Jurnal Perlindungan Tanaman Indonesia, Vol. 27, No. 2, 2023: 93–102
DOI: 10.22146/jpti.77784
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Potential of Compost Enriched with *Bacillus velezensis* B-27 and *Bacillus cereus* RC76 for the Management of Twisted Disease on Shallots

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ABSTRACT

Shallot (*Allium cepa* var. *aggregatum*.) is a horticultural plant that is widely consumed in the world. However, the productivity of shallots in Indonesia is still relatively low, if compared to the actual optimum production potential of shallot. Shallot cultivation in Indonesia often experiences many problems. One of the problems is twisted diseases caused by *Fusarium* sp. This research aimed to study the effect of the application of organic material enriched with Bacillus in suppressing the development of twisted disease of shallot and increasing yield of shallot. This study was arranged in Randomized Complete Block Design (RCBD) with 5 treatments namely (A) compost + *Bacillus velezensis* isolate B-27, (B) compost + *Bacillus cereus* isolate RC76, (C) *B. velezensis* isolate B-27+*B. cereus* isolate RC76+compost, (D) compost + *Trichoderma asperellum* and (E) control (compost 1 ton/ha) with 5 replications on glasshouse treatment and 3 replications on field treatment. The results showed that the combination of *B. velezensis* in compost effectively reduced the incidence of twisted disease, the number of *Fusarium* spp. colonies, and the number of infected bulbs by *Fusarium* sp. Besides, the combination of compost with microbial agents showed better results than compost single treatment.

Keywords: *Bacillus* spp.; compost; shallot; *Trichoderma*; twisted disease

INTRODUCTION

Shallots (*Allium cepa* var. *aggregatum* L.) is a species of the *Allium* plant genus that is widely cultivated and is an important crop. Shallots have a high economic value and have become a necessity for the Indonesian causing their demand continually increase respective to population growth. Although the majority comes from Asia, shallot is now widely cultivated in various countries and climates, ranging from tropical to temperate regions (Marrelli *et al.*, 2018). In 2019, the global shallot planted area is estimated at 5,192,651 ha producing 99,968,016 tons (FAO, 2020). The increase in demand for shallots occurred in Indonesia with an average increase in national consumption in 2014−2018 reaching 2.88% per year (Manurung, 2019). This consumption growth cannot be supported by low national productivity where often the supply is not sufficient to meet national needs (Yanuarti & Afsari, 2016).

Shallot plants require a lot of nutrients in the planting process. To reduce the use of chemical fertilizers, the use of other nutritional sources as well as biological fertilizers (biofertilizers) is expected to meet the nutritional deficiencies of shallot plants. Organic fertilizers are known to help the availability of nutrients and increase the efficiency of chemical fertilizers in shallot cultivation. Application of Biological Control Agent (BCA) is an alternative solution to be used safely in controlling plant diseases. One of the Bacillus isolates belonging to the Laboratory of Plant Disease Universitas Gadjah Mada, namely *Bacillus velezensis* B-27 after being investigated was able to increase plant height, number of leaves, number of tillers and bulb weight in shallot plants. However, *B. velezensis* has not been able to reduce the intensity of disease in shallots, one of the causes is thought to be because it was carried out in the off-season (Rahma *et al.*, 2020). Therefore, this study was conducted to confirm the ability of *B. velezensis* enriched compost.

Another type of bacteria, namely *Bacillus cereus*, was shown to produce Indole Acetic Acid (IAA) (Zhou *et al.*, 2021). The formation of a clear zone on National Botanical Research Institute's Phosphate (NBRIP) media around the colony is also an indication of the activity of phosphate-solubilizing bacteria (Ibáñez *et al.*, 2021). Another collection, namely *B. cereus* RC76, the results of the in vitro antagonism test of isolates against *Ralstonia syzygii* subsp. *syzygii* on Yeast Peptone Agar (YPA) medium also showed that among the tested bacteria could produce inhibition zones. This indicates that antibiosis is one of the antagonistic mechanisms of *B. cereus* bacteria in controlling *R. syzygii* subsp. *syzygii* on cloves (Dwimartina *et al.*, 2017). However, the ability of *B. cereus* RC76 bacteria to suppress plant diseases still needs to be investigated further, especially in field conditions.

Trichoderma sp. has been widely used as a biocontrol agent to manage plant diseases and control plant pathogenic fungi, including Fusarium species. It is known that *Trichoderma asperellum* can be used effectively in the management of shallot disease caused by *Fusarium* sp. such as twisted disease, basal rot disease, damping off disease and increases plant growth (Darsan *et al.*, 2016; Gunaratna *et al.*, 2020; Wulan *et al.*, 2022). Díaz-Gutiérrez *et al.* (2021) revealed that *T. asperellum* is known to produce phytohormones (salicylic and jasmonic acids) and to secrete cell wall degrading enzymes (chitinase and cellulase). This ability can be related to the activity of its antagonists and mycoparasites so it has the potential as a biological controller of Fusarium. In this study, *T. asperellum* used was produced and recommended by the The Biological Laboratory, Pakem, Sleman, so it was used as a treatment comparison.

In shallots, macronutrients availability in the soil rapidly declines as they are absorbed by plants. The enrichment of organic matter with useful microbes can support these microbes to utilize organic carbon for their heterotrophic metabolism (Eiler *et al.*, 2003). Enrichment of organic matter by inoculation of certain microbes has been shown to be able to produce the presence of macronutrients and soil microbes that are beneficial for plants (Berger *et al.*, 2013; Lasmini *et al.*, 2018). Hindersah *et al.*, (2021) also added that organic fertilizer enriched with a consortium of microbes was able to increase several growth parameters, including the number of nutrients in the soil and the yield of shallots. This experiment was conducted to evaluate the potential of compost enriched with *B. velezensis* B-27 and *B. cereus* RC76 as a source of organic matter and for the management of twisted disease and shallot yield.

MATERIALS AND METHODS

Research Location

The research was carried out in a greenhouse and the field. Greenhouse research was conducted at the Laboratory of Plant Disease and Greenhouses, Faculty of Agriculture, Universitas Gadjah Mada. The field research was conducted in a shallot cultivation area located in the coastal area, Parangtritis, Yogyakarta from February until April 2021.

Analysis of Needs for Bacterial Suspension and *T. asperellum*

The density of the bacterial suspension of *B. velezensis* B-27 and *B. cereus* RC76 bacteria used was 10^8 CFU/ml with an OD (optical density) of 0.5 and the wavelength (λ) of 600 nm. Both bacteria and *T. asperellum* were prepared in suspension form for treatment. Referring to Sumardiyono *et al.* (2007), the suspension requirements of bacteria and *Trichoderma* were 12.5 ml of the suspension for every 50 g of compost. In the greenhouse experiment, the use of compost was calculated based on the polybag area of 0.16 m^2, and each polybag used 16 g of compost. In the field experiment, each treatment used 1 kg of compost/plot, so the suspension requirement for each plot was 250 ml of suspension/kg of compost.

Preparation of *Bacillus* spp. and *Trichoderma* Isolates

The antagonist bacteria used was *B. velezensis* B-27 *and B. cereus* RC76. The bacterial isolates were collection of the Plant Disease Laboratory, Universitas Gadjah Mada. Both isolates were cultured in Yeast Peptone Agar (YPA) media (Yeast extract 0.5%, polypeptone 1%, agar 1.5%) and then incubated at room temperature for 48 hours. Isolate of *T. asperellum* as a comparison treatment was obtained from the collection of the Biological Laboratory, Pakem, Sleman in the form of preparations on corn media.

Producing of Bacterial Suspensions and *T. asperellum*

B. velezensis B-27 and *B. cereus* RC76 were harvested using ose, then suspended in sterile water (dH_2O) according to the required amount. The density of the suspension was then calculated using a spectrophotometer for a density of 10^8 CFU/ml with an OD (optical density) of 0.5 at λ of 600 nm and homogenize.

The amount of *T. asperellum* following the recommended dose of compost: *T. asperellum* = 500:1 (w/w). In the greenhouse experiment, for every 16 g of compost, a suspension of 0.032 g of *T. asperellum* was prepared which was dissolved in 4 ml of sterile water (dH_2O). In the field treatment, each treatment plot needed 1 kg of compost. Thus, 2 g of *T. asperellum* were weighed and 250 ml of sterile water was added to each treatment plot. The suspension was then shaken and allowed to stand for a while. The suspension was filtered before use to remove the remaining corn residue.

Compost Enrichment with Bacteria or *T. asperellum*

Each suspension for each type of treatment was put into different sprayer bottles. Compost was placed on a tray, and enrichment was done by spraying the suspension (16 grams compost/40 ml suspension) into the compost aseptically. The enriched fertilizer was then mixed well, then incubated for 24 hours. The types of enrichment treatment were:
A : compost + isolate B-27
B : compost + isolate *B. cereus* RC76
C : compost + isolate *B. velezensis* B-27 + *B. cereus* RC76
D : compost + *Trichoderma asperellum*
E : control (compost 1 ton/ha)

Greenhouse Experiment

Planting medium for the greenhouse experiment was 5 kg of sterile soil per pot. Shallots were planted in polybags (5 plants per pot). The research design used was Completely Randomized Block Design (CRBD) consisting of 5 treatments and 5 blocks as replication resulting in 25 experimental units and each experimental unit consisted of 5 plants.

Two days before planting, the media was mixed with enriched compost according to the treatment. The shallot planted were Tajuk variety obtained from farmers from Bantul. Two weeks after planting, plants were inoculated with 25 mL of *Fusarium acutatum* spore suspension with a density of 10^6 spores/ml. The *F. acutatum* used was a collection from the UGM plant disease laboratory. *F. acutatum* was previously cultured on a PDA medium for 48 hours at room temperature. Inoculation was done by pouring *Fusarium* suspension on the soil where the shallots were planted.

Field Preparation

The field preparation consisted of soil tillage and making plots of beds. Beds were made in the size of 10×1 m^2 of 0.5 m height and 0.5 m distance between the beds. Planting distances were 15×15 cm. Fertilization was also carried out using NPK fertilizer 4 days after planting. Farming practices followed common practices of local farmers. The seeds in the form of tubers used were obtained from local farmer.

Application of Enriched Compost Fertilizer

The application was made two days before planting by spreading the compost on the fields. Fertilization was done the day before planting using compost at a dose of 1 ton/ha or 1 kg/10 m^2 which has been enriched with bacterial suspension of *B. cereus* B-27, RC76 isolates, a mixture of both *Bacillus* isolates and Trichoderma.

Observation and Data Collection

Observation was done on incidence of twisted disease, the population of Fusarium in the soil, and infected bulb by Fusarium. The agronomic parameters observed included plant height, fresh weight, production weight, and tuber dry weight.

Observation of twisted disease incidence. Twisted disease is a systemic disease making it more important to observe disease incidence than disease intensity because symptom can be observed in all parts of the leaves. Disease incidence observation was carried out by counting plants showing twisted symptom in each plot. In each planting plot, there were 125 plant samples. Disease incidence was observed once a week from 1 week after planting until the plants were 6 weeks old. The incidence of shallot disease was calculated using a formula that refers to Lestiyani *et al.* (2016) as follows:

$$\text{Disease Incidence (DI)} = \frac{n}{N} \times 100\%$$

Where n = number of diseased plants, N = total number of plants.

Total *Fusarium* population calculation in soil. Prepared Komada media which is a *Fusarium*-specific media was used in this study (Sun, 1978). As much as one gram of soil was sampled from both greenhouse and field treatments, with 3 replications for each treatment. The soil was suspended in sterile water with a dilution of 10^{-2}, then 100 microliters was poured into a solid medium. Observations were made by counting the number of white fungal colonies of *Fusarium* sp. at 4 days after incubation on media.

Observation of bulb infected by *Fusarium* sp. The bulb samples were taken from the harvested shallot plants. The bulbs were then washed with running water, surfaces were sterilized with 70% ethanol, and cut into small pieces. These small pieces were then immersed in 0.5% NaOCl, soaked in sterile water, and then placed in a petridish that already contained Komada medium (Sun, 1978). One petridish contained 3 small pieces of shallot. The number of pieces that show fungal growth were then counted.

Data Analysis

The data were analyzed using ANOVA to determine the differences between treatments. If there was a difference, then Duncan's test was carried out at a 95% confidence level. In addition, the data was also analyzed descriptively and displayed in the form of graphs and images and included in the report.

RESULTS AND DISCUSSION

Twisted Disease Suppression of Shallot

In accordance with Lestiyani *et al.* (2016), twisted disease shallots showed specific symptoms including the production of longer pseudostems and pale green twisted leaves without wilting. On the other hand, the pathogen that caused twisted disease could sometimes cause bulb rot symptom. Furthermore, infected bulbs were smaller than healthy bulbs. Based on the results of this research, compost enriched with both *B. velezensis* B-27 and *B. cereus* RC76 were able to significantly reduce the incidence of twisted disease in the greenhouse compared to the control and treatment of compost enriched with *T. asperellum*. However, in the field treatment, only compost enriched with *B. velezensis* B-27 significantly reduced the incidence of twisted disease (Table 1).

Compost enriched with *B. cereus* RC76 showed the ability to suppress twisted incidence in the greenhouse but did not have a significantly different effect when applied in the field (Table 1). This is presumably because *B. cereus* requires more certain conditions for its growth. The environmental conditions in the field experiment and the greenhouse were different. The biological control effectiveness is highly dependent on the successful introduction of biocontrol agents used with their environment (de Weert & Bloemberg, 2006). Several abiotic factors have considerable impacts on the introduction success of microorganisms into plant roots including the soil environment, soil structure and moisture content, soil temperature and soil pH (Zhang *et al.,* 2000).

Compost is thought to affect nutrients and communities in the soil. However, the combination of compost with *B. velezensis* or *B. cereus* showed better results when compared to the combination of compost with *T. asperellum* or a single application of compost. This showed that individual application of compost often gives inconsistent results for disease management. Shen *et al.* (2013) have studied that inoculation of compost or enrichment with rhizosphere microbes is known to be more efficient in controlling soil-borne pathogens than the use of microbes or compost alone. Recently, a combination of microbes and compost has also been studied in the management of diseases caused by *Fusarium* (Fu *et al.*, 2016, 2017; Huang *et al.*, 2019). Enrichment of compost with beneficial microbes is carried out as an effort to increase the benefits of compost in increasing soil fertility (Andrade *et al.*, 2021).

Table 1. The effect of compost enriched with *Bacillus velezensis* B-27 and *Bacillus cereus* RC76 on incidence of twisted disease and bulb infected by *Fusarium* sp.

Treatment	Glasshouse Experiment		Field Experiment	
	Disease Incidence (%)	Percentage of Infected Bulb (%)	Disease Incidence (%)	Percentage of Infected Bulb (%)
A	68.77 [b]	0 [d]	18.40 [b]	0
B	71.50 [b]	0 [d]	32.26 [a]	0
C	71.22 [b]	44 [c]	28.00 [ab]	0
D	85.54 [a]	75 [b]	38.40 [a]	0
E	93.84 [a]	100 [a]	36.00 [a]	0

Remark: (A) compost + isolate *Bacillus velezensis* B-27, (B) compost + isolate *Bacillus cereus* RC76, (C) compost + isolate *B. velezensis* B-27+ *B. cereus* RC76, (D) compost + *Trichoderma* sp., (E) control (compost) 1 ton/ha. Data analysis using DMRT, data notation followed by the same letter shows a non-significant difference at the 95% confidence level.

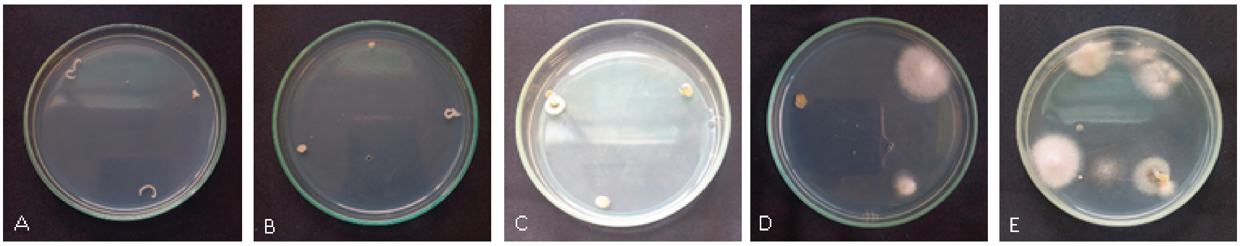

Figure 1. The results of the test of infected bulb from the greenhouse experiment on Komada's medium; (A) compost + isolate *Bacillus velezensis* B-27, (B) compost + isolate *Bacillus cereus* RC76, (C) compost + isolate *B. velezensis* B-27+ *B. cereus* RC76, (D) compost + *Trichoderma* sp., (E) control (compost) 1 ton/ha

Research by Ye *et al.* (2021) found that *B. velezensis* produced a type of antifungal peptide called as fengycin. Fengycin suppresses mycelium growth of the pathogenic fungus *F. solani* that causes basal stem rot disease in passion fruit plants. Further studies revealed that fungycin increased the permeability of the mycelial membrane of *F. solani*, caused swelling at the tip of the hyphae, and caused abnormal accumulation of nucleic acid and chitin (Xu *et al.*, 2016). In addition, *B. velezensis* is known to produce secondary metabolites in the form of iturin, surfactin, oxydifficidin, bacillibactin, L-di hydroanticapsin, and azelaic acid which play an important role in suppressing pathogens (Nifakos *et al.*, 2021). The secondary metabolites produced by *B. velezensis* can also trigger the induction of systemic resistance in plants so that plants are resistant to pathogen attack (Rabbee *et al.*, 2019).

Based on research by Wang *et al.* (2018), *B. cereus* strain BCM2 bacteria colonized roots and rhizosphere of tomato plants better in soils with neutral pH, compared to alkaline or acid soils. In addition, 25% water content in the soil showed optimum population of *B. cereus* in the rhizosphere. Meanwhile, at higher water content, the population decreases. However, it is necessary to conduct further studies on this matter.

Based on the results of isolation of harvested bulb on Komada's medium, field experiment no Fusarium fungus successful grew from all treatments. Meanwhile, in the greenhouse experiment microbe enriched compost showed better results when compared to the control (Table 1; Figure 1). In addition, results of isolating bulbs on Komada's medium showed that the combination of compost with *B. velezensis* or *B. cereus* showed that no *Fusarium* fungus growth. Whereas in the control, more *Fusarium* growth compared other treatments. Based on these results, compost enriched with microbes could suppress tuber-borne pathogens, especially in the greenhouse experiment.

Results also showed that the application of compost enriched with *T. asperellum* did not show optimal results compared to other treatments in reducing the incidence of twisted disease and in suppressing the infection of *Fusarium* sp. in greenhouse and field trials. One possibility was that the ratio between *Trichoderma* and compost was not effective for the management of twisted disease in shallots. In addition, it was suspected that *T. asperellum* isolate was not effective in combination with compost. This is in line with Singh *et al.* (2020) that showed *Trichoderma* sp. was only able to suppress disease under limited nutritional conditions. It has been reported that *Trichoderma* spp. does not attack *R. solani* in the presence of compost due to the availability of cellulose for *Trichoderma*. In line with this, Pal and McSpadden Gardener *et al.* (2006) also revealed that at a lower amount of cellulose, the gene that regulates the synthesis of chitinase, in *Trichoderma* spp. activated so that enzymes for parasitic activity are then produced.

Effect of Compost Enriched with Rhizosphere Bacteria on the Number of Fusarium Colonies in Soil

Treatment of compost enriched with *B. velezensis* and *B. cereus* or a mixture of both in the greenhouse were able to significantly suppress the number of *Fusarium* spp. colonies. compared to controls. In addition, in the field experiment all treatments decrease the number of colonies significantly different from the control, but *B. velezensis* significantly showed the best results (Table 2).

This was consistent with previous research by Wu *et al.* (2020) who stated that in suppressing Fusarium, *B. velezensis* was found to affect the soil environment in various ways. *B. velezensis* increases peroxidase levels, which increases plant defense, and shows better yields when combined with compost. Furthermore, *B. velezensis* rebuilds the microbial community damaged by the pathogen and further increases the degree of disease suppression along with the compost. *B. velezensis* also improves the functional properties of the soil, including those related to defense mechanisms, and these properties are further enhanced by the combination of compost. *B. velezensis* strain has good potential in promoting plant growth.

Isolation results from shallot rhizosphere soil samples on Komada's medium showed that the treatment of compost enriched with *Bacillus* spp. in the greenhouse were able to significantly suppress the number of *Fusarium* spp. colonies compared to control (Figure 2). Treatment of compost enriched with *T. asperellum* was also known to reduce the number of Fusarium colonies in postharvest soil. It can be seen in Figure 2a that the smallest number of Fusarium colonies was in the compost treatment enriched with *B. velezensis* that implies that that this treatment showed the best results compared to the other treatments. While the most numerous Fusarium colonies were in the control (Figure 2e).

Along with previous tests, environmental factors (soil texture, temperature, humidity, radiation) in the field affect each isolate differently and therefore, it is important to choose microbes accordingly environmental situations. This is in line with Lahlali *et al.* (2022) which stated that environmental factors had significant impacts on the success of biocontrol programs. Small changes in temperature changed the concentration of microbes. Moreover, the virulence of agents decreased when they were exposed to UV light from the sun.

The Effect Compost Enriched with Rhizosphere Bacteria on Shallot Yield

Results showed that there was significant difference between the control plant height with other treatments in the field. However, on other parameters,

Table 2. The effect of compost enriched with *Bacillus velezensis* B-27 and *Bacillus cereus* RC76 on total population of Fusarium colonies in soil after harvest

Treatment	Glasshouse Experiment (cfu/ml)	Field Experiment (cfu/ml)
A	4.66×10^{2} c	4.66×10^{2} d
B	6.33×10^{2} c	1.06×10^{3} c
C	9.66×10^{2} c	1.66×10^{3} b
D	3.63×10^{3} b	1.73×10^{3} b
E	1.53×10^{4} a	1.90×10^{3} a

Remark: (A) compost + isolate *Bacillus velezensis* B-27, (B) compost + isolate *Bacillus cereus* RC76, (C) compost + isolate *Bacillus velezensis* B-27+ *Bacillus cereus* RC76, (D) compost + *Trichoderma* sp. (E) control (compost) 1 ton/ha. Data analysis using DMRT, data notation followed by the same letter shows a non-significant difference at the 95% confidence level

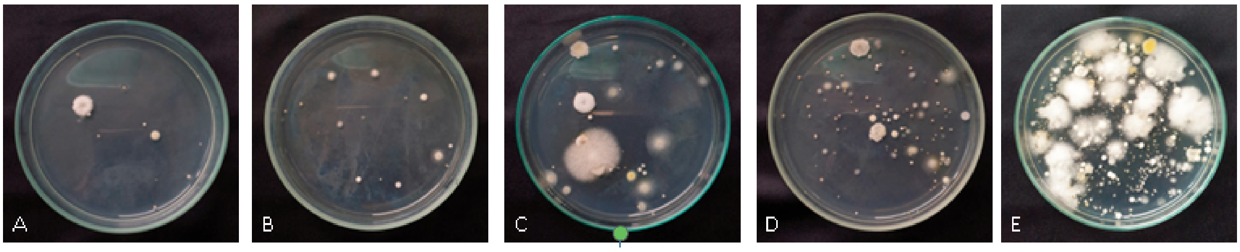

Figure 2. The isolation result of Fusarium from shallot rhizosphere soil samples in the greenhouse experiment on Komada's medium; (A) compost + isolate *Bacillus velezensis* B-27, (B) compost + isolate *Bacillus cereus* RC76, (C) compost + isolate *B. velezensis* B-27+ *B. cereus* RC76, (D) compost + *Trichoderma* sp., (E) control (compost) 1 ton/ha. Data analysis using DMRT, data notation followed by the same letter shows a non-significant difference at the 95% confidence level

Table 3. The effect of compost enriched with *Bacillus velezensis* B-27 and *Bacillus cereus* RC76 on yield of shallots in the glasshouse and field experiment

Treatment	Glasshouse Experiment				Field Experiment			
	Plant Height (cm)	Wet Weight (grams)	Production Weight (grams)	Dry Weight (grams)	Plant Height (cm)	Wet Weight (grams)	Production Weight (grams)	Dry Weight (grams)
A	29.34 a	8.59 ab	6.16 ab	5.19 ab	26.77 a	496.66 a	356.33 a	300.33 a
B	25.77 a	11.98 a	8.46 a	6.73 a	25.13 ab	524.33 a	367.33 a	289.00 a
C	25.86 a	7.61 ab	5.40 ab	4.08 ab	25.23 ab	410.66 a	293.33 a	227.33 a
D	28.10 a	6.46 ab	4.77 ab	3.88 ab	25.48 ab	470.66 a	349.00 a	285.00 a
E	26.63 a	5.12 b	3.70 b	2.77 b	21.32 b	489.33 a	351.83 a	265.66 a

Remark: (A) compost + isolate *Bacillus velezensis* B-27, (B) compost + isolate *Bacillus cereus* RC76, (C) compost + isolate *B. velezensis* B-27+ *B. cereus* RC76, (D) compost + *Trichoderma* sp. (E) control (compost) 1 ton/ha. Data was analyzed using DMRT, data followed by the same letter shows a non-significant difference at the 95% confidence level

compost enriched with *B. velezensis* did not show any significant difference. However, application of compost enriched with *B. cereus* showed a significant difference when compared to the control in the greenhouse in terms of fresh weight, production weight, and dry weight (Table 3). This is also similar with findings from Zhou *et al.* (2021) who stated that *B. cereus* could be used as a growth-promoting microbe in rice plants by producing Indole Acetic Acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophores, and phosphate-solubilizing and major enzymes. IAA and ACC deaminase have important properties as plant growth promoters (Velmurugan *et al.*, 2015). ACC deaminases can synergistically interact with IAA and enhance plant growth. IAA is a phytohormone, which help regulate plant root growth by stimulating root cell proliferation and elongation but has yet no clear effects on bacteria (Lwin *et al.*, 2012). ACC is a precursor for ethylene, one of the most important plant growth regulatory hormones (Bernard, 2005; Glick, 2014; Torbaghan *et al.*, 2016).

Results also implied that compost enriched with *B. velezenzis* had an important effect on shallot plants resistance against twisted disease and suppression its pathogens. Meanwhile, compost enriched with *B. cereus* had the ability to increase crop yield, especially in greenhouses. However, why compost enriched with *B. cereus* cannot suppress disease incidence, still needs further research.

CONCLUSION

The results showed that compost enriched with *B. velenzensis* had an important effect on the resistance of shallot plants to twisted disease and suppression of *Fusarium* sp. the causal agent of twisted disease. Meanwhile, compost enriched with *B. cereus* had the ability to increase the yield of shallots, especially in the greenhouse experiment. However, why compost enriched with *B. cereus* could not suppress disease incidence still needs further research.

ACKNOWLEDGEMENT

The authors would like to thank the Faculty of Agriculture, Universitas Gadjah Mada for funding this research in collaboration with the ACIAR Vegies Australia research project. We also thank the Laboratory of Plant Diseases, Faculty of Agriculture, Universitas Gadjah Mada, which has assisted in the process of collecting data for this research.

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