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RESEARCH PAPER

Enrichment of organic material with *Trichoderma asperellum* for the management of twisted disease on shallot

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ABSTRACT

Shallots are a type of plant used as a flavoring agent and are often affected by twisted disease caused by *Fusarium* spp. This study aims to determine the ability of organic material enriched by *Trichoderma asperellum* in controlling twisted disease, increasing production, and suppressing the population of *Fusarium* spp. in the soil. The research showed that compost and chicken manure enriched with *T. asperellum* in a ratio (200:1) were able to reduce the disease incidence compared to controls, in addition to increasing production and suppressing the population of *Fusarium* spp. in the soil. The colonies of *Fusarium* spp. in the soil were correlated with disease incidence in each treatment. The highest incidence of twisted disease was followed by the number of *Fusarium* spp. colonies. The microbial composition did not affect disease suppression when observed using a dependent method using PCR-RISA. The microbial composition with the dependent method using PCR RISA did not affect the suppression of the twisted disease in the shallot plants.

Key words: Fusarium, organic material, *T. asperellum*

INTRODUCTION

Shallot (*Allium cepa*) is a widely cultivated plant, extensively grown by farmers. It is a horticultural commodity commonly used as a flavoring ingredient. Although shallot production has been increasing annually, it still falls short of meeting the demand in Indonesia (Central Agency on Statistics, 2021). Efforts to boost shallot production and productivity are essential to meet the community's needs. However, a significant obstacle to achieving this goal is the presence of pests and diseases, with twisted disease being one of the severe conditions that affect shallots, leading to considerable losses.

Fusarium attack shallots during the rainy season, leading to twisted disease (Wibowo et al., 2023). The pathogen, *Fusarium* spp., is a soil-borne pathogen characterized by purplish-white, slightly orange-white, cream, or white-like cotton colonies. *Fusarium* spp. takes the form of thread-like structures, is branched, lacks chlorophyll, and and has a cell wall containing chitin and cellulase. Shallots infected with *Fusarium* spp. exhibit symptoms such as twisted leaves, a change in leaf color to pale green and yellowish, smaller and fewer bulbs, inability to produce bulbs, and in advanced stages, it can result in plant death. These symptoms align with those documented by Kaeni et al. (2014).

The application of organic material and *Trichoderma asperellum* has been reported to have the capability to suppress the development of twisted disease. Decomposed remains of plants, animals, and humans are added to the soil as organic material to provide nutrients. The use of organic material has a dual role: first, as a carrier for biocontrol agents, and second, as a source of nutrients for plants and biocontrol agents (Hoitink & Boehm, 1999). Organic material is needed in the soil; in addition to provide nutrients for microbes, organic material can also increase the activity of microorganisms in suppressing pathogens in the soil (Boehm et al., 1997). The plant growth media added with compost was able to suppress the development of *Fusarium* (Bonanomi et al., 2010). Organic materials that can be used are chicken manure and compost. Chicken manure contains many nutrients, and a recommended dose for shallots is 20 tons/ha (Samadi & Cahyono, 2005), while compost is recommended at a dose of 1 ton/ha (Santoso et al., 2005).

In addition to using organic materials, adding biological control agents such as *T. asperellum* to organic materials can also suppress the development of twisted disease in shallot plants. *T. asperellum*

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is a fungus that is antagonistic and is commonly found in the soil. *T. asperellum* generally has green colonies, septate mycelium, and conidia with a round or oval shape. According to Hidayati et al. (2019), *T. asperellum*, with a dose of 700 kg/ha, was able to increase the wet weight of shallot bulbs per clump. Research conducted by Deden & Umiyati (2017) stated that the application of *T. asperellum* can suppress the development of twisted disease in shallots. Besides being able to suppress twisted disease, *T. asperellum* can also suppress *Pyricularia oryzae* (Hidayat et al., 2014).

MATERIALS AND METHODS

Research Site. This research was conducted in the farmer's field in Samiran, Kretek District, Bantul Regency, from March to April 2021.

Experimental Design. The experiment was carried out using a completely randomized block design (RCBD) method, which consisted of 6 treatments, then each treatment was repeated three times so that 18 plots were obtained. The treatments tested include:

Treatment A : Control (Compost + Fungicide with the active ingredient iprodione),
Treatment B : Compost + *T. asperellum* (200:1),
Treatment C : Compost + *T. asperellum* (500:1),
Treatment D : Control (Chicken manure + Fungicide with the active ingredient iprodione),
Treatment E : Chicken manure + *T. asperellum* (200:1),
Treatment F : Chicken manure + *T. asperellum* (500:1).

Preparation of *T. asperellum*. Isolates of *T. asperellum* was collection from the Biological Agency Laboratory of the Department of Agriculture and Food Security Special Region of Yogyakarta. The isolates were cultured in 250 g of corn medium for 14 days until the fungal mycelium filled the corn medium or until the corn medium was green.

Soil Tillage. Soil tillage in the field experiment was carried out one day before applying organic material to break the soil and obtain loose soil conditions. Plant remains and weeds are removed from the planting area. The land was leveled, and the beds were made with a size of 1 × 10 m, a height of 30 cm and a 40 cm distance between plots.

***T. asperellum* Application on Organic Material.** The application of *T. asperellum* on organic material was made a day before being applied to the field. Applying *T. asperellum* on organic material was done by mixing *T. asperellum* evenly with organic material. *T. asperellum* was applied to organic material in two different ratios: 200:1 and 500:1. In the ratio of 200:1.1 kg of *T. asperellum* was added to 200 kg of organic material, while in the ratio of 500:1.1 kg of *T. asperellum* was added to 500 kg of organic material. For each plot in the field experiment, *T. asperellum* with a ratio of 200:1 was applied at 100 g per plot and *T. asperellum* with a ratio of 500:1 was applied at 40 g/plot.

The Population of *Fusarium* sp. in the Soil. Observation of the population of *Fusarium* sp. in the soil was carried out before planting, 28 days after planting, and 42 days after planting. The observation aimed to determine changes in the population density of *Fusarium* sp. in the soil by taking soil samples from the planting area and creating a suspension. Soil sampling involved collecting 10 g of soil from five different points within each plot. The collected soil was then decomposed, and 10 g of decomposed soil were used to create a suspension.

The suspension was prepared by homogenizing 10 g of decomposed soil with 90 mL of sterile distilled water, resulting in a 10^{-1} dilution. From this dilution, 1 mL was transferred to a test tube containing 9 mL of sterile distilled water, and homogenized to produce a 10^{-2} dilution. Similarly, from the 10^{-2} dilution, 1 mL was transferred to a test tube containing 9 mL of sterile distilled water, and homogenized to create a 10^{-3} dilution. The obtained suspension was then spread on Komada medium, a specific medium for Fusarium, using 50 µL and incubated for seven days at room temperature. After incubation, the colony density was calculated using the Total Plate Count (TPC) method, considering each living and developing cell in a petri dish as a colony (Arantika et al., 2019).

Organic Material Application. The organic materials consist of compost at a rate of 1 ton/ha (Santoso et al., 2007) and chicken manure at a rate of up to 20 tons/ha (Samadi & Cahyono, 2005). The required amount of compost for each planting plot in the field experiment is 1 kg, and the required amount of chicken manure is 20 kg. Organic materials enriched with *T. asperellum* in the field experiment were applied one day after tillage and three days before planting. The plot of land used in the experiment measures 1 × 10 m. Organic material was applied by thoroughly mixing it with the planting medium until it was evenly distributed. The

organic material must be ripe and dry after a 14-day fermentation process, characterized by the absence of any foul smell in the compost and chicken manure. Fungicide application was carried out using Iprodione 50 WP fungicide, applied every two weeks by spraying it on the plants.

Planting of Shallot. The planting of shallots was carried out three days after the application of organic material. One day before planting, watering was carried out until the field capacity was in place to maintain moisture in the soil. The shallots were planted by inserting the bulbs into the soil at a distance of 20 × 20 cm for each bulb.

Maintenance of Shallot. The maintenance of shallot plants in the field consisted of watering and weeding. Watering is done every morning or evening until the plants are 50 days after planting; then, watering is adjusted to the planting environment. Manual weeding is carried out according to the condition of the weeds in the crop (Samadi & Cahyono, 2005). No other fertilizers, except organic material, are added to the field.

Harvesting of Shallot. Harvesting of shallots in the field was carried out when the shallots were old enough with a plant age of 50–60 days after planting (Fatmawaty et al., 2015). The characteristics of onion plants that are ready to be harvested are that the plants fall and turn yellow, the bulbs become dry, and the neck of the stem becomes soft.

Bulbs Health Test. The bulb health test was carried out on healthy shallot bulbs obtained from the previous harvest. The harvested shallot bulbs were cut at the base, the bulbs were cultured on Komada media for seven days in the incubator to determine whether *Fusarium* spp. was present.

Observation Parameter. The parameters observed in this study were disease incidence, shallot plant height, fresh weight, dry shallot weight, shallot bulb weight, and bulb health test. The observation of disease incidence was carried out by counting the number of symptomatic plants and the total number of plants at 7-day interval using the following formula:

$$DI = \frac{n}{N} \times 100\%$$

DI = Disease incidence (%);
n = Number of symptomatic plants;
N = Total number of plants.

Data Analysis. The data were analyzed using Analysis of Variance (ANOVA) with a 95% confidence level in Statistical Package for the Social Sciences (SPSS). Observation of plant height was carried out by measuring the plant height with a ruler at 7-day intervals. Observation of fresh weight, dry weight, and bulb weight were conducted using a digital scale. Bulb health testing was carried out by isolating the harvested shallot bulbs on PDA media, then incubating for 7 days and observing the fungus that grows on the PDA media. To prepare the harvested shallot bulbs for this process, they are sterilized by soaking them in a 1% hypoclorite solution for 1 min, then rinsing with sterile distilled water and soaking in 70% alcohol for 30 s. Subsequently, they are rinsed again with sterile distilled water. Finally, the bulb pieces are aseptically placed on PDA medium.

Analysis of Microbial Diversity in Soil with PCR-RISA.

DNA Extraction. Soil samples were taken 28 days after planting and suspended with a density of 10^{-3}. The suspension was prepared by homogenizing 1 g of decomposed soil with 9 mL of sterile distilled water, resulting in a 10^{-1} dilution. From this dilution, 1 mL was transferred to a test tube containing 9 mL of sterile distilled water and homogenized to produce a 10^{-2} dilution. Similarly, from the 10^{-2} dilution, 1 ml was transferred to a test tube containing 9 ml of sterile distilled water and homogenized to create a 10^{-3} dilution. The suspension obtained was then plated on 50 µL of SEA (Soil Extract Agar) and incubated for 48 hours. Bacterial colonies that grew in SEA were then extracted for DNA (Joko et al., 2012). DNA Extraction and purification are based on a commercial protocol manual kit (Wizard Genomic DNA Purification Kit) (Promega, USA).

PCR-RISA. The analysis of microbial diversity in the soil was conducted in the field tests. Microbial diversity was assessed when the plants were 28 days after planting to determine the variety of microbes in the soil. Bacterial DNA was amplified in a total volume of 25 µL using the universal primers S926f (50-CTYAAAKGAATTGACGG-30) and L189r (50-TACTGAGATGYTTMARTTC-30) attached to positions 910 to 926 of the 16S rRNA gene and positions 189 to 207 of the 23S rRNA gene (Joko et al., 2012). PCR was carried out with 12.5 µL PCR mix, 1 µL forward and reverse primers, 1 µL DNA (ng/µL), and Milli-Q water. The PCR conditions were predenatured at 95 ºC for 2 min, denatured at 94 ºC

for 30 s for 30 cycles, annealed at 47 ºC for 30 s at 30 cycles, and extended at 72 ºC for 30 min at 30 cycles. After 30 cycles, there was an elongation for 5 min at 72 ºC and then cooled at 48 ºC. The PCR results were then added to a 2% agarose gel to which ethidium bromide had been added and electrophoresed at 70 volts for 120 min with BioRad Mini Ready Sub-Cell GT Horizontal Electrophoresis Cell.

RESULTS AND DISCUSSION

Disease Incidence. The results showed that the application of compost enriched with *T. asperellum* in a ratio of (200:1) reduced the disease incidence in shallot plants, reaching 27.69% compared to the controls (Table 1). The treatment of chicken manure enriched with *T. asperellum* in a ratio of (200:1) reduced the disease incidence in shallot plants, reaching 23.58%. This result aligns with the research conducted by Deden & Umiyati (2017), who stated that the addition of *T. asperellum* reduced the incidence of twisted disease in melon plants compared to the control treatment without *T. asperellum* application.

Plant Height of Shallot. The plant height of shallots in all treatments was not significantly different; adding organic material enriched with *Trichoderma* did not yield significantly different results from the control treatment (Table 1). According to research conducted by Hafri et al. (2020), *T. asperellum* was able to increase the height of shallot plants, and the application of *T. asperellum* promotes shallot growth. As a plant growth-promoting rhizobacteria (PGPR), *T. asperellum* could act as biocontrol agent against of pathogens, stimulate root growth, solubilize nutrients, induce systemic resistance (ISR), and enhanced stress tolerance. The application of *T. asperellum* as a PGPR involves introducing the fungus into the soil or rhizosphere of shallot plants. This can be achieved through various methods, such as seed treatment, root dipping, or soil application. The increase in the height of shallot plants by *T. asperellum* and its application as a Plant Growth-Promoting Rhizobacteria (PGPR) is primarily attributed to several mechanisms that this fungus employs to promote plant growth and health. *Trichoderma* can stimulate the vegetative growth of shallots (Sudantha & Suwardji, 2021).

Fresh Weight of Shallot Plants. The application of compost enriched with *T. asperellum* with a ratio (200:1) showed significantly different results in the fresh weight of shallot plants reaching 785.67 g compared to the control (Table 1). The treatment of compost enriched with *T. asperellum* with a ratio of 200:1, the treatment of compost enriched with a ratio of 500:1, and the treatment of chicken manure enriched with a ratio of 200:1 showed the results that were not significantly different. These three treatments differed significantly from the control (chicken manure and fungicide). Mixing organic materials with *Trichoderma* can have several beneficial effects on plants. Organic materials are rich in organic matter and nutrients. When combined with *Trichoderma*, they create an environment where nutrient release and uptake by plants are optimized. *Trichoderma* can improve the breakdown of organic materials, making

Table 1. Effect of application of organic material enriched with *T.asperellum* at 42 days after planting

Code	Treatment	Disease incidence (%)	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)	Bulb weight (g)
A	Control (Compost + Fungicide)	27.18 cd	29.41 a	689.00 bc	555.33 b	501.17 c
B	Compost + *T. asperellum* (200:1)	21.28 a	30.48 a	785.67 a	685.00 a	600.97 a
C	Compost + *T. asperellum* (500:1)	24.62 bc	29.22 a	728.33 ab	631.67 a	533.97 bc
D	Control (Chicken manure + Fungicide)	27.69 d	28.62 a	619.67 c	545.00 b	496.90 c
E	Chicken manure + *T. asperellum* (200:1)	23.58 ab	26.55 a	724.00 ab	635.33 a	574.00 ab
F	Chicken manure + *T. asperellum* (500:1)	24.87 cd	26.57 a	698.33 bc	632.33 a	527.63 bc

Numbers followed by the same letter in the column show no significant difference.

nutrients more accessible to plant roots. Known for its biocontrol properties, *Trichoderma*, when applied in combination with organic materials, can help protect plants from soilborne pathogens and root diseases. *Trichoderma* outcompetes pathogens for resources and produces antifungal compounds, reducing the risk of disease.

Based on plant incidence, the application of chicken manure and fungicides as a control treatment had the highest incidence of disease, which could affect the fresh weight of shallot plants. This result is in line with Hidayati et al. (2019), where the addition of *T. asperellum* suppresses twisted disease in shallot plants, allowing plants to grow optimally. This optimal growth can influence the fresh weight of the shallot plant. The yield of fresh weight of plants with the addition of *T. asperellum*-enriched organic material showed significantly different results compared to the control.

Dry Weight of Shallot Plants. The application of organic material enriched with *T. asperellum* showed significantly different results from the control treatment. The treatment, including compost + *T. asperellum* with a ratio of 200:1, compost + *T. asperellum* with a ratio of 500:1, chicken manure + *T. asperellum* with a ratio of 200:1, and chicken manure + *T. asperellum* with a ratio of 500:1, were able to increase the dry weight of shallot plants compared to the control. Besides increasing the fresh weight of shallot plants, adding *T. asperellum* can also increase the dry weight of shallot plants (Hidayati et al., 2019). The dry weight yield of plants with the addition of *T. asperellum*-enriched organic material showed significantly different results compared to the control.

Trichoderma is known to promote plant growth by enhancing root development, increasing the number of leaves, and improving plant health. This enhanced growth can lead to larger shallot bulbs and increased bulb weight. *Trichoderma* can improve nutrient uptake by plants, and this enhanced nutrient absorption likely played a role in the increased growth and yield of shallot bulbs.

Shallot Bulb Weight. The application of compost enriched with *T. asperellum* in a ratio of 200:1 and the treatment of chicken manure enriched with *T. asperellum* in a ratio of 200:1 were not significantly different, and both treatments showed different results when compared to the control. The treatment of compost enriched with *T. asperellum* in a ratio of 500:1 showed that there was no significantly different from the treatment of chicken manure enriched with *T. asperellum* in a ratio of 500:1, both treatments showed no significant differences compared to the control. Based on the results, the treatment of compost enriched with *T. asperellum* in a ratio of 200:1 and chicken manure enriched with *T. asperellum* in a ratio of 200:1 increased the production of shallots compared to the control. Organic material enriched with *T. asperellum* can increase the production of shallot by improving soil health with increase in the availability of nutrients and organic material. Organic material enriched with *T. asperellum* can also improve the nutrient uptake and yield of edible amaranth under the field condition (Lyu & Huang, 2022).

Shallot Health Test. Shallots are planted with seeds, and the initial step to control twisted disease is to utilize healthy seeds. Healthy seeds are essential for the production of robust and disease-resistant plants (Fadhilah et al., 2014). The health assessment of shallot bulbs is crucial to determine whether they are infected by the *Fusarium* spp. fungus. Bulbs that are free from infection serve as the foundation for healthy plant growth.

The health testing process involves isolating shallot bulbs planted in the field to evaluate potential infections caused by pathogenic agents present on the bulbs. This isolation procedure includes cutting the base of the bulbs, placing them on Komada media, and then allowing them to grow in an incubator for 5–7 days at room temperature.

Farmers commonly use shallot bulbs harvested in the previous season as seeds for the upcoming planting season. In the conducted health test, no growth of *Fusarium* spp. was observed in any of the treatments (Figure 1). The treatments involving compost and chicken manure enriched with *T. asperellum* exhibited no significant differences compared to the control.

The healthy bulbs obtained from the harvest, being free from infection in the previous planting, can be confidently used as seeds for the next growing season. These results indicate that the bulbs produced on the ground were not infected by *Fusarium* spp., the pathogen responsible for twisted disease, and that the fungus was not transmitted through the bulbs.

Population Density of *Fusarium* spp. in the Soil. The number of *Fusarium* spp. colonies at 28 days after planting and 42 days after planting did not exhibit significantly different results. Therefore, it can be concluded that the quantity of *Fusarium* spp. in the soil remained consistent, showing neither a decrease nor

an increase. These findings imply that the treatment involving compost enriched with *T. asperellum* at a ratio of (200:1) at both 28 and 42 days after planting significantly different in its ability to suppress the number of *Fusarium* spp. colonies in the soil when compared to the control (Table 2).

The number of *Fusarium* spp. colonies in the soil is closely linked to the observed disease incidence in each treatment. The highest incidence of mole disease was associated with the greatest number of *Fusarium* spp. colonies, particularly evident in the control treatment involving chicken manure application and fungicide treatment. Conversely, the lowest incidence of mole disease correlated with a lower number of *Fusarium* spp. colonies, notably observed in the compost treatment enriched with *Trichoderma* at a ratio of 200:1.

The twisted disease caused by *Fusarium* spp. is a pathogen capable of surviving in the soil through a complex biological process involving multiple factors. *Fusarium* spp. produces chlamydospores and microsclerotia, resilient structures enabling them to persist in the soil (Agrios, 2005). *Fusarium* spp. can be found in infected plant tissue, dead plant residues, or the soil. The population density of *Fusarium* spp. in the soil can be determined by isolating soil samples and cultivating them on specific media, such as Komada. According to the table, the *Fusarium* spp. decreased in the soil 28 days after planting in all treatments compared to the control. The addition of organic material enriched by *T. asperellum* suppressed *Fusarium* spp. in the soil. However, at 42 days after planting, the population of Fusarium spp. remained the same as the population 28 days after planting. These results align with research conducted by Bonanomi et al. (2010), which stated that plant growth media added with compost could suppress the population of *Fusarium* spp. in the soil.

While *Trichoderma* spp. is known for its potential to reduce *Fusarium* spp. populations and combat *Fusarium*-related plant diseases, there can be instances where *Trichoderma* may not effectively decrease *Fusarium*. The effectiveness of *Trichoderma* in controlling *Fusarium* can be influenced by various factors, including environmental conditions and the ability of *Trichoderma* to establish beneficial interactions with the host plant. Therefore, while *Trichoderma* has the potential to reduce *Fusarium* populations and diseases, its effectiveness may vary,

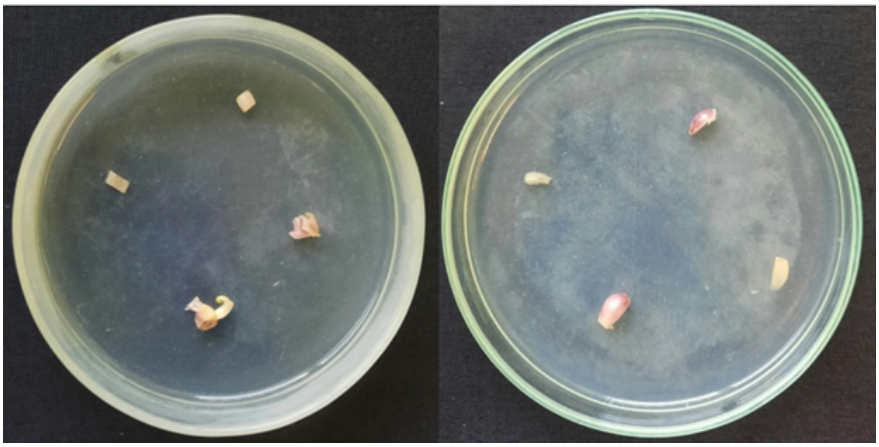

Figure 1. Testing bulb health on Komada media after 7 days of incubation.

Table 2. Observation of the population density of *Fusarium* spp. in the soil at before planting, 28 days after planting and 42 days after planting

Code	Treatment	Fusarium colony number (CFU/g)		
		Before planting	28 days after planting	42 days after planting
A	Control (Compost + Fungicide)	1.3×10^4	1.3×10^4 c	1.3×10^4 c
B	Compost + *T. asperellum* (200:1)		1.1×10^4 a	1.1×10^4 a
C	Compost + *T. asperellum* (500:1)		1.2×10^4 b	1.2×10^4 b
D	Control (Chicken manure + Fungicide)		1.3×10^4 c	1.3×10^4 c
E	Chicken manure + *T. asperellum* (200:1)		1.2×10^4 b	1.2×10^4 b
F	Chicken manure + *T. asperellum* (500:1)		1.2×10^4 b	1.2×10^4 b

Numbers followed by the same letter in the column show no significant difference.

and selecting the right *Trichoderma* strains and optimizing environmental conditions are critical factors in achieving successful biocontrol (Harman et al., 2004).

Analysis of Microbial Diversity in Soil with PCR-RISA. The rhizosphere is the part of the soil close to plant roots and serves as a site for microbial interactions with plants, influencing plant growth and crop yields. The microbial population in the soil, particularly in the rhizosphere, varies, with soils rich in organic material having higher microbial populations and diversity. Microbes in the soil play a significant role in soil health and productivity (Sudarma et al., 2012).

The diversity of soil microbes can be assessed based on DNA composition. Electrophoresis of each sample revealed distinct band patterns. All treatments exhibited different DNA bands, indicating variations in each treatment (Figure 2). The control treatment (chicken manure and fungicide) and chicken manure enriched with *T. asperellum* at a ratio of 500:1 showed the most diverse results, suggesting that this treatment is effective in enhancing rhizobacterial diversity. The compost enriched with *T. asperellum* at a ratio of 500:1 and chicken manure enriched with *T. asperellum* at a ratio of 200:1 exhibited comparatively lower diversity. These outcomes suggest that these treatments may not be conducive to the growth of rhizobacteria and may not enhance rhizobacterial diversity. There are two prominent groups of rhizobacterial communities in the soil.

The control treatment (compost and fungicide) and the compost treatment enriched with *T. asperellum* at a ratio of (200:1) exhibited 100% similarity (Figure 3). The control treatment (compost and fungicide) and the compost treatment enriched with *T. asperellum* at

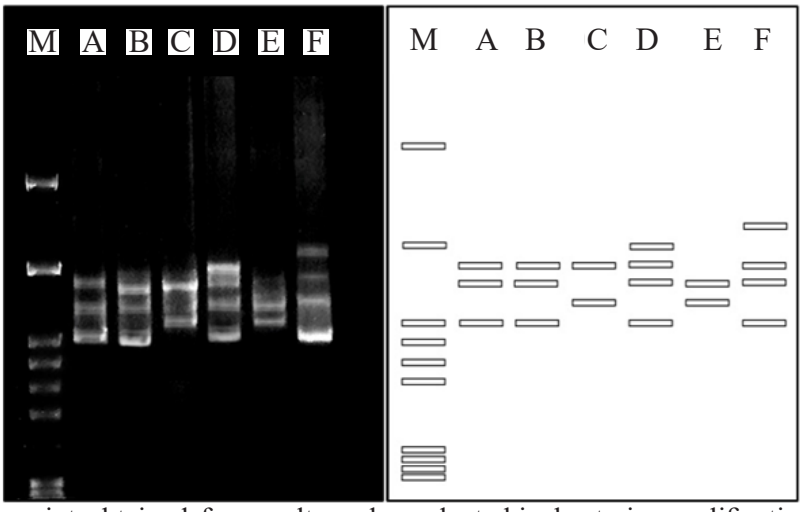

Figure 2. DNA fingerprint obtained from culture dependent rhizobacteria amplification. M=1 kb marker, A= indicates Control (Compost + Fungicide); B= indicates Compost + *T.asperellum* (200:1); C= indicates Compost + *T.asperellum* (500:1); D= indicates control (Chicken Manure + *T.asperellum*), E= indicates Manure Chicken + *T.asperellum* (200:1); F= indicates Chicken Manure + *T.asperellum* (500:1).

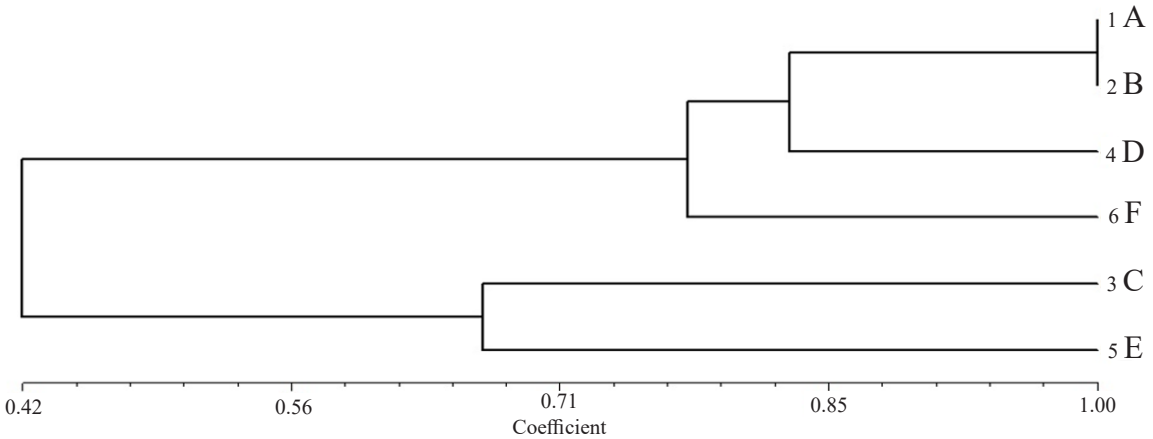

Figure 3. Species grouping dendogram based on kinship with the NTSyS-PC 2.IT program.

a ratio of 200:1 shared 80% similarity with the control treatment (chicken manure and fungicide). The control treatment (compost and fungicide), the compost treatment enriched with *T. asperellum* at a ratio of 200:1, and the control treatment (chicken manure and fungicide) had 78% similarity. The treatment of compost enriched with *T. asperellum* at a ratio of 500:1 and chicken manure enriched with *T. asperellum* at a ratio of 200:1 demonstrated 66.8% similarity. The treatment of compost + fungicide, the treatment of compost enriched with *T. asperellum* at a ratio of 200:1, the treatment of chicken manure + fungicide, and the treatment of chicken manure enriched with *T. asperellum* at a ratio of 500:1 showed the lowest similarity with the treatment of compost enriched with *T. asperellum* at a ratio of 500:1 and the treatment of chicken manure enriched with *T. asperellum* at a ratio of 200:1, which was 42%.

The microbial composition analyzed with the dependent method using PCR-RISA did not impact the suppression of twisted disease in shallot plants. This indicates that compost and chicken manure enriched with *T. asperellum* at a ratio of 200:1 showed no different results from the control treatment. The analysis of microbial diversity in the soil using the dependent method has drawbacks; only 1% of microbes can be cultured on artificial media, and 99% cannot be cultured. Observing the diversity of microbial communities in the soil, such as bacteria, cannot be carried out using conventional methods with cultivation (Valley et al., 2009).

CONCLUSION

The application of compost and chicken manure enriched with *T. asperellum* in a ratio of 200:1 can reduce the incidence of twisted disease, but it is unable to improve agronomic parameters and shallot production. It is effective in suppressing the population of Fusarium spp. in the soil but has no effect on microbial populations in the soil when analyzed using the dependent method with PCR-RISA.

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AUTHORS' CONTRIBUTIONS

HNAI is the first author for her contribution to this writing manuscript. As the corresponding author, AW contributed as a proofreader and revised this manuscript. TJ perform data collection and analysis. SS planned the design of the experiment. SH also planned the design of the experiment. The authors provided response and comments on the research flow, data analysis and interpretation as well as shape of the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that there is no potential conflict of interest.

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