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RESEARCH PAPER

Preliminary performance screening of microbial consortia on fusarium basal rot control and shallot growth

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ABSTRACT

Developing an effective biocontrol consortium requires comprehensive assessment to ensure that the selected microbial combinations can provide both strong disease suppression and plant growth-promoting effects. This study aimed to evaluate the performance of four biocontrol consortia composed of indigenous microbes from Bantul Regency, Indonesia, in suppressing Fusarium basal rot (FBR) and promoting the growth of shallot (*Allium cepa* var. *aggregatum*) cv. *Bauji*. Three indigenous isolates were used: *Trichoderma asperellum* strain PBt1, *Bacillus cereus* strain PBt2, and *B. cereus* strain PBt3. Four consortia were formulated by combining two or three of these isolates, designated as Consortia A, B, C, and D. The biocontrol activity against *Fusarium solani* DRB-1 was evaluated for both single isolates and consortia. A greenhouse experiment was conducted using a Completely Randomized Design with two inoculation timings (before planting and early vegetative stage) and five replicates. The performance of each consortium was assessed based on FBR severity and shallot growth parameters. Results showed that Consortia B (*T. asperellum* PBt1 + *B. cereus* PBt3) applied before planting achieved the highest FBR reduction (34.8%) at 42 days after planting (DAP). Moreover, this consortium significantly enhanced shallot yield, as reflected by increased bulb weight and number. These findings suggest that Consortia B has strong potential to improve both FBR management efficacy and shallot productivity.

Keywords: Allium, bacillus, biological control, sustainable, Trichoderma

INTRODUCTION

Fusarium basal rot (FBR) is one of the major diseases infecting many *Allium* species, including economically important crops such as shallot, onion, leek, and garlic. The economic losses caused by this disease vary among crop depending on the developmental stage at which infection occurs. FBR typically reduces plant vigor and induces rot symptoms on roots, basal stems, and bulbs (Degani et al., 2022). The disease can be caused by a single *Fusarium* species or a species complex (Tirado-Ramírez et al., 2019). The occurrence of FBR in *Allium* species is associated with different *Fusarium* species depending on the host species and geographical factors (Le et al., 2021; Sharma et al., 2024). Furthermore, the pathogenicity of these *Fusarium* species is strongly influenced by host susceptibility and geographic origin (Galván et al., 2008; Le et al., 2021; Taylor et al., 2016), resulting in diverse infection modes and making disease management particularly challenging.

In Indonesia, FBR has been reported to cause significant yield losses in shallot cultivation, ranging from 50% to 100% (Herlina et al., 2021). Several studies have shown variations in FBR incidence across regions in Java, including Brebes (32.1%), Probolinggo (80%), and Bantul (33.9–80%) (Aisyah et al., 2022; Nurcahyanti & Sholeh, 2023; Supyani et al., 2021; Wibowo et al., 2023). These differences may be related to variations in the *Fusarium* species infecting shallots in each area. To date, several *Fusarium* species have been identified as FBR causal agents in Indonesia, including *F. oxysporum* f.sp. *cepae, F. solani, F. acutatum, F. proliferatum, F. verticillioides,* and *F. pallidoroseum* (Cahyaningrum et al., 2020; Herlina et al., 2021; Lestiyani et al., 2014; Marianah et

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al., 2024). Management of FBR is particularly difficult because *Fusarium* species are soilborne pathogens. Their survival structures, chlamydospores, allow them to persist in the soil for long periods as saprophytes (Grünwald et al., 2000; Lucas, 2006).

Various management strategies have been proposed to mitigate FBR incidence, including the use of biological control agents (Sharma et al., 2024). Biological control (biocontrol) offers an environmentally friendly alternative to chemical pesticides, as it minimizes negative environmental impacts (Jamil et al., 2021; Kalman et al., 2020; Lecomte et al., 2016). Among the numerous biocontrol agents, *Trichoderma* and *Bacillus* species have been widely recognized for their effectiveness in suppressing *Fusarium*-induced diseases in *Allium* crops (Bunbury-Blanchette & Walker, 2019; Kara et al., 2023; Karim et al., 2022; Shin et al., 2023). However, Karim et al. (2022) noted that the antifungal activity of *Trichoderma* and *Bacillus* can be enhanced when used in consortium with other compatible microorganisms. The use of microbial consortia can improve disease suppression due to synergistic interactions and multiple modes of action (Wong et al., 2019).

The application of multi-species consortia has proven more effective in controlling *Fusarium* wilt in several crops, such as chickpea (Ankati et al., 2021) and banana (Kaleh et al., 2023; Prigigallo et al., 2022). Similarly, promising results have been reported for FBR management in *Allium* species, including garlic, onion, and shallot (Dewi et al., 2025; Diabankana et al., 2024; Krestini et al., 2020; Sari et al., 2023). A microbial consortium combining *Trichoderma*, *Bacillus*, and *Pseudomonas* was effective in suppressing FBR in shallot fields located in coastal areas of Bantul Regency, Yogyakarta, Indonesia (Sari et al., 2023). However, that consortium was developed using non-indigenous microorganisms. To enhance biocontrol efficacy, the source of microbial strains is critical, as indigenous microbes typically exhibit better adaptability and bioactivity under local conditions. Moreover, there are currently no registered microbial consortium products for FBR management in shallots in Indonesia, highlighting the need to develop effective indigenous-based biocontrol formulations. Therefore, this study aimed to evaluate the performance of several potential microbial consortia composed of indigenous isolates from Bantul, Yogyakarta, in suppressing FBR infection and promoting shallot growth.

MATERIAL AND METODS

Research Site. This study was conducted in the Laboratory of Agrobiotechnology and greenhouse facilities, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, Indonesia, from June 2021 to April 2022.

Preparation of Microbes Isolates. The fungal pathogen used in this study was *Fusarium solani* strain DRB-1, isolated from FBR-infected shallots in Bantul Regency (Aisyah et al., 2022). Molecular characterization using internal transcribed spacer (ITS1–4) primers confirmed its identity as *F. solani*. The isolate was cultured on Potato Dextrose Agar (PDA) and incubated at room temperature (29 ± 2 ºC) under dark conditions.

Three indigenous biocontrol isolates were obtained from the in-house collection of the Laboratory of Plant Pests and Diseases Observation, Pandak, Bantul, Indonesia. All isolates were molecularly characterized using 16S rRNA (for bacteria) and ITS (for fungi) to confirm their species identify (Table 1). The bacterial isolates were grown on Nutrient Agar (NA) for 48 h at room temperature (29 ± 2 ºC), while the fungal isolate was maintained using the same culture procedure as *F. solani* DRB-1.

In-vitro Antagonistic Assay of Single Biocontrol Isolates. The antagonistic activity of each indigenous isolates was evaluated in vitro to determine its ability to suppres *F. solani* DRB-1 growth. The assay followed a Completely Randomized Design (CRD) and was conducted using a dual culture technique as described by Aisyah et al. (2016). Each biocontrol isolate (Table 1) was co-cultured with *F. solani* DRB-1 on PDA and incubated for 7 days at room temperature (29 ± 2 ºC).

Fungal colony diameter was measured daily for 7 days, and the percentage of growth inhibition was calculated using the formula proposed by Islam et al. (2012):

Table 1. Details of biocontrol isolates used in this study

Isolate code	Source of collection (Location)	Identified species
PBt1	Shallot rhizosphere (Pandak, Bantul)	*Trichoderma asperellum*
PBt2	Shallot rhizosphere (Pandak, Bantul)	*Bacillus cereus*
PBt3	Shallot rhizosphere (Pandak, Bantul)	*Bacillus cereus*

$$I = \frac{dUf - dTf}{dUf} \times 100(\%)$$

I (%) = Inhibition;
dU_f = Diameter of *F. solani* DRB-1 in control (non-antagonistic) medium;
dTf = Diameter of *F. solani* DRB-1 in antagonistic medium.

Each treatment consisted of five replicates. The control consisted of *F. solani* DRB-1 cultured alone on PDA without biocontrol isolates.

In-vitro Compatibility Screening for Consortia Assembly. Compatibility among biocontrol isolates was tested to determine potential combinations for consortium formulation. The experiment was designed in completely randomized design (CRD) with four types of consortia as follows:
Consortium A: *T. asperellum* PBt1 + *B. cereus* PBt2;
Consortium B: *T. asperellum* PBt1 + *B. cereus* PBt3;
Consortium C: *B. cereus* PBt2 + *B. cereus* PBt3;
Consortium D: *T. asperellum* PBt1 + *B. cereus* PBt2 + *B. cereus* PBt3.

Compatibility was tested using a modified dual culture method (Aisyah et al., 2016). Isolates composing each consortium were positioned 3 cm apart from the fungal pathogen, as illustrated in Figure 1, and incubated for 7 days at 29 ± 2 ºC under dark conditions. *F. solani* DRB-1 cultured alone served as control. Fungal growth inhibition was recorded daily using the same procedure described above. A consortium was defined as compatible when its fungal suppression rate exceeded that of the individual isolates.

Preparation of Shallot Plants. Shallot (*Allium cepa* var. *aggregatum*) cv. *Bauji* bulbs were used as planting material. Regosol soil was fumigated with 5% formalin, sealed for 7 days, and then aerated for another 7 days. The treated soil was filled into polybags (35 × 35 cm) and mixed with manure (44.9 g/polybag) and SP-36 fertilizer (1.83 g/polybag). One bulb was planted per polybag and maintained for 60 days with routine watering. Urea (0.815 g/polybag) and KCl (0.625 g/polybag) fertilizers were applied at 15 and 30 days after planting. Observed growth parameters included plant height, leaf width, leaf number, plant weight, bulb weight, and bulb number.

Preparation of Microbial Inoculum Using Alternative Media. Before the in-planta assay, both pathogen and biocontrol isolates were propagated in alternative media. *T. asperellum* PBt1 was cultured on corn medium for 14 days until the conidial density reached 10^7 conidia/mL. *B. cereus* strains PBt2 and PBt3 were grown in sugar–potato broth for 18 h to obtain a concentration of 10^6 CFU/mL. *F. solani* DRB-1 inoculum was prepared in corn–meal sand medium (100 g sand and 5.6 g corn flour) and incubated for 3 weeks until the spore density reached 10^7 spores/mL.

In-planta Biocontrol Assay of Consortia. The in-planta biocontrol experiment was conducted using a CRD with two inoculation timings: 1. Before planting (7 days before planting); 2. Early vegetative (7 days after planting; DAP).

Each treatment was replicated three times, with each replicate consisting of ten plants. All isolates composing a consortium were applied simultaneously according to the designated inoculation time.

For the before planting treatment, 20 mL of *T. asperellum* PBt1 suspension (10^7 conidia/ml) and 10 mL of each *B. cereus* suspension (10^6 CFU/ml) were poured into each polybag 7 days prior to planting. For

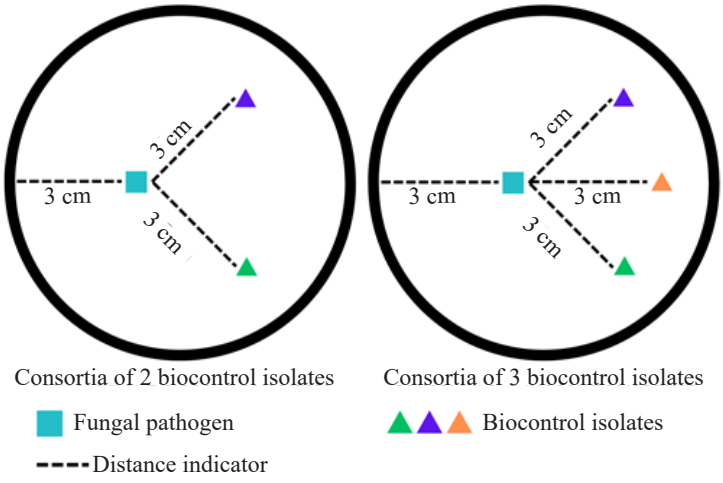

Figure 1. Schematic illustration of the dual culture assay procedure conducted in this study.

the early vegetative treatment (7 DAP), the suspensions were applied near the root zone and lightly covered with soil. Pathogen inoculation (20 mL of *F. solani* DRB-1 suspension, 10^7 spores/mL) was performed at 7 DAP via soil drenching near the root zone.

Control plants were inoculated with the pathogen only, without biocontrol application. Disease symptoms were monitored biweekly, based on the appearance of characteristic leaf twisting (Table 2). Disease severity was calculated according to the following formula:

$$DS\ (\%) = \frac{\sum(n_i \times v_i)}{N \times V} \times 100$$

DS (%) = Disease Saverity;
n_i = Number of plants with symptom score v_i;
N = Total number of plants observed;
V = The highest symptom score.

Statistical Analysis. All data were analyzed using analysis of variance (ANOVA) in SPSS® version 23.0 (IBM, USA). Mean comparisons were performed using Duncan's New Multiple Range Test (DNMRT) at $p < 0.05$. Results are presented as mean ± standard deviation, with statistical notations indicating significant differences among treatments.

RESULTS AND DISCUSSION

Confirmation on Strain Compatibility for Consortia Assembly. Microbial compatibility is one of the key factors determining the efficacy of microbial consortia in reducing the severity of plant diseases. Based on in vitro screening, the growth inhibition produced by *T. asperellum* PBt1 increased significantly when applied as part of a consortium (61.4 ± 6.07%) compared to its single application (55.6 ± 1.96%) (Figure 2). In terms of compatibility, the consortium composed of *T. asperellum* PBt1 and *B. cereus* PBt2 exhibited a higher synergistic interaction compared to its pairing with *B. cereus* PBt3. Interestingly, the multi-strain consortium consisting of both *B. cereus* isolates (PBt2 + PBt3) also enhanced antagonistic activity against *F. solani* DRB-1, reaching a suppression level comparable to that of *T. asperellum* PBt1 applied alone.

Under greenhouse conditions, each consortium exhibited distinct compatibility patterns. Among all treatments, Consortium B (*T. asperellum* PBt1 + *B. cereus* PBt3) showed the lowest FBR severity (34.8 ± 5.02%) (Figure 3), indicating high efficacy in disease suppression. In addition, the multi-species Consortium A also resulted in a significant reduction in FBR severity (43.6 ± 6.23%) compared to the control (56.8 ± 4.60%) (Figure 3). In contrast, the application of single biocontrol isolates was less effective in controlling FBR infection under in planta conditions.

In recent decades, the development of biocontrol consortia has increasingly focused on artificial or synthetic assembly approaches, in which consortium members are deliberately selected based on their desired functional traits. This strategy emphasizes both the biocontrol potential of each isolate and its level of compatibility with other microbial partners within the consortium. The combination of compatible microbes in a consortium often leads to greater success in managing plant diseases and enhancing plant defense mechanisms (Izquierdo-García et al., 2021; Kumar et al., 2021; Panchalingam et al., 2022; Wong et al., 2019).

The selection of *Trichoderma* and *Bacillus* genera in this study was based on their well-documented biocontrol potential, as reported in previous studies (Ganuza et al., 2018; Giordano et al., 2023; He et al., 2019; Karuppiah et al., 2022; Li et al., 2020). Specifically, both *T. asperellum* and *B. cereus* have been recognized as effective antagonists against *Fusarium*-related diseases (Báez-Astorga et al., 2022; He et al., 2019; Karuppiah et al., 2022; Madriz-Ordeñana et al., 2022; Pazarlar et al., 2022; Ramírez et al., 2022; Zhang et al., 2020). Moreover, previous studies have demonstrated that consortia composed of *Trichoderma* and *Bacillus* species exhibit strong compatibility, resulting in synergistic effects that enhance the management of *Fusarium* diseases (Izquierdo-García et al., 2021; Izquierdo-García et al., 2020; Jangir et al., 2019; Liu et al., 2022; Prigigallo et al., 2022).

Table 2. Scoring system used to assess FBR symptoms on shallot leaves

Score	Symptom description
0	No leaf twisting
1	≤ 10% leaf twisting
2	11–30% leaf twisting
3	31–75% leaf twisting
4	> 75% leaf twisting

Adjustment of Biocontrol Consortia Inoculation Time on FBR Disease Development. The efficacy of microbial consortia as biocontrol agents is influenced by various environmental factors, including the timing of inoculation and the growth stage of the plant at the time of application. The present study revealed that the degree of FBR suppression by the same consortium could vary depending on the inoculation time under greenhouse conditions. As shown in Figure 4, consortia applied before planting exhibited higher biocontrol efficacy than those applied at the early vegetative stage (7 DAP). The pre-planting inoculation likely facilitated better microbial adaptation to the rhizosphere environment, thereby enhancing disease suppression. However, as the plants matured, the performance of this inoculation strategy became less stable in some consortia (Consortia A and B), as indicated by increased FBR severity between 45 and 60 DAP (Figure 4).

Conversely, consortia inoculated at the early vegetative stage were generally less effective in suppressing FBR infection. Inoculation at this stage resulted in more severe infections in younger plants (15 and 30 DAP) (Figure 4). Because both the pathogen and the consortia were introduced simultaneously, direct competition at the infection site likely hindered proper establishment of the applied biocontrol agents, reducing their survival and colonization success. Nonetheless, FBR severity in older plants (60 DAP) remained lower for the vegetative-stage inoculation compared to the pre-planting treatment, except for Consortium A (Figure 4).

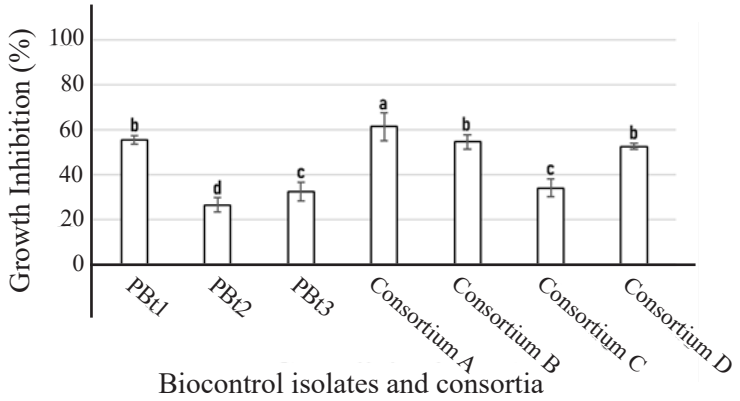

Figure 2. Comparison of *in vitro* suppression by single isolates and consortia against *Fusarium solani* DRB-1 after 7 days of incubation. Data represent mean values from five replicates, with standard deviation (SD) shown as error bars. Bars sharing the same lowercase letter are not significantly different according to DNMRT at $p < 0.05$. PBt1 = *Trichoderma asperellum* PBt1; PBt2 = *Bacillus cereus* PBt2; PBt3 = *Bacillus cereus* PBt3; Consortium A = PBt1 + PBt2; Consortium B = PBt1 + PBt3; Consortium C = PBt2 + PBt3; Consortium D = PBt1 + PBt2 + PBt3.

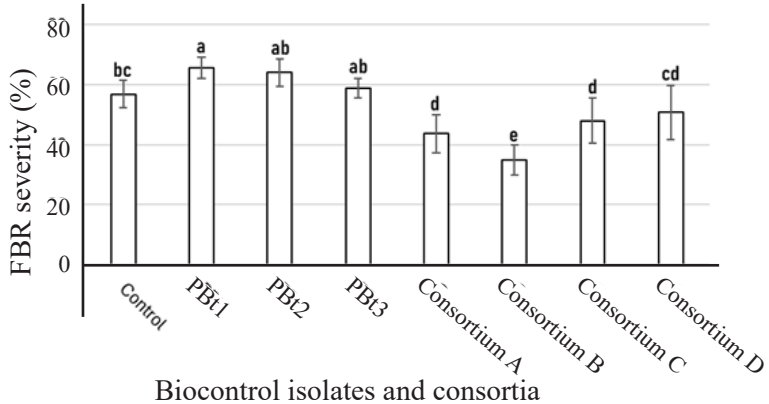

Figure 3. Comparison of Fusarium basal rot (FBR) severity resulting from the *in planta* application of single isolates and consortia. Data was collected at 42 days after planting. Values represent the mean of 50 representative plants ± SD. Bars sharing the same lowercase letter are not significantly different according to DNMRT at $p < 0.05$. PBt1 = *Trichoderma asperellum* PBt1; PBt2 = *Bacillus cereus* PBt2; PBt3 = *Bacillus cereus* PBt3; Consortium A = PBt1 + PBt2; Consortium B = PBt1 + PBt3; Consortium C = PBt2 + PBt3; Consortium D = PBt1 + PBt2 + PBt3.

The suppression efficacy of FBR disease among consortia applied before planting varied across different consortium types (Figure 5). Compared with the control, Consortium B (*T. asperellum* PBt1 + *B. cereus* PBt3) achieved the highest suppression, with the lowest FBR severity recorded at 45 DAP (34.8 ± 5.02%) and 60 DAP (51.2 ± 7.56%) (Figure 5). However, when the same consortium was applied at the early vegetative stage, a decline in performance was observed (Figure 5). Overall, these findings suggest that the inoculation time had no significant impact on the relative efficacy between the two consortium types tested.

The observed instability in biocontrol performance following pre-planting inoculation might be attributed to the use of fumigated soil as the growth medium during testing. Fumigated soil typically exhibits low microbial diversity, which may hinder the establishment and persistence of the introduced consortia, thereby reducing their periodic efficacy. This hypothesis aligns with previous reports emphasizing the crucial role of soil microbial diversity in maintaining soil health and quality (Fierer et al., 2021; Zhao et al., 2018). Furthermore, interactions between introduced consortia and native soil microbes are fundamental to plant growth-promoting effects (Bhatt et al., 2021). The application of exogenous microbial consortia can modify the soil microbiome, stimulating beneficial native microorganisms and reinforcing the positive effects of inoculated biocontrol agents (Jangir et al., 2019; Stummer et al., 2022).

Such community-based interactions form the underlying principle of microbial consortia technology—combining a diverse array of microbial species to produce holistic benefits for plant growth and disease suppression (Nunes et al., 2024; Palmieri et al., 2017; Ram et al., 2022). Considering the limitations of the present study, further investigation using non-fumigated (living) soil is needed to better evaluate consortium performance and its effects on the native soil microbiome.

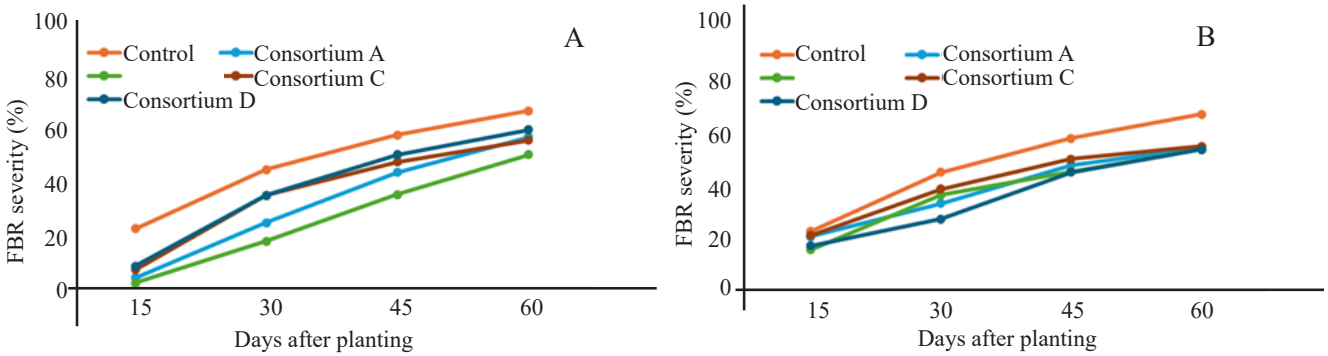

Figure 4. Comparison of the dynamics of Fusarium basal rot (FBR) development among various biocontrol consortia. A. Applied before planting; B. At the early vegetative stage (7 DAP). Data shown are mean values from 50 representative plants. Consortium A = PBt1 + PBt2; Consortium B = PBt1 + PBt3; Consortium C = PBt2 + PBt3; Consortium D = PBt1 + PBt2 + PBt3; Control = No biocontrol applied.

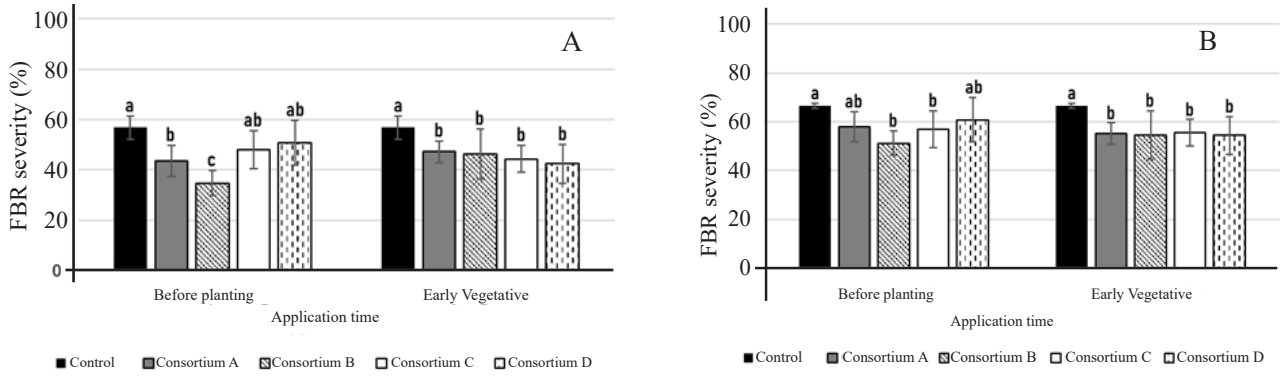

Figure 5. Comparison of Fusarium basal rot (FBR) severity resulting from the *in planta* application of various biocontrol consortia applied at two different periods (before planting and early vegetative). A. 45 DAP; B. 60 DAP. Data shown are mean values from 50 representative plants, followed by SD values as error bars. Bars with the same lowercase letter within the same application time are not significantly different based on DNMRT at $p < 0.05$. Consortium A = PBt1 + PBt2; Consortium B = PBt1 + PBt3; Consortiaum C = PBt2 + PBt3; Consortium D = PBt1 + PBt2 + PBt3; Control = No biocontrol applied.

Another aspect that warrants further exploration is the plant response to consortium inoculation, particularly regarding plant–microbe compatibility that may contribute to enhanced disease suppression. Because both the pathogen and the biocontrol microbes in this study are soil-borne, the rhizosphere serves as the primary site for plant–microbe interactions, which can exert both beneficial and detrimental effects on plant growth. Cai et al. (2021) reported that the plant rhizosphere harbors a multitude of symbiotic microorganisms with diverse ecological and physiological roles. The combined activity of these microbes can influence plant physiological and metabolic processes. Similarly, Zhalnina et al. (2018) highlighted that rhizosphere microbial communities are shaped by intricate feedback mechanisms among plants, microbes, and their environment. Plants, in turn, significantly affect the composition of the rhizosphere microbiota, with healthy plants often enriching populations of beneficial antagonists (Meng et al., 2019; Shen et al., 2014). Other studies have also demonstrated that the soil microbiome contributes to disease suppression through plant root exudates that selectively stimulate beneficial microbial populations (Li et al., 2014; Rosenzweig et al., 2012; Shen et al., 2015).

Impact of Biocontrol Consortia Inoculation on Shallot Growth. Inoculation of biocontrol consortia at different times resulted in varied effects on shallot growth parameters. Regarding plant height, no significant difference was observed between consortia applied before planting and those applied at the early vegetative stage (Figure 6), indicating that inoculation timing did not directly influence vertical growth. In contrast, a significant increase in leaf number was recorded at 21 DAP in plants treated with Consortium A (*T. asperellum* PBt1 + *B. cereus* PBt2) and Consortium C (*B. cereus* PBt2 + *B. cereus* PBt3) applied before planting (Figure 7). Compared with the control, these results suggest that pre-planting inoculation of certain consortia can stimulate early leaf development in shallots. However, this growth-promoting effect diminished in older plants, as evidenced by leaf loss at later stages. Unlike pre-planting inoculation, consortia applied at the early vegetative stage showed no significant differences in leaf number compared with the control, except for Consortium D (Figure 7). Consistent with these findings, Resti & Liswarni (2021) reported that bacteria consortia inoculation in shallots did not significantly increase leaf number. Conversely, Sutariati et al. (2021) found that consortia of indigenous rhizobacteria successfully enhanced leaf production in shallot var. *Wakatobi*.

Interestingly, an increase in leaf area was observed at 42 DAP in plants treated with three consortia containing *B. cereus* PBt3 applied before planting. Leaf area from Consortia B, C, and D exceeded that of the control and Consortium A (Figure 8). Conversely, when these three consortia were applied at the early vegetative stage, both fresh and dry weights of plants at 42 DAP increased significantly compared to the control and Consortium A treatments (Figure 9). These findings suggest that the synergistic interaction between *T. asperellum* PBt1 and *B. cereus* PBt3 influenced shallot growth and FBR suppression differently depending on the inoculation time. Pre-planting application effectively reduced disease incidence and promoted early vegetative growth, whereas application at the early vegetative stage appeared to help maintain plant quality under FBR infection. However, the specific mechanisms by which

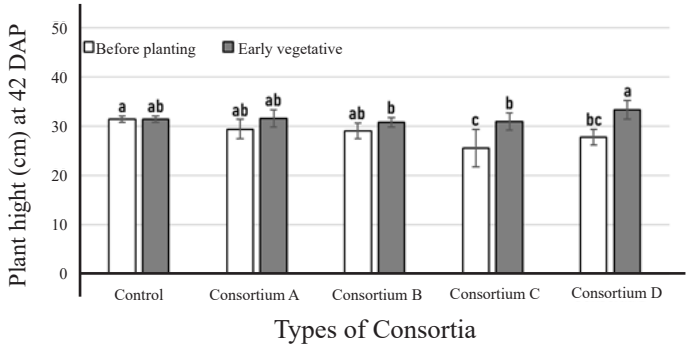

Figure 6. Comparison of shallot plant height at 42 DAP among various biocontrol consortia applied before planting and at early vegetative (7 DAP). Data shown are mean values from 30 representative plants, followed by SD values as error bar. Bars with the same color followed by the same lowercase letter are not significantly different based on DNMRT at $p < 0.05$. Consortium A = PBt1 + PBt2; Consortium B = PBt1 + PBt3; Consortiaum C = PBt2 + PBt3; Consortium D = PBt1 + PBt2 + PBt3; Control = No biocontrol applied.

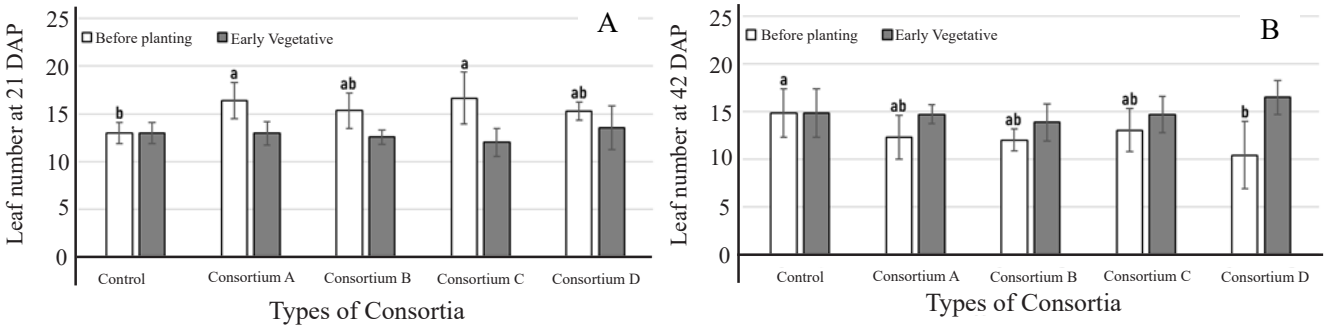

Figure 7. Comparison of the number of shallot leaves among various biocontrol consortia applied before planting and at early vegetative (7 DAP). A. 21 DAP; B. 42 DAP. Data shown are mean values from 30 representative plants followed by SD values as error bars. Bars with the same color followed by the same lowercase letter are not significantly different based on DNMRT at $p < 0.05$. Consortium A = PBt1 + PBt2; Consortium B = PBt1 + PBt3; Consortiaum C = PBt2 + PBt3; Consortium D = PBt1 + PBt2 + PBt3; Control = No biocontrol applied.

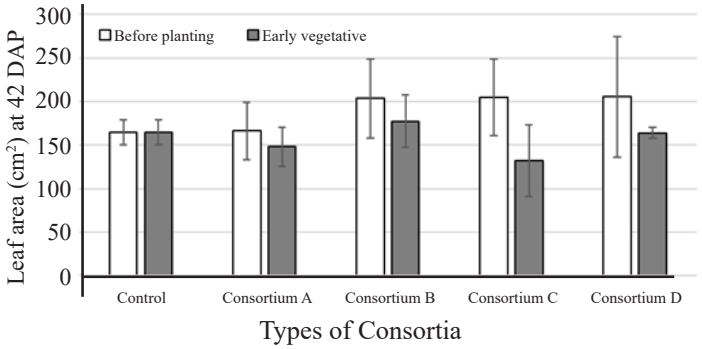

Figure 8. Comparison of shallot leaf area at 42 DAP among various biocontrol consortia applied before planting and at early vegetative (7 DAP). Data shown are mean values from 15 representative plants, followed by SD values as error bars. Consortium A = PBt1 + PBt2; Consortium B = PBt1 + PBt3; Consortiaum C = PBt2 + PBt3; Consortium D = PBt1 + PBt2 + PBt3; Control = No biocontrol applied.

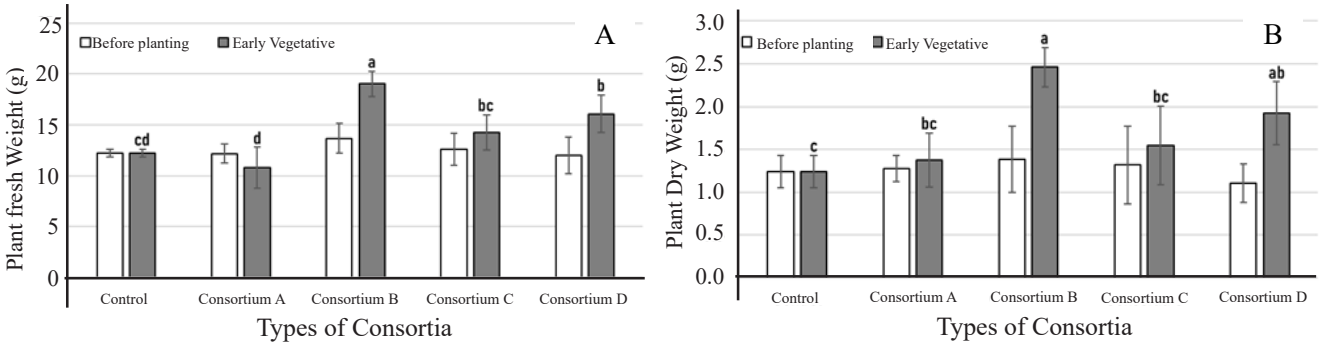

Figure 9. Comparison of plant fresh (A) and dry weight (B) at 42 DAP among various biocontrol consortia applied before planting and at early vegetative (7 DAP). Data shown are mean values from 15 representative plants followed by SD values as error bars. Bars with the same color followed by the same lowercase letters are not significantly different based on DNMRT at $p < 0.05$. Consortium A = PBt1 + PBt2; Consortium B = PBt1 + PBt3; Consortiaum C = PBt2 + PBt3; Consortium D = PBt1 + PBt2 + PBt3; Control = No biocontrol applied.

T. asperellum PBt1 and *B. cereus* PBt3 suppress FBR and promote growth remain to be elucidated.

Both *Bacillus* and *Trichoderma* species have been widely reported as effective biocontrol agents against various plant diseases, including *Fusarium* (Anbalagan et al., 2024; Ankati et al., 2021; Asrul, 2023; Bunbury-Blanchette & Walker, 2019; Dewi et al., 2025; Jamil et al., 2021; Kaleh et al., 2023; Modrzewska et al., 2022). According to Saxena et al. (2020), the antagonistic activity of *Bacillus* species

relies on multiple mechanisms, including antimicrobial compound production, induced systemic resistance, and biofilm formation. In contrast, *Trichoderma* species suppress pathogens through niche competition, mycoparasitism, antibiosis, and the production of plant growth–promoting metabolites (El-Komy et al., 2022; Guzmán-Guzmán et al., 2023; Kaur et al., 2022; Nofal et al., 2021; Rao et al., 2022; Romera et al., 2019). The FBR suppression achieved by the *T. asperellum* PBt1 and *B. cereus* PBt3 consortium (Figure 4) in this study is likely associated with one or more of these mechanisms, contributing to the improved growth performance observed in Figures 8 and 9.

The use of microbial consortia to manage Fusarium basal rot in *Allium* species has been well documented (Dewi et al., 2025; Diabankana et al., 2024; Krestini et al., 2020; Sari et al., 2023). This strategy is generally more effective than single-strain applications, as microbes naturally coexist and interact within complex communities (Bhatia et al., 2018; Vacheron et al., 2013). Multi-species consortia can offer diverse modes of action, resulting in stronger disease suppression and enhanced plant productivity (Sarma et al., 2015). For instance, Dewi et al. (2025) demonstrated that a consortium combining *B. amyloliquefaciens* B1 and arbuscular mycorrhizal fungi (AMF) effectively mitigated FBR infection in garlic through synergistic mechanisms of direct antagonism and root colonization, improving both disease resistance and plant growth. Similarly, the combination of *T. asperellum* PBt1 and *B. cereus* PBt3 (Consortium B) in the present study reduced FBR severity and improved shallot growth, raising important questions about the mutualistic interactions between these microbes and the host plant that underlie their biocontrol efficacy.

One key factor determining the success of microbial consortia is compatibility among isolates, which shapes the type of interaction within the consortium. High compatibility promotes synergistic effects, enhancing both disease suppression and growth promotion (Minchev et al., 2021). However, ensuring microbial compatibility under *in planta* conditions remains challenging, as efficacy often varies across growth parameters. Izquierdo-García et al. (2021) quantified synergistic interactions among 10 microbial consortia and found that only 17% exhibited measurable synergism under field conditions. Although multi-strain consortia generally show higher efficacy, this advantage occurs only when constituent microbes provide complementary modes of action (Sylla et al., 2013). In this study, consortium composition was based on individual biocontrol performance in single-isolate assays; some combinations did not enhance performance when combined, consistent with findings by Izquierdo-García et al. (2021). Hence, investigating inter-isolate interactions is essential, as negative interactions among members may compromise overall consortium efficacy (Sarma et al., 2015).

Consortia inoculated at different growth stages also had varied effects on shallot yield. When applied before planting, Consortium A produced the highest oven-dried (1.7 ± 0.25 g) and sun-dried bulb weights (8.6 ± 0.73 g) among all treatments (Table 3). However, its efficacy declined when applied at the early vegetative stage, where Consortium B–treated plants yielded the heaviest bulbs (Table 3). Unlike bulb weight, the multi-strain Consortium C applied before planting produced the highest bulb number (5.6 ± 0.55) (Table 4), while early vegetative inoculation had no significant effect on this parameter.

Overall, this study highlights that FBR suppression by biocontrol consortia also influenced shallot growth and yield in diverse ways. The superior FBR suppression by Consortium B (Figure 5) likely contributed to enhanced bulb quantity and quality (Tables 3 and 4). This result suggests that Consortium B created a synergistic interaction that effectively suppressed the disease while maintaining plant vigor, particularly in bulb development. These findings align with Resti & Liswarni (2021), who reported increased bulb dry weight following inoculation with bacterial endophyte consortia. The enhanced bulb growth in this study may also be related to the soil treatment method used, suggesting that the bulbs benefited more directly from microbial activity than aboveground tissues. Nevertheless, the level of FBR suppression achieved here was lower than that reported by Sutariati et al. (2021), who used a seed-soaking method for inoculation. Future studies should therefore compare different inoculation techniques to optimize consortium establishment and performance in controlling FBR infection.

CONCLUSION

This study successfully demonstrated the potential of a microbial consortium comprising *Trichoderma asperellum* PBt1 and *Bacillus cereus* PBt3 in suppressing Fusarium basal rot (FBR) disease in shallots under greenhouse conditions. The consortium not only reduced FBR severity but also positively influenced shallot growth and yield parameters. However, the specific mechanisms

underlying this biocontrol activity—particularly the individual contribution of each microbial species to FBR suppression and plant growth promotion—remain to be elucidated. Furthermore, the compatibility of this consortium with the native soil microbiome and the host plant requires further investigation. Future studies should focus on evaluating its performance in living soil systems to better understand microbial interactions and synergistic effects that enhance plant health and disease resistance. Such insights will be essential to validate and optimize the practical use of this consortium for improving shallot productivity in sustainable agricultural systems.

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AUTHORS' CONTRIBUTIONS

According to the CRediT contributorship taxonomy, SNA performed the roles of conceptualization, funding acquisition, data curation, formal analysis, methodology, validation, visualization, writing – original draft, and writing – review and editing. KKA, DRS, ABS, and SWP performed the experimental works, data collection, preparation of figures and tables, and project administration. AA performed the roles of methodology, funding acquisition, supervision. TH performed the roles of methodology, formal analysis, supervision, writing – review and editing. ES performed the roles of funding acquisition and methodology. JAH performed the roles of visualization, data analysis, writing – review and editing.

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