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Eco friendly management of onion (*Allium cepa* L. var. *aggregatum* Don.) purple blotch incited by *Alternaria porri*

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Abstract

The biocontrol agents *Bacillus subtilis* (Bbv 57) and *Trichoderma viride* (TNAU Tv 1) were tested in combination against onion purple blotch disease under both pot culture and field conditions. In the 2020-21 pot culture experiment, bulbs treated with *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (5 g each per kg) and plants sprayed with 0.2% *B. subtilis* (Bbv 57) at 30 and 50 DAS showed a significant reduction in purple blotch incidence, with a PDI of 15.55, second only to chemical treatments. In contrast, higher disease incidence was recorded in other treatments and the untreated control (PDI: 34.63). Yield data revealed an increased bulb yield of 16.8 g per plant in the same combination treatment, compared to 10.2 g per plant in the untreated control. Similar trends were observed in the 2021-22 experiment. Pooled data from three field trials confirmed that the combination treatment resulted in the lowest purple blotch incidence (PDI: 16.02), comparable to chemical treatments, while the control exhibited the highest incidence (PDI: 32.01). Additionally, this treatment led to a significantly higher yield (8.03 t ha^{-1}) compared to the control (3.25 t ha^{-1}). Hence, the combined application of *B. subtilis* (Bbv 57) and *T. viride* (TNAU Tv 1) at 5 g each per kg of onion bulbs, along with foliar sprays of 0.2% *B. subtilis* (Bbv 57) at 30 and 50 DAS, effectively reduces purple blotch incidence and enhances onion yield.

Keywords: *Alternaria porri, Bacillus subtilis,* biological control, onion, purple blotch, *Trichoderma viride*

Introduction

The small onion, also known as the common onion (*Allium cepa* var. aggregatum), is an important vegetable and spice widely cultivated around the world. It is the second most produced crop globally, following tomatoes (Khade *et al*. 2022). India is the second-largest onion-producing country, after China (FAOSTAT, 2021). Onion bulbs are rich in phytochemicals, especially flavonoids and sulfur compounds; hence, they possess medicinal properties that are used in some pharmaceutical preparations (Pareek *et al*. 2017). Often referred to as the 'Queen of the Kitchen,' it is an indispensable ingredient in daily food preparations. Onion production is affected by various biotic and

abiotic factors, with the foliar disease purple blotch, caused by *Alternaria* species, being one of the most destructive, leading to significant losses in bulb yield. This disease occurs widely on onions and other *Allium* species in various countries around the world (Kareem *et al*. 2012). The pathogen causes symptoms on all parts of onion crop including bulb (Mansha *et al*. 2019). Symptoms initiate as small, sunken whitish specks of 2 – 3 mm at leaf tips and progress towards the base. In severe infections, specks enlarge in size, turning into brown to purplish lesions covered with spores, causing the leaves to dry out (Agha 2022). The disease girdles the stalk, leading to failures in seed production, delays in bulb maturity, and making the plant vulnerable to other fungal infections (Mishra *et al*. 2014). In India, onions are cultivated throughout the year and are heavily infected with purple blotch, with infection rates reaching up to 85% (Tripathy *et al*. 2013, Veeraghanti *et al*. 2017). Several *Alternaria* species, such as *Alternaria allii, A. porri, A. tenuis, A. tenuissima, A. alternata* and *A. arborescens*, have been reported as the causes of purple blotch disease (Nolla 1927; Bock 1964; Simmons, 1999; Shahnaz *et al*. 2013). With regard to the management of purple blotch disease, chemical fungicides are widely used (Mandi *et al*. 2020). However, the excessive and continuous use of these chemicals has led to a resurgence in the pathogen population and the deterioration of the agroecosystem (Rial-Otero *et al*. 2005). Moreover, residual toxicity has become a significant emerging issue concerning the consumption of onion bulbs in regular food preparations (Kalsoom *et al*. 2019). Consequently, an alternative approach is needed to produce pesticide-free bulbs and create a pollution-free environment. In recent decades, biological control of plant diseases has gained importance (Thilagavathi *et al*. 2021; Itelima *et al*. 2018) and become popular among farmers as a powerful alternative to synthetic pesticides. It is a potential non-chemical and eco-friendly method of plant disease management that reduces the inoculum levels of pathogens. Biological control using beneficial bacteria and fungi continues to be a promising approach for managing crop diseases of economic importance. Plant beneficial bacteria suppress disease-causing pathogens both directly-through the production of antimicrobial compounds, including lipopeptides, and the secretion of hydrolytic enzymes and-indirectly, by conferring biotic stress tolerance to plants via induced systemic resistance (ISR) (Miljaković *et al*. 2020). With regard to beneficial fungi, Trichoderma sp. is capable of enhancing plant growth by producing growth hormones and making soil nutrients available to plants, while also suppressing disease-causing phytopathogens through various mechanisms such as mycoparasitism, antibiosis, competition for space and/or nutrients, and the production of lytic enzymes—or indirectly, through the induction of plant defences (Al-Ani 2018). However, the application of a single biological control agent may not be effective in all agroecosystems and may lead to inconsistent performance in disease control. To overcome this, an approach with combined application of biocontrol agents may demonstrates a more consistent expression of beneficial traits across a wider range of soil conditions and may be effective against a larger number of plant pathogens than biocontrol strains applied individually. According to Maketon *et al*. (2008), the combined use of *Trichoderma* and *Bacillus* significantly reduces the occurrence of plant diseases. Hence, the present study aimed to manage onion purple blotch disease using a combination of talc-based formulations of *B. subtilis* (Bbv 57) and *T. viride* (TNAU Tv 1) under pot culture and field conditions.

Material and methods

Isolation of pathogen

Purple blotch-infected onion leaves were collected from the field and transported to the laboratory for the isolation of the Alternaria species. The blotched areas of the leaves were cut into 0.5 cm pieces, surface sterilized with 5% (v/v) sodium hypochlorite for 3 minutes, and then washed three times with sterile distilled water before being blot-dried. The

pieces were subsequently transferred to Petri dishes containing potato dextrose agar (PDA) medium and incubated at 28 ± 2 °C with a 12-hour photoperiod to promote fungal growth (Muhae Ud Din *et al*. 2024). After seven days, individual hyphal tips from the fungal mycelium growing out of the leaf pieces were marked and transferred separately to new PDA plates (Rangaswami 1972). The pure cultures of the fungus were then examined under a microscope for their morphological characteristics.

Pathogenicity (Koch postulates)

The pure culture of the purple blotch pathogen, *Alternaria* was used to evaluate its pathogenicity on the susceptible onion cultivar CO (On) 5 which scored maximum disease rating scale of 5 under field conditions at Perambalur district. A total of 10 ml of sterile distilled water was added to a 14-day-old *Alternaria* culture grown on potato dextrose agar (PDA). The resulting spore suspension was adjusted to 10^8 cfu conidia per ml. The potted onion plants, 30 days after sowing (DAS), were sprayed with the conidial suspension and then immediately covered with a polythene bag for 12 hours to maintain a saturated atmosphere, prevent the drying of the inoculum droplets, and promote infection (Baltzakis *et al*. 2024). The plants were regularly observed for the development of symptoms. The pathogen was re-isolated on PDA and examined under a microscope.

Preparation of talc-based formulations of biocontrol agents

The mother cultures of the bacterial and fungal biocontrol agents, *Bacillus subtilis* (Bbv 57) and *Trichoderma viride* (TNAU Tv 1), respectively, were obtained from the Department of Plant Pathology at Tamil Nadu Agricultural University (TNAU), Coimbatore. These cultures are commercial strains that are mass-multiplied and supplied to farmers. The bacterial biocontrol agent *B. subtilis* (Bbv 57) was grown on a Petri plate containing nutrient agar medium for two days at room temperature (28 ± 2°C). A loopful of bacterial culture was inoculated into sterilized nutrient broth and incubated in a rotary shaker at 150 rpm for 72 hours at room temperature. The formulation was prepared by mixing 400ml of bacterial broth suspension containing 9 ± 10^8cfu ml^{-1}, one kg of carrier material (talc powder), 15 g of calcium carbonate (to adjust the pH to neutral) and 5 g of carboxy methyl cellulose (adhesive) under sterile conditions (Vidhyasekaran and Muthamilan 1995). The formulation for the fungal biocontrol agent, *T. viride* (TNAU Tv 1) was prepared by culturing it in molasses-yeast broth (30 ml molasses; 5 g yeast; and water to a total volume of 1000 ml). The sterile broth was inoculated with an actively growing mycelial disc (9 mm) and incubated for 15 days. The biomass (3 ± 10^8cfu ml^{-1}), along with the medium, was incorporated into the sterile talc powder carrier material at the rate of 50 ml of suspension per 100 g of talc powder and thoroughly mixed with 500 mg CMC as reported earlier by Ramakrishnan *et al*. (1994). The mixture was shade-dried for 12 h and stored in polythene bags.

Evaluating the effect of biocontrol agents on the incidence of onion purple blotch under pot culture conditions

The pot culture experiment was conducted at the Horticultural College and Research Institute for Women (HC & RI (W)), TNAU in Tiruchirappalli (10°75'N; 78°61'E) during December 2020 and 2021. There were seven treatments, each replicated three times in a completely randomized design (CRD). Onion bulbs of cultivar CO (On) 5 were treated with 5 grams each of *B. subtilis* (Bbv 57) and T. viride (TNAU Tv 1) per kilogram, along with untreated bulbs, which were sown in pots. Five plants per pot with 30 cm dia were maintained. Soil application of 1 gram each of *B. subtilis* and *T. viride* (TNAU Tv 1) per pot was performed at the time of sowing. Foliar sprays of *B. subtilis* (Bbv 57) (0.2%) were applied twice at 30 and 50 DAS. Chemical sprays were performed using Mancozeb (0.2%) and a mixture of Metiram + Pyraclostrobin (0.2%) on the same days. An untreated control was maintained without any treatments. A spore suspension of *Alternaria*

sp. (10^8 cfu conidia ml^{-1}) was sprayed on all treatments at 30 DAS. The treatments were observed regularly for disease development and disease ratings were done based on the 0 – 5 rating scale mentioned by Manu *et al*. (2014). Per cent disease index (PDI) was calculated using the following formula,

= (Sum of individual disease ratings / total number of leaves observed) x (100 / maximum score)

Bulb weight or yield of each treatment was recorded.

Evaluating the effect of biocontrol agents against onion purple blotch under field conditions

A field trial was conducted during 2020-21 rabi season at Padalur village, Perambalur district, Tamil Nadu (10o89'N; 78o65'E). Second and third trials were conducted at the HC & RI (W), TNAU, Tiruchirappalli during 2021-22 and 2022-23 rabi seasons. Locations were receiving same weather conditions. Talc based formulations of biocontrol agents were used. The experiments were designed with seven treatments, each replicated three times in a Randomized Block Design (RBD). Onion bulbs of cultivar CO (On) 5 were sown in plots measuring 4 x 3 meters, with a spacing of 20 x 12 cm. For treatments involving bulb treatment, 5 g each of *B. subtilis* (Bbv 57) and *T. viride* (TNAU Tv 1) were applied per kilogram of seed bulbs. Soil applications of 1.25 kg each of *B. subtilis* (Bbv 57) and *T. viride* (TNAU Tv 1) per hectare were done at the time of sowing. Foliar sprays of *B. subtilis* (Bbv 57) at a concentration of 0.2% were applied at 30 and 50 DAS. Fungicidal sprays were also administered on these dates. An untreated control was maintained without any treatments. The plots were regularly monitored from 15 days after planting for the occurrence of purple blotch disease. The PDI was calculated as described above, using the individual disease ratings recorded from twenty randomly selected leaves in each plot. Additionally, the bulb yield (fresh weight) was recorded for all treatments. Pooled analysis of data from all three trials was performed.

The impact of biocontrol agents on the shelf life of onion bulbs

The onion bulbs treated with talc-based bio formulations of *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) at each 5 g per kg and bulbs treated with talc alone (untreated control) were stored for 30 days. Observed for discoloration, spoilage and germination percentage after planting on soil.

Statistical analysis

The present experimental data were analysed using analysis of variance (ANOVA) by Agres Statistical Software Package Version 3.01 (Agres, 1994). The least significant difference (LSD) analysis was performed to separate the group mean when ANOVAs were significant at $p = 0.05$.

Results and discussion

Microscopic examination of the pathogen

The pure culture of the fungus isolated from onion purple blotch was examined under a light microscope (Figure 1a). The results revealed that the mycelia and conidia were septate and light brown in color. The conidia were typically muriform (brick-like) in shape and possessed a beak at one end. These observations were in accordance with the findings of Yar *et al*. (2024). Hence, the isolated fungus was confirmed as *Alternaria porri* (Figure 1b).

Establishing pathogenicity

Onion plants CO (On) 5 sprayed with a conidial suspension of *A. porri* were observed for typical purple blotch symptoms. The results indicated the presence of small whitish dots on the leaves, accompanied by irregular chlorotic areas, particularly at the leaf tips after 10 days of inoculation. These white dots rapidly expanded into elongated brown to purple blotches several centimeters in length. Concentric black velvety rings appeared in the purplish and chlorotic regions. As the disease progresses, the leaves die from the tip downward, breaking at the point of infection and hanging limply. The symptoms observed were consistent with

Figure 1a. Pure culture of *Alternaria porri* isolated from infected onion leaves in the Petri Plate
Figure 1b. Microscopic view (40 x) of conidia of *A. porri*

Fig. 1. Pure culture and conidia of *A. porri*

the original symptoms. The fungus isolated from the infected areas was examined under a microscope, and the conidial characteristics matched those of the original culture. These findings confirm the pathogenicity of the fungus and proved the Koch postulates for the fungus, *A. porri*. Similarly, Muhae-Ud-Din *et al*. (2024) proved the pathogenicity of the *Alternaria* species by spraying conidial suspension on the onion plants.

The effect of biocontrol agents on artificially developed onion purple blotch disease

In the present study, the results from the pot culture experiment conducted in December 2020 indicated that the chemical treatments Mancozeb at 0.2% and Metiram + Pyraclostrobin at 0.2%, applied as sprays at 30 and 50 days after sowing (DAS), significantly reduced the occurrences of purple blotch by 8.33% and 8.89%, respectively. Following this, the treatment involving bulb application of *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) at 5g each per kg along with foliar spray of 0.2 % B. subtilis (Bbv 57) applied at 30 and 50 DAS showed a substantial reduction in purple blotch incidence (PDI: 15.55) than other treatments and untreated control (PDI: 34.63). The same combination treatment resulted in an increased bulb yield of 16.8 g per plant compared to the untreated control (10.2 g plant^{-1}) under pot culture conditions. This reflects a 55.10% reduction in purple blotch and a 64.71% increase in bulb yield per plant with the combination treatment that included both bulb treatment and foliar spray compared to the untreated control (Table 1).

Same trends were observed in the experiment repeated in December 2021. The combination treatment resulted in a 57.4% reduction in the incidence of purple blotch, with a PDI of 15.37 compared to the control treatment, which had a PDI of 36.11. In terms of yield, the same treatment combination led to 117.21 % increase in bulb yield per plant (26.5 g plant^{-1}) as against untreated control (12.2 g plant^{-1}) under pot culture conditions (Table 2).

The findings of Izquierdo-García *et al*. (2020) revealed the potential biocontrol effect of a combination of *T. virens* and *B. velezensis* in controlling Fusarium wilt in cape gooseberry under greenhouse conditions. Similarly, the results of Chien *et al*. (2020) demonstrated that the application of *B. amyloliquefaciens* and *T. asperellum*-either through foliar spray, growth medium application, or both has the potential to control bacterial spot on tomatoes. According to Chethana *et al*. (2012) and Mishra and Gupta (2012), onion seeds treated with *T. harzianum* showed reduced purple blotch incidence and increased bulb yield.

Field evaluation of biocontrol agents for their effectiveness against onion purple blotch

The results from the field experiment conducted during 2020 - 21 in Padalur village, Perambalur district, indicated that, next to

Table 1. Efficacy of biocontrol agents on the incidence of onion purple blotch under pot culture conditions (2020-21)

Trt. No.	Treatment	Purple blotch (PDI)	Per cent decrease over control	Yield (g plant^{-1})	Per cent increase over control
T_1	Bulb treatment with *Bacillus subtilis* (Bbv 57) + *Trichoderma viride* (TNAU Tv 1) (5g each / kg)	21.8 (27.7)	37.05	13.5	32.35
T_2	Soil application of *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (1g each / pot)	28.33 (32.16)	18.19	11.3	10.78
T_3	Bulb treatment with *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (5g each / kg) + Foliar spray of 0.2% *B. subtilis* (Bbv 57) at 30 & 50 DAS	15.55 (23.33)	55.10	16.8	64.71
T_4	Soil application of *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (1g each / pot) + Foliar spray of 0.2% *B. subtilis* (Bbv 57) at 30 & 50 DAS	21.48 (27.61)	37.97	13.8	35.29
T_5	Spray of Mancozeb at 0.2% at 30 & 50 DAS	8.33 (16.78)	75.95	14.7	44.12
T_6	Spray of Metiram + Pyraclostrobin 0.2% at 30 & 50 DAS	8.89 (17.35)	74.33	14.9	46.08
T_7	Untreated control	34.63 (36.1)	-	10.2	-
	LSD (P<0.05)	5.62	-	1.52	-

All the values are mean of three replications and analyzed by one-way ANOVA using Agres Statistical Software Package Version 3.01 (Agres, 1994). Values in parenthesis were arcsine transformed. PDI: Per cent disease index. LSD: least significant difference.

Table 2. Efficacy of biocontrol agents on the incidence of onion purple blotch under pot culture conditions (2021-22)

Trt. No.	Treatment	Purple blotch (PDI)	Per cent decrease over control	Yield (g plant^{-1})	Per cent increase over control
T_1	Bulb treatment with *Bacillus subtilis* (Bbv 57) + *Trichoderma viride* (TNAU Tv 1) (5g each / kg)	22.78 (28.5)	36.9	17.7	45.08
T_2	Soil application of *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (1g each / pot)	28.52 (32.28)	21.0	13.2	8.20
T_3	Bulb treatment with *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (5g each / kg) + Foliar spray of 0.2% *B. subtilis* (Bbv 57) at 30 & 50 DAS	15.37 (23.08)	57.4	26.5	117.21
T_4	Soil application of *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (1g each / pot) + Foliar spray of 0.2% *B. subtilis* (Bbv 57) at 30 & 50 DAS	21.66 (27.74)	40.0	20.8	70.49
T_5	Spray of mancozeb @ 0.2% at 30 & 50 DAS	10.18 (18.61)	71.8	13.7	12.30
T_6	Spray of Metiram + Pyraclostrobin 0.2% at 30 & 50 DAS	9.81 (18.25)	72.8	14.2	16.39
T_7	Untreated control	36.11 (36.94)	-	12.2	-
	CD (P<0.05)	5.13	-	4.69	-

All the values are mean of three replications and analyzed by one-way ANOVA using Agres Statistical Software Package Version 3.01 (Agres, 1994). Values in parenthesis were arcsine transformed. PDI: Per cent disease index. LSD: least significant difference

chemical treatments, the minimum incidence of purple blotch (PDI: 11.67) was observed in onion plants treated with a bulb application of *B. subtilis* (Bbv 57) and *T. viride* (TNAU Tv 1) (5 g each per kg), along with a foliar spray of 0.2% B. subtilis (Bbv 57) at 30 and 50 DAS. This combination outperformed other treatments and the control, which showed a PDI of 19.44. In terms of yield, the same combination treatment resulted in an increase of 7.81 t ha^{-1}, while the control yielded significantly less at 2.48 t ha^{-1} (Figure 2).

Similar trends were observed in the field experiments conducted during the 2021-22 and 2022-23 periods (Tables 3). The results from the pooled data analysis of field trials demonstrated that 50.0 % reduction in purple blotch incidence (PDI: 16.02) was observed in the onion plants receiving bulb treatment with *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (5g each / kg) and foliar spray of 0.2% B. subtilis (Bbv 57) at 30 and 50 DAS over control. This combination treatment was on par with the chemical treatments. In contrast to this, the control treatment showed maximum purple blotch incidence (PDI: 32.01). There was 147% increased yield of 8. 03 t ha^{-1} was observed in the same combination treatment as against control which showed significantly reduced yield of 3.25 t ha^{-1}. The cost-benefit ratio of the combination treatment (1:5.98) was higher than that of the control (Table 3).

In this combination treatment, *B. subtilis* (Bbv 57) sprayed on the onion plants may directly

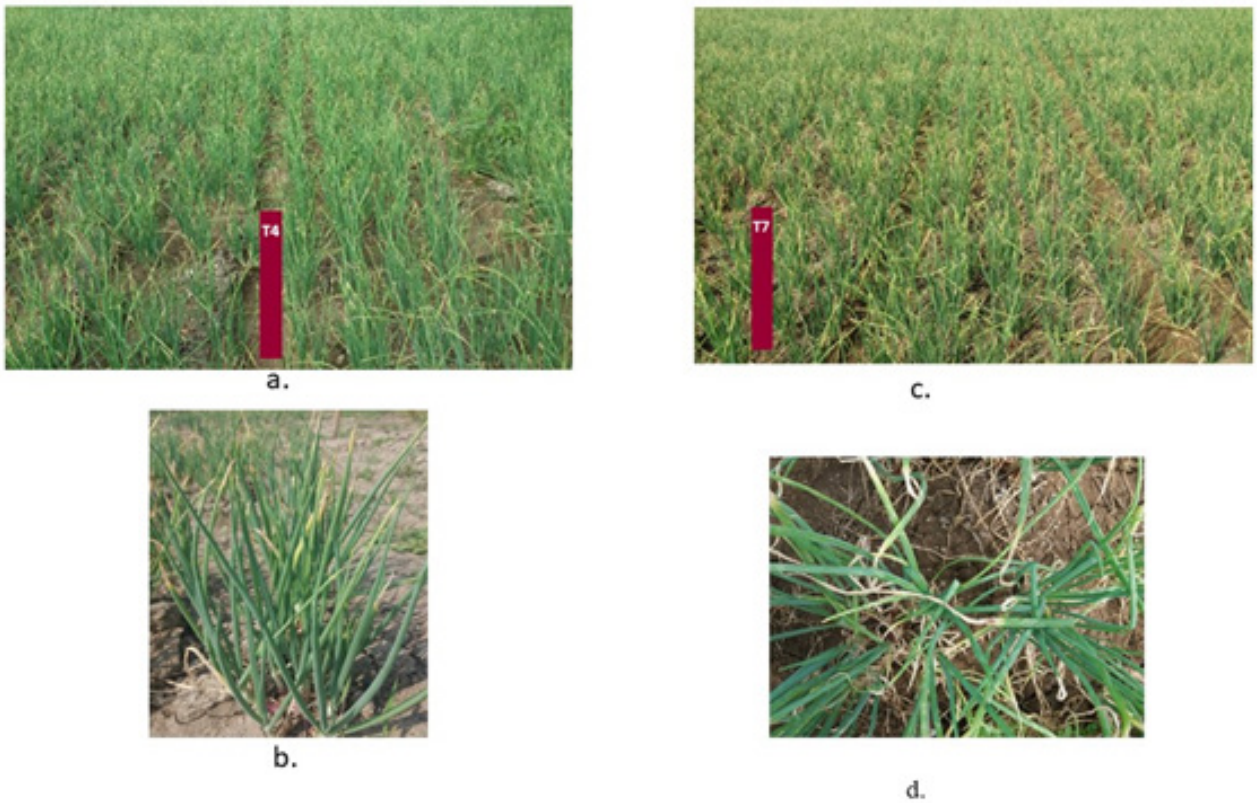

Fig. 2. Evaluating the effect of biocontrol agents on the incidence of onion purple blotch under field conditions.

a. Treatment 4. Bulb treatment with *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (5g each/kg) + Foliar spray of 0.2% *B. subtilis* (Bbv 57) at 30 & 50 DAS;

b. Onion plants of the treatment 3.;

c. Treatment 7. Untreated control.

d. Onion plants with purple blotch lesions showing darkness due to coverage of conidial mass in the treatment 7.

inhibit the growth of the fungal pathogen by producing antimicrobial compounds such as surfactins, fengycins, bacillomycin-D, bacillaene, macrolactin, difficidin, bacillibactin, and bacilysin (Santoso *et al*. 2020; Praveena *et al*. 2022; Kim *et al*. 2022), and by producing hydrolytic enzymes, such as chitinase, β-glucanase, protease, cellulase and lipase. These enzymes are able to hydrolyze specific cell wall components including chitin, glucan, proteins, cellulose, and lipids, leading to the disintegration of cell wall matrices and a loss of protective and functional properties (Moon *et al*. 2021; Won *et al*. 2021; Ajuna *et al*. 2023). Further, the reduction in purple blotch may be attributed to the complementary mechanisms of action that lower the inoculum level of *A. porri* in the pre-treated bulbs and in the soil after planting. Both *B. subtilis* (Bbv 57) and *T. viride* (TNAU Tv 1) might inhibit the growth of *A. porri* by rapidly multiplying to occupy space, thereby limiting available sites for pathogen colonization, and by utilizing nutrients to starve the pathogen population (Yao *et al*. 2023; Shafi *et al*. 2017). *B. subtilis* (Bbv 57) might inhibit fungal growth by producing antimicrobial compounds as mentioned earlier.

Additionally, the synergistic effect of both *Bacillus* (Bbv 57) and *Trichoderma* (TNAU Tv 1) may enhance induced systemic resistance (ISR) in onions against *A. porri*. According to the initial report by Bigirimana *et al*. (1997), root colonization by *Trichoderma* reduces the symptoms of foliar pathogens through ISR. Co-inoculation of *Bacillus* and *Trichoderma* leads to the formation of biofilms on the root surface and induces systemic resistance in crop plants synergistically (Zhou *et al*. 2021). In the present study, the combined mechanisms of action of both *B. subtilis* (Bbv 57) and *T. viride* (TNAU Tv 1) showed enhanced biocontrol efficiency against onion purple blotch disease under both pot culture and field conditions.

Regarding yield, increased bulb weight was observed in the treatment that combined bulb treatment of *B. subtilis* (Bbv 57) and *T. viride* (TNAU Tv 1) (5 g each per kg) and foliar spray with 0.2% *B. subtilis* (Bbv 57) at 30 and 50 DAS. *Trichoderma* spp. are described as growth-promoting fungi and thereby yield because they enhance the availability of nutrients and minerals (Fe, N, P) for plants and produce phytohormones (Martínez-Medina *et al*. 2011). Phytohormones produced by *Bacillus* spp. include indole acetic acid (IAA), cytokinins (CKs), gibberellins (GAs), and abscisic acid (ABA), which directly improve plant growth (Odoh 2017; Poveda *et al*. 2021) and thereby yield. Thus, in the present study, both *B. subtilis* and *T. viride* played significant roles in increasing onion bulb weight and yield under both pot culture and field conditions. According to Younes *et al*. (2023), the onion bulbs pretreated with the bioinoculants, *T. album* and *B. megaterium*, improves plant growth, nutritional qualities, antioxidant properties, and yield potentials under field conditions in comparison with control plants. Although chemical fungicides reduced disease occurrence, they had no influence on bulb yield, whereas biocontrol agents showed positive effects in enhancing the yield.

Impact of *B. subtilis* + *T. viride* treatment on onion bulb storage and germination: Onion bulbs treated with *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) exhibited no signs of spoilage by saprophytic microorganisms and maintained their health after storage. Notably, these bulbs demonstrated a high germination rate of greater than 90% when planted in soil. In contrast, untreated onion bulbs displayed discoloration, spoilage, and the emission of foul odors due to the growth of saprophytic microorganisms and insect damage. These bulbs exhibited a low germination rate of less than 10% when planted in soil (Figure 3).

In conclusion, the findings of this experiment revealed that the treatment that combined bulb treatment of *B. subtilis* (Bbv 57) and *T. viride* (TNAU Tv 1) (5 g each per kg) and foliar spray of 0.2% *B. subtilis* (Bbv 57) at 30 and 50 DAS, resulted in reduced purple blotch occurrence and increased onion bulb yield under both pot culture and field conditions

Table 3. Field evaluation of biocontrol agents on the incidence of onion purple blotch

Trt. No.	Treatment	Field trial 1 (2020-21)		Field trial 2 (2021-22)		Field trial 3 (2022-23)		Pooled data				
		Purple blotch (PDI)	Yield (t ha^{-1})	Purple blotch (PDI)	Yield (t ha^{-1})	Purple blotch (PDI)	Yield (t ha^{-1})	Purple blotch (PDI)	Per cent decrease over control	Yield (t ha^{-1})	Per cent increase over control	Cost benefit ratio
T1	Bulb treatment with *Bacillus subtilis* (Bbv 57) + *Trichoderma viride* (TNAU Tv 1) (5g each / kg)	14.63 (22.49)	7.08	22.96 (28.63)	5.73	25.18 (30.12)	5.03	20.92	34.7	5.95	83.08	1 : 4.4
T2	Soil application of *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (1g each / pot)	18.70 (25.62)	4.06	28.89 (32.51)	4.31	29.07 (32.63)	4.29	25.55	20.2	4.22	29.85	1 : 2.3
T3	Bulb treatment with *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (5g each / kg) + Foliar spray of 0.2% *B. subtilis* (Bbv 57) at 30 & 50 DAS	11.67 (19.98)	7.81	17.97 (25.08)	7.35	18.41 (25.66)	8.93	16.02	50.0	8.03	147.08	1 : 5.98
T4	Soil application of *B. subtilis* (Bbv 57) + *T. viride* (TNAU Tv 1) (1g each / pot) + Foliar spray of 0.2% *B. subtilis* (Bbv 57) at 30 & 50 DAS	13.89 (21.88)	6.88	22.59 (28.38)	5.65	25.00 (30.00)	4.69	20.49	36.0	5.74	76.62	1 : 3.6
T5	Spray of Mancozeb @ 0.2% at 30 & 50 DAS	9.07 (17.53)	7.07	14.26 (22.19)	4.57	14.63 (22.49)	4.88	12.65	60.5	5.51	69.54	1 : 4.56
T6	Spray of Metiram + Pyraclostrobin 0.2% at 30 & 50 DAS	9.44 (17.89)	7.07	14.81 (22.63)	4.68	14.81 (22.63)	4.64	13.02	59.3	5.46	68.00	1 : 4.68
T7	Untreated control	19.44 (26.16)	2.48	37.6 (37.82)	3.57	39.00 (38.65)	3.71	32.01	-	3.25	-	1 : 1.55
	LSD (P<0.05)	1.85	0.71	2.89	0.27	3.5	0.3	4.4	-	1.8	-	-

All the values are mean of three plots (replications) and analyzed by one-way ANOVA using Agres Statistical Software Package Version 3.01 (Agres, 1994). PDI: Per cent disease index. PDI is the mean of observations in twenty plants Values in parenthesis were arcsine transformed. LSD: least significant difference

References

Ajuna H B, Lim H I, Moon J H, Won S J, Choub V, Choi S I, Yun J Y & Ahn Y S 2023 The Prospect of hydrolytic enzymes from *Bacillus* species in the biological control of pests and diseases in forest and fruit tree production. Int. J. Mol. Sci. 24 (23): 16889.

Agha M 2022 Using some systemic resistance inducers to protect onion plants from purple blotch disease. Middle East J. Agric. Res. 11: 638–648.

Agres 1994 Statistical Software Version 3.01. Pascal International Software Solutions, USA.

Al-Ani L K T 2018 *Trichoderma*: Beneficial role in sustainable agriculture by plant disease management. In: Egamberdieva, D., Ahmad, P. (eds) Plant microbiome: Stress response. Microorganisms for sustainability. Springer, Singapore. 5: 105-126.

Baltzakis I, Nindya S, Rahmayani R, Thomas J E, van der Werf W & Struik P C 2024 Pathogenicity of Single and Combined Inoculations of *Alternaria* spp. on Potato. Potato Res. https://doi.org/10.1007/s11540-024-09739-8.

Bock KR. 1964. Purple blotch (*Alternaria porri*) of onion in Kenya. Ann. Appl. Biol. 54: 303–311.

Bigirimana J, De Mejer G, Poppe J, Elad Y & Hofte M 1997 Induction of systemic resistance on bean (*Phaseolus vulgaris*) by *Trichoderma harzianum*. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent. 62: 1001–1007.

Chethana B S, Ganeshan G, Rao A S & Bellishree K 2012 *In vitro* evaluation of plant extracts, bioagents and fungicides against *Alternaria porri* (Ellis) Cif., causing purple blotch disease of onion. Pest Manag. Hort. Ecosyst. 18: 194–19.

Chien Y C & Huang C H 2020 Biocontrol of bacterial spot-on tomato by foliar spray and growth medium application of *Bacillus amyloliquefaciens* and *Trichoderma asperellum*. Eur. J. Plant Pathol. 156: 995–1003.

FAOSTAT Onion Production, Area and Productivity 2021 Available online: http://www.fao.org/faostat/en/#data/QC

Fayzalla S, Metwally A & Sadat M 2011 Effect of some fungicides, bioagents and essential oils for controlling purple blotch disease of onion. J. Plant Prot. Pathol. 2 : 663-676.

Harman G, Herrera-Estrella A, Horwitz B & Lorito M 2012 Special issue: *Trichoderma* - from Basic Biology to Biotechnology. Microbiology (Reading, England). 158: 1 - 2.

Itelima J U, Bang W J, Onyimba I A & Oj E 2018 A review: biofertilizer; a key player in enhancing soil fertility and crop productivity. J. Microbiol Biotechnol Rep. 2 (1): 22–28.

Izquierdo García L, Cotes A & Moreno C 2021 Screening for effective microbial consortia against Fusarium wilt of cape gooseberry (*Physalis peruviana*). Bio Control. 66 10.1007.

Izquierdo-García L F, González-Almario A, Cotes A M & Moreno-Velandia C A 2020 *Trichoderma virens* Gl006 and *Bacillus velezensis* Bs006: A compatible interaction controlling *Fusarium* wilt of cape gooseberry. Sci. Rep. 10 (1): 1–13.

Kalsoom R, Haider M S & Chohan S 2020 Phytochemical analysis and antifungal activity of some medicinal plants against *Alternaria* species isolated from onion. J. Anim. Plant Sci. 30: 454–460.

Khade Y P, Salunkhe S R, Manjunathagowda D C, Sinhasane S R, MahidarGowd T Y, Mahajan V & Singh M 2022 Molecular characterization of short-day onion genotypes by intron length polymorphic (ILP) markers. Genet. Resour. Crop Evol. 69: 2077–2086.

Kareem M A, Murthy K V M K, Nadaf H A & Waseem M A 2012 Effect of temperature, relative humidity and light on lesion length due to *Alternaria porri* in onion. Asian J. Environ. Sci. 7: 47–49.

Kim Y S, Lee Y, Cheon W, Park J, Kwon H T, Balaraju K, Kim J, Yoon Y J & Jeon Y 2021 Characterization of *Bacillus velezensis* AK-0 as a biocontrol agent against apple bitter rot caused by Colletotrichum gloeosporioides. Sci. Rep. 11(1): 626.

Leta A & Selvaraj T 2013 Evaluation of Arbuscular Mycorrhizal Fungi and *Trichoderma* Species for the Control of Onion White Rot (*Sclerotium cepivorum* Berk). J. Plant Pathol Microbiol. 4: 159.

Maketon M, Apisitsantikul J & Siriraweekul C 2008 Greenhouse evaluation of *Bacillus subtilis* AP-01 and *Trichoderma harzianum* AP-001 in controlling tobacco diseases. Braz. J. Microbiol. 39: 296-300.

Mandi N, Nayak B S, Sahoo B B, Prasad G & Khanda C 2020 Efficacy of novel fungicides against purple blotch in onion (*Allium cepa* L.) in the western undulating zone of Odisha, India.

Int. J. Curr. Microbiol. Appl. Sci. 9: 1970–1976.

Mansha M Z, Sahi S T, Habib A & Ahmed S 2019 Variations in yield and nutritional profile of onion germplasm under the influence of purple blotch disease. Int. J. Agric. Biol. 21: 120–124.

Manu D, Chandrakar A K, Chandrakar C K, Dewangan C K & Talukdar D. 2014. Evaluation of fungicides for control of *Alternaria* blight (*Alternaria porri*) of garlic. J. Plant Dis. Sci. 9: 108–111.

Martínez-Medina A, Roldán A & Pascual J A 2011 Interaction between arbuscular mycorrhizal fungi and *Trichoderma harzianum* under conventional and low input fertilization field condition in melon crops: growth response and Fusarium wilt biocontrol. Appl. Soil Ecol. 47: 98–105.

Miljaković D, Marinković J & Balešević-Tubić S 2020 The Significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms. 7: 1037.

Mishra R K & Gupta RP 2012 *In-vitro* evaluation of plant extracts, bio-agents and fungicides against Purple Blotch and Stemphylium blight of onion. J. Med. Plant Res. 6: 5840–5843.

Moon J H, Won S J, Maung C E H, Choi J H, Choi S I, Ajuna H B & Ahn Y S 2021 *Bacillus velezensis* CE 100 inhibits root rot diseases (*Phytophthora* spp.) and promotes growth of Japanese cypress (*Chamaecyparis obtusa* Endlicher) seedlings. Microorganisms. 9: 821.

Muhae-Ud-Din G, Kalsoom R, Yang Z, Chohan S, Haider M S, Ahmed D & Abid M 2024 Identification of *Alternaria* species associated with purple blotch disease of onions in Southern Punjab of Pakistan and evaluation of plant-based fungicides for disease control. Trop. Plant Pathol. 49: 601–611.

Nolla JAB 1927 A new *Alternaria* disease of onions (*Allium cepa* L.). Phytopathology. 17: 115–132.

Odoh C K 2017 Plant growth promoting rhizobacteria (PGPR): A bioprotectant bioinoculant for sustainable agrobiology. A review. Int. J. adv. res. biol. sci. 4: 123-142.

Praveena R, Srekha K, Revathy R, Srinivasan V, Sarathambal C, Priya George, Subila K P & Dinesh R 2022 New rhizobacteria strains with effective antimycotic compounds against rhizome rot pathogens and identification of genes encoding antimicrobial peptides. Rhizosphere. 22: 100515.

Pareek S, Sagar NA, Sharma S & Kumar V 2017 Onion (*Allium cepa* L.). Fruit and vegetable phytochemicals: chemistry and human health. – 2nd ed. Hoboken, NJ: Wiley Blackwell, 1145-62.

Poveda J 2021 Trichoderma as biocontrol agent against pests: New uses for a mycoparasite. Biol. Control. 159, Article 104634.

Ramakrishnan G, Jeyarajan R & Dinakaran D 1994 Talc based formulation of *Trichoderma viride* for biocontrol of *Macrophomina phaseolina*. J. Biol. Control. 8: 41-44.

Rangaswami G 1972 Diseases of crop plants in India. Prentice Hall of India Pvt. Ltd., New Delhi, 520.

Rial-Otero R, Arias-Estévez M, López-Periago J, Cancho-Grande B & Simal-Gandara J 2005 Variation in concentrations of the fungicides tebuconazole and dichlofluanid following successive applications to greenhouse-grown lettuces. J. Agric. Food Chem. 53: 4471-5.

Santoso I, Fadhilah Q G, Maryanto A E & Yasman 2020 The Potency of *Bacillus siamensis* LDR as biocontrol agent against fungal phytopathogen. Adv. Biol. Sci. Res. 14: 463 - 468.

Shafi J, Tian H & Ji M 2017 *Bacillus* species as versatile weapons for plant pathogens: A review. Biotechnol. Biotechnol. Equip. 31: 446 - 459.

Shahnaz E, Razdan V K E H, Rezwi S, Rather T R, Gupta S, & Andrabi M. 2013. Integrated disease management of foliar blight disease of onion: A case study of application of confounded factorials. J. Agric. Sci. 5: 17-22.

Simmons E G 1999 *Alternaria* themes and variations (236–243): host-specific toxin producers. Mycotaxon. 70: 325–369.

Thilagavathi R, Nakkeeran S, Balachandar D, Raguchander T & Samiyappan R. 2021. Bacterial community analysis on *Sclerotium*-suppressive soil. Arch. Microbiol. 203: 4539–4548. https://doi.org/10.1007/s00203-021-02426-z

Tripathy P, Priyadarshini A, Das S K, Sahoo B B & Dash D K 2013 Evaluation of onion (*Allium cepa L.*) genotypes for tolerance to thrips (*Thrips tabaci* L.) and purple blotch [*Alternaria*

porri (Ellis) Cif]. Int. J. Bio-Resource Stress Manag. 4 (4): 561.

Vahidinasab M, Lilge L, Reinfurt A, Pfannstiel J, Henkel M, Morabbi Heravi K & Hausmann R 2020 Construction and description of a constitutive plipastatin mono-producing *Bacillus subtilis*. Microb. cell fact. 19: 205.

Veeraghanti K S, Naik B G & Hegde K T 2017 Management of Purple blotch disease of onion under field condition. J. Pharmacogn. Phytochem. 6 (6): 1768–1769.

Vidhyasekaran P & Muthamilan M 1995 Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Dis. 79: 782-786.

Won S J, Moon J H, Ajuna H B, Choi S I, Maung C E H, Lee S & Ahn Y S 2021 Biological control of leaf blight disease caused by *Pestalotiopsis maculans* and growth promotion of *Quercus acutissima* Carruth container seedlings using *Bacillus velezensis* CE 100. Int. J. Mol. Sci. 22: 11296.

Yao X, Guo H, Zhang K, Zhao M, Ruan J & Chen J. 2023. *Trichoderma* and its role in biological control of plant fungal and nematode disease. Review article. Sec. Microbe and Virus Interactions with Plants. Front. microbiol. 14.

Yar A, Ullah R, Khan MN, Iqbal M, Ercisli S, Kaplan A, Olana DD, Javed MA, Mohamed EI, Baowidan S, Harakeh S & Moulay M. 2024. Unveiling the germination patterns of *Alternaria porri* (Ellis) by using regression analysis and hydrothermal time modeling. Sci Rep. 14 (1): 25694. doi: 10.1038/s41598-024-76050-4. PMID: 39465273; PMCID: PMC11514278.

Younes N A, Anik T R, Rahman M M, Wardany A A & Dawood M F A 2023 Tran LP, Abdel Latef AAH, Mostofa MG. Effects of microbial biostimulants (T*richoderma album* and *Bacillus megaterium*) on growth, quality attributes, and yield of onion under field conditions. Heliyon 2, 9(3): e14203.

Zhou Y, Yang L, Wang J, Guo L & Huang J 2021 Synergistic effect between *Trichoderma virens* and *Bacillus velezensis* on the control of tomato bacterial wilt disease. Horticulture 7: 439.