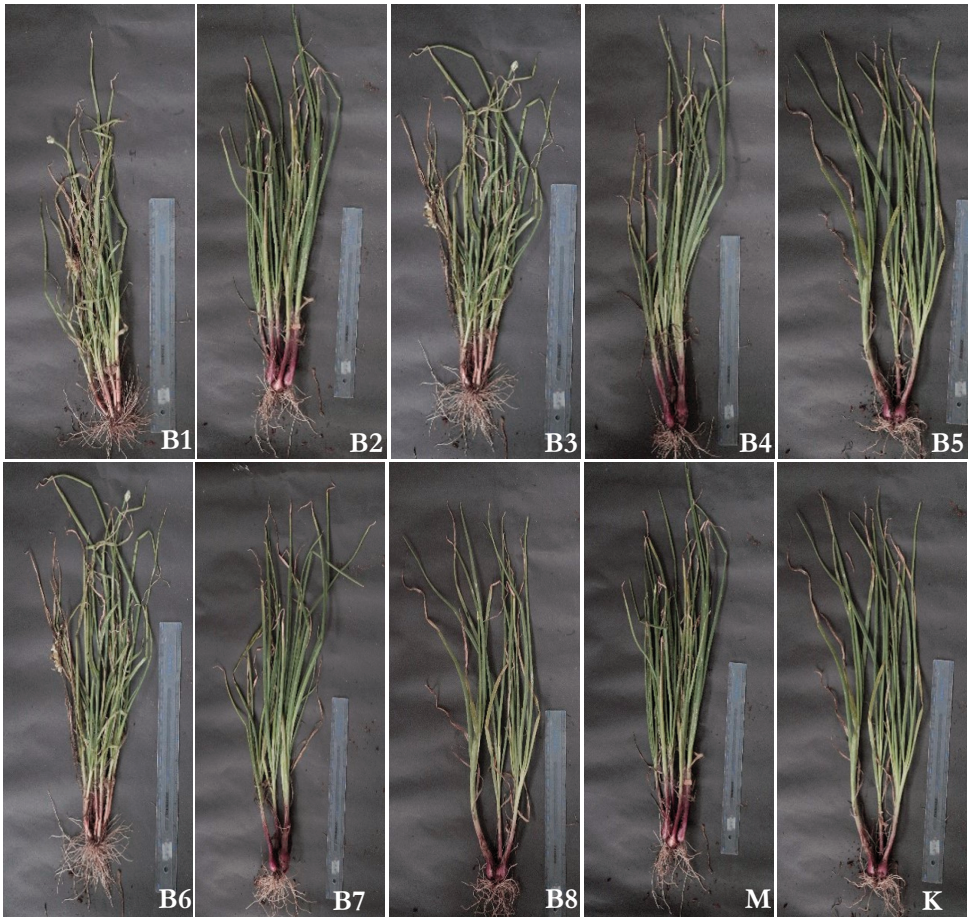

Figure 1. Shallot plant growth treated with *Bacillus* spp. and mycorrhizal fungi after 6th week after planting (B1 = isolate TL-9; B2 = isolate BRB Tl; B3 = isolate EP-3; B4 = isolate RC-76; B5 = isolate A-11; B6 = isolate KP-B51; B7 = isolate A-9; B8 = isolate B-27; M= mycorrhizal fungi; K= without mycorrhizal fungi and *Bacillus*)

Table 6. Phenolic content form shallot samples treated with *Bacillus* and mycorrhizal fungi at 6th week after planting

Bacillus isolate and mycorrhizal fungi	Total Phenol (mg Gallic Acid/g sample)
TL-9	0.84bc
BRB TI	0.83bc
EP-3	0.85bc
RC-76	0.84bc
A-11	0.80abc
KP-B51	0.76a
A-9	0.76a
B-27	0.86c
Mycorrhizal fungi	0.79a
Control	0.86c
Sig. (*p*)	0.124

Notes: Numbers followed by different notations in the same column group showed significant differences according to Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

Table 7. Phosphorus content form shallot samples treated with *Bacillus* and mycorrhizal fungi at 6th week after planting

Mycorrhiza	Phosphorus Total (mg/kg)
Mycorrhiza 1	403.61a
Mycorrhiza 2	295.42a
Mycorrhiza 3	450.63a
Control 1	372.87a
Control 2	263.13a
Control 3	391.91a
Sig. (*p*)	0.647

Notes: Numbers followed by different letters show significant differences according to Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

Analysis of Phosphorus Content

Phosphate analysis was conducted to determine nutrients absorption after treatment of mycorrhizae, especially phosphorus. Based on Table 7, the total value of phosphate content was not significantly different from the control. The reason is still not known for certain and requires further research with the addition of the frequency of mycorrhizal treatment to confirm the results of this study.

CONCLUSION

All eight isolates of *Bacillus* spp. and mycorrhizal fungi did not reduce twisted disease incidence and intensity on shallot production during off-season planting. However, it could improve number of leaves. Phenolic content significantly decreased for treatment B6, B7, and M. Meanwhile phosphorus content was not significantly affected in shallot samples after BCA treatment.

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