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PLANT PATHOLOGY | ORIGINAL ARTICLE

Field Management of Stemphylium Blight of Garlic by Combined Application of Bio-Agents, Inducers and Fungicide

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ABSTRACT

Garlic (*Allium sativum)*, one of the most important spice crops in Bangladesh, is affected by a variety of fungal diseases. Among them, Stemphylium blight is the topmost disease which limits the quality and quantity of both vegetative growth as well as yield of garlic. The study was carried out under both *in vitro* and *in vivo* conditions to evaluate the efficacy of both individual and integrated application of bioagents, inducers like salicylic acid and chitosan with chemical fungicides, Iprodione (Rovral 50 WP) against Stemphylium blight of garlic. The *in-vitro* antagonism test was conducted using five strains of each of the *Bacillus subtilis* and *Pseudomonas fluorescens*, where the highest percent inhibition of mycelium growth was found in *Pseudomonas fluorescens* strain Psf3 (57.04 %) and *Bacillus subtilis* strain Bacs4 (54.44 %). A total of 10 treatments were applied in the field. Treatment T_6 (Rovral @ 0.2 % + *Pseudomonas fluorescens* strain Psf3 @ 10 mL (10^6 CFU/ml) / 5 plant) showed the lowest disease severity (38.4 %). This treatment also exhibited promising result in both the vegetative and yield parameters of garlic under field conditions. The highest bulb yield (6.23 ton/ ha) was found in T_6, surpassing all individual treatments. This finding denoted the positive synergistic effect of combined application of Rovral 0.2 % and *Pseudomonas fluorescens* strain Psf3 for sustainable management of Stemphylium blight. The combined application of chemical and bioagents is more feasible and practical for managing Stemphylium blight disease of garlic as well as for getting better yield in field condition.

1. Introduction

Garlic (*Allium sativum* L.) is a perennial bulbous crop, having significant importance for its use as spice and condiment (Singh et al. 2023). Though it is primarily used for its culinary purpose, garlic holds potential medicinal values. It possesses antibiotic and antiseptic action, thanks to the presence of volatile oil and Sulphur compound (Bhatwalkar et al. 2021). Furthermore, recommended amount of garlic also helps to control blood pressure, serum lipid levels, prevent cancer and cardiovascular disease, and has antioxidant properties (Hossain et al. 2023). In Bangladesh garlic is considered as the third most important spice crop after chilli and onion considering both acreage and production (BBS 2023). According to the report of BBS (2023) annual production of garlic is 7.65 metric tons/ha, however it is still severely low compared to some other major garlic producing countries like China, Egypt, Jordan, India, Tajikistan where annual production is 2-3 times higher than Bangladesh (Hasan and Khalequzzaman 2015). As a result, consumer demand of garlic far surpasses the production capacity of Bangladesh and we need to import from neighboring country (Kaysar et al. 2023). A number of factors is responsible for this low production but pathogenic, especially fungal diseases are considered as the major cause (Gálvez et al. 2016; Akter et al. 2021). Although a number of pathogens from the genus *Aspergillus, Penicillium, Sclerotium, Fusarium* are associated with garlic. Leaf blight by *Stemphylium vesicarium* is considered as the most serious foliar disease which can cause upto 70% yield loss (Gálvez et al. 2016). Most common traditional practice for controlling of Stemphylium blight is the use of fungicide especially of tebuconazole and procymidone group (Pandey and Singh 2024). It is also reported that development of resistance of *Stemphylium* spp popularly used procymidone group has already occurred which makes it no longer effective as earlier cases (Chen et al. 2021). Although some new fungicide group like iprodione, prochloraz, pyraclorstrobin

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are reported to successfully control this disease (Gálvez et al. 2016). This is not a long-term solution, as new fungicides still present several limitations, including environmental pollution, the development of highly resistant pathogenic strains, and toxic effects on human health, food, and non-target organisms. A suitable alternative, which is the use of beneficial biological control agents (BCA) is now a days catches the attention of many researchers (Elsharkawy et al. 2022). Various beneficial bacteria like *Pseudomonas* sp. and *Bacillus* sp are reported to possess multiple mechanism like parasitism, secretion of lytic enzyme, secondary metabolites, competition for space and nutrients for suppressing opponent pathogen growth along with provide protective biofilm for plant protection (Azeem et al. 2020).

Use of bioagent although solves the issue regarding damage to environment degradation and health concerns, but they are not as effective as fungicides, possess lower shelf life and their efficacy is highly dependent on environment (Ji et al. 2019). Another type of chemicals known as plant resistance inducers like chitosan and salicylic acid are now being studied by many scientists to alleviate pathogenic damage and induce resistance to host crop (Urban et al. 2022). These inducers are also now considered as part of biocontrol mechanism in a broader sense as they upregulate defense response without hampering surroundings and some are derivatives of living organisms (Raymaekers et al. 2020). Chitosan treatment affects defense related genes and activates defensive proteins, and salicylic acid participates is signal transduction and activates defense reactions in plants against pathogenic aggression (Ons et al. 2020; Elsharkawy et al. 2022).

Now a days IPM (Integrated Pest Management) consists of a combination of biological treatment with lowered dose and frequency of fungicide than traditional rates are getting attention for sustainable solution of pathogen control. This strategy falls under maximum residue limit (MRLs) programme of EU, while achieving significant disease control (Ons et al. 2020). Considering these facts, this study focused on the combined application of beneficial bioagent and resistance inducer with lowered dose of chemical fungicide to achieve satisfactory control of Stemphylium blight of garlic.

2. Materials and Methods

2.1. Collection and multiplication of *Stemphylium vesicarium* and antagonistic bacteria

Both the pathogen responsible for Stemphylium blight, *Stemphylium vesicarium* (OR923574) and antagonistic *Pseudomonas fluorescens* and *Bacillus subtilis* strains were collected from Bio-signaling, Bioformulation and Bio-active compounds (BBB) laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. The collected pathogen was isolated previously from infected garlic plant and later it was cultured on PDA media at 25 ± 1° C for 10–12 days (Zapata-Sarmiento et al. 2020). The bacteria were cultured on King's B Broth and Nutrient Broth and incubated at 28° C for 1 day and at 25° C for 2 days respectively (Mohamed and Moharam, 2023; Chakraborty et al. 2024).

2.2. Pathogenicity test

Healthy garlic cloves were surface sterilized with 1% Clorox (NaOCl) solution for 3 minutes and rinsed several times with distilled water before drying in shade (Dėnė et al. 2024). The cloves were sown in sterilized soils in pots. Conidial suspension of the pathogen *Stemphylium vesicarium* was prepared by scrapping the mycelia of the fully grown pathogen on 12-15 days old PDA plate and mixing with sterile distilled water (Hassan et al. 2020). For inoculation of the pathogen a modified method stated by Salaria et al. (2020) was followed. The concentration was adjusted at 10^4 spores/ml. When the garlic plants got to four leaf stage, the aerial part of the plants were sprayed with conidial suspension till run off. The pathogenicity tests were confirmed by observing the typical symptoms of Stemphylium blight after 7-10 days.

2.3. *In vitro* antagonism test of selected antagonistic bacteria against *Stemphylium vesicarium* by dual culture assay

A 5 mm mycelial disc was separated from 14 days old culture of *Stemphylium vesicarium* and placed at the middle of a fresh PDA plate. Then a loopful of antagonistic bacteria was streaked around the pathogen at a triangular shape. The control plate contained only *Stemphylium vescarium*. Finally, the PDA plate containing both pathogen and bioagents were incubated at 25 ± 1° C till the control plate was fully covered by mycelial growth of *Stemphylium vesicarium*. Finally, the suppressive effect of the selected antagonists was determined by the following formula (Murmu and Saha 2021).

$$\text{Pathogen growth suppression (\%)} = \frac{C-T}{C}$$

Where, C = Mycelial growth of pathogen in control plate; T = Mycelial growth of pathogen in antagonist inoculated plate.

2.4. Preparation of inducers

Two different plant resistance inducers were used in this experiment, *i.e.:* Chitosan and Salicylic acid. A concentration of 500 ppm and 36 ppm was maintained for these inducers respectively (Islam et al. 2025). Chitosan solution was prepared by mixing 0.15 g chitosan in small amounts of water at 40° C then acetic acid (1%) was added drop by drop for solubility. Finally distilled water was added, and volume was adjusted upto 300 ml for getting desired concentration (do Amaral Sobral et al. 2022). For preparation of salicylic acid (36 ppm) protocol stated by Xi et al. (2021) was used with slight modifications. At first 0.0108 g of salicylic acid was taken and mixed in 15 ml, (98 % ethanol). The ethanol was added drop by drop and mixed well with the solute. Finally, the volume was adjusted to 300 ml with addition of distilled water for preparation of final concentration as 36 ppm.

2.5. Preparation of fungicide

Rovral (Iprodione) @ 0.2 % was used in this experiment. 0.2g fungicide was weighed and mixed with 100 ml distilled water for getting desired concentration.

2.6. Field preparation and sowing of seeds

For field experiment, BINA-rashun 1 variety was selected for its high yield potential and tolerance against Stemphylium blight. Seeds were sown in Plant pathology field, Bangladesh Agricultural University, Mymensingh. The soil type was sandy loam, 6.8 pH and well fertilized. Garlic cloves were surface sterilized with 0.5% clorox for 1 minutes and rinsed in distill water for removing all traces of sterilizing chemical. Then the cloves were sown in the field at November 2022. The climate of the locality is winter season and characterized by low temperature (15-25 °C) and low rainfall (0-8 inch) during the rabi season. Total 2400 sq. inch area was allocated for each plot and each treatment consisted of 3 replications or 3 plots. Clove to clove distance and line to line distance were 6 inches and 8 inches respectively in each plot. The number of garlic plant in one line was 10 and the number of lines were 5 in each plot. So, the total number of plants were 50 in each plot.

2.7. Application of treatments

The following treatments were applied by foliar spray with the first spray at 40 DAS and two more subsequent sprays at 15 days interval, following a modified method of Pandey et al. (2023a).

T_0 = Control

T_1 = *Pseudomonas fluorescens* strain Psf3 @ 10 mL per 5 plants

T_2 = *Bacillus subtilis* strain Bacs4 @ 10 mL per 5 plants

T_3 = Salicylic Acid @ 36 *ppm*

T_4 = Chitosan @ 500 ppm

T_5 = Rovral @ 0.2%

T_6 = Rovral @ 0.2% + *Pseudomonas fluorescens* strain Psf3 @ 10 mL per 5 plants

T_7 = Rovral @ 0.2% + Salicylic Acid @ 36 ppm

T_8 = Rovral @ 0.2% + Chitosan @ 500 ppm

T_9 = Rovral @ 0.2% + *Bacillus subtilis* strain Bacs4 @ 10 mL per 5 plants

2.8. Assessment of disease incidence and disease severity

Disease incidence is the ratio of infected plants to total plants. Disease incidence was determined by the following formula stated by Hassan et al. (2018).

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of observed plants}} \text{ x } 100$$

As for disease severity, a 0-5 scale was followed as stated by Salaria et al. (2022). The scale is listed below:

0 = No infection

1 = 1-10 % infected area in leaves at the top portion

2 = 11-20 % infected area in leaves with purplish spots

3 = 21-40 % infected area in leaves with purple spots with outer border of

4 = 41-75 % infected area in leaves with breaks at the center

5 = Above 75 % infected area in leaves with drying of the leaves

Finally, disease severity was determined by the following formula stated by Salaria et al. (2022).

$$\text{Disease Severity} = \frac{\sum (\text{Scale rating x No. of plants in the particular scale})}{\text{Maximum scale rating x Total no. of plants}}$$

2.9. Data collection

Data on vegetative parameters *i.e.*: Plant height, no. of leaves/ plant, leaf length, leaf sheath length, root length, fresh weight and dry weight of whole plant and bulb were collected for the first time at 30 DAS before application of any treatments for ensuring the homogenous quality of each treatment. Subsequent data on vegetative parameters were taken at 50 and 70 DAS. Disease incidence and severity recordings were taken at 50, 70 and 90 DAS. Data on bulb yield were taken at 110 DAS. For each parameter and treatments, three replications were maintained. Data were analyzed by following Tukey's test using Statistics 10 software.

3. Results

3.1. Confirmation of pathogen by pathogenicity test

At 7 days after inoculation typical Stemphylium blight symptoms like development small whitish spots on the leaves and presence of necrotic area at the tip area. Eventually the spots enlarged and turned elongated water with white-brown spots. Our findings were similar with the symptoms stated by Gálvez et al. (2016) thus confirming that the collected pathogen was responsible for Stemphylium blight (Figure 1).

Figure 1. Characteristics symptoms of stemphylium blight disease on garlic seedlings upon inoculation with *Stemphylium vesicarium*

Table 1. Evaluation of the bacterial antagonist for their growth suppression efficacy against *Stemphylium vesicarium*

Mycelial growth of *Stemphylium vesicarium* against *Pseudomonas fluorescens* strains				**Mycelial growth of *Stemphylium vesicarium* against *Bacillus subtilis* strains**			
Treatments	**5 DAI**	**10 DAI**	**15 DAI**	**Treatments**	**5 DAI**	**10 DAI**	**15 DAI**
Control	36.50 a	67.50 a	90.00 a	Control	36.50 a	67.50 a	90.00 a
Psf1	28.00 cd	40.50 d	46.50 d	Bacs1	31.50 bcd	46.33 c	52.50 cd
Psf2	31.50 bc	47.83 c	54.16 c	Bacs2	33.50 abc	49.00 bc	58.00 bc
Psf3	27.00 d	33.50 e	38.66 e	Bacs3	35.00 ab	53.50 b	62.00 b
Psf4	35.16 ab	56.16 b	64.83 b	Bacs4	29.00 d	36.00 d	41.00 e
Psf5	34.50 ab	51.50 bc	61.66 b	Bacs5	30.00 cd	40.50 d	48.00 d
SE	1.34	1.75	1.41	SE	1.26	1.46	1.72
CV (%)	4.49	5.89	4.73	CV (%)	4.22	4.89	5.76

P=0.05; CV= Coefficient of variation; SE = Standard Error; Treatments are analysed based on their mean data. Treatments with similar letter are statistically similar. Here, T_0 = Control, T_1 = *Pseudomonas fluorescens* strain Psf3 @ 10 mL /5 plant, T_2 = *Bacillus subtilis* strain Bacs4 @ 10 mL /5 plant, T_3 = Salicylic Acid @ 36 *ppm,* T_4 = Chitosan @ 500 ppm, T_5 = Rovral @ 0.2%, T_6 = Rovral @ 0.2% + *Pseudomonas fluorescens* strain Psf3 @ 10 mL /5 plant, T_7 = Rovral @ 0.2% + Salicylic Acid @ 36 ppm and T_8 = Rovral @ 0.2% + Chitosan @ 500 ppm, T_9 = Rovral @ 0.2% + *Bacillus subtilis* strain Bacs4 @ 10 mL per 5 plants

3.2. Evaluation of antagonistic effect of the selected bacteria by dual culture assay

Data on dual culture assay on antagonists against *Stemphylium vesicarium* was collected at 5, 10 and 15 DAI (Days after inoculation). Against both *Pseudomonas fluorescens* and *Bacillus subtilis* strains, highest mycelial growth was observed in control or uninoculated plates. The effect of different bioagents appeared more distinct and significantly different from one another as the days progressed. At 15 DAI against *Pseudomonas fluorescens* strains, lowest mycelial growth was observed as 38.66 mm against *Pseudomonas fluorescens* strain Psf3 (MN256389). Thus suppressing 57.04 % of mycelial growth compared to control. Among the *Bacillus subtilis* strains maximum suppression of mycelial growth (54.44 %) was found against *Bacillus subtilis* strain Bacs4 (MN252545). Only 41.00 mm of growth diameter of the pathogen. However, the efficacy of *Pseudomonas fluorescens was* slightly higher than *Bacillus subtilis* regarding suppression of pathogen *in vitro* (Table 1 and Figure 2, 3).

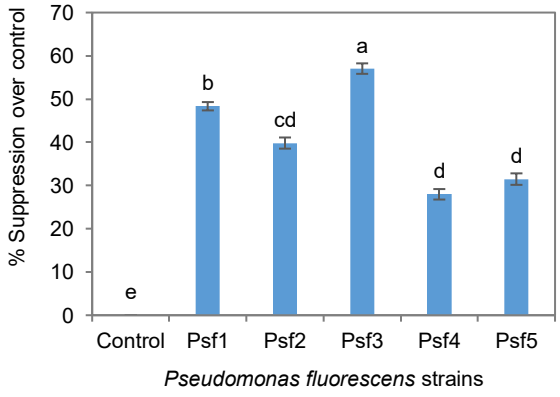

Figure 2. *In vitro* growth suppression efficacy of different *Pseudomonas fluorescens* strains against *Stemphylium vesicarium*

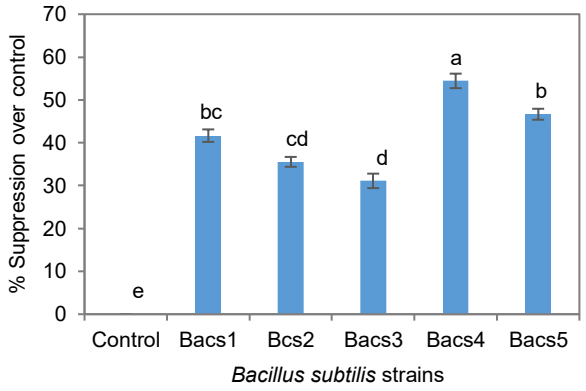

Figure 3. *In vitro* growth suppression efficacy of different *Bacillus subtilis* strains against *Stemphylium vesicarium*

3.3. Effect of different treatments on disease incidence (DI %) and disease severity (DS%) of Stemphylium blight of garlic

Disease incidence and severity was determined at 50, 70 and 90 DAS (Days after sowing). Both of these parameters increased with the progress of growing period of the plants. The range of disease incidence varied from (12.00 % - 42.66 %), (37.33 % - 72.00 %) and (49.33 % - 94.66 %) at 50, 70 and 90 DAS respectively. The range for severity was (19.86 % - 29.06 %), (34.26 % - 50.66 %) and (38.4 % - 60.4 %) at three different data collection periods. In every period, lowest disease severity (19.86 %, 34.26 % and 38.4 %) was observed in T_6 (Rovral @ 0.2 % + *Pseudomonas fluorescens* strain Psf3 @ 10 mL /5 plant) followed by T_9 (Rovral @ 0.2 % + *Bacillus subtilis* strain Bacs4 @ 10 mL /5 plant) (Figure 4,5).

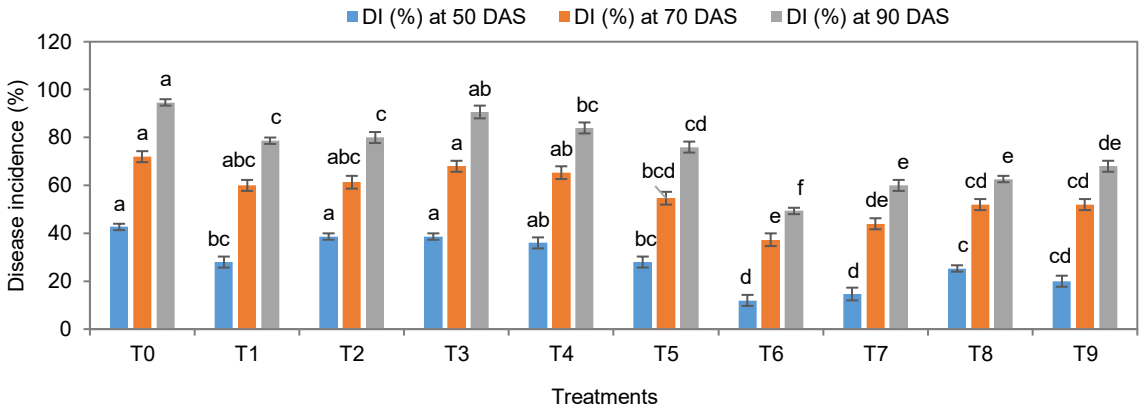

Figure 4. Disease incidence (%) of Stemphylium blight of garlic upon inoculation of different treatments at 50, 70 and 90 DAS. T_0 = Control, T_1 = *Pseudomonas fluorescens* strain Psf3 @ 10 mL /5 plant, T_2 = *Bacillus subtilis* strain Bacs4 @ 10 mL /5 plant, T_3 = Salicylic Acid @ 36 *ppm,* T_4 = Chitosan @ 500 ppm, T_5 = Rovral @ 0.2%, T_6 = Rovral @ 0.2% + *Pseudomonas fluorescens* strain Psf3 @ 10 mL /5 plant, T_7 = Rovral @ 0.2% + Salicylic Acid @ 36 ppm and T_8 = Rovral @ 0.2% + Chitosan @ 500 ppm, T_9 = Rovral @ 0.2% + *Bacillus subtilis* strain Bacs4 @ 10 mL per 5 plants

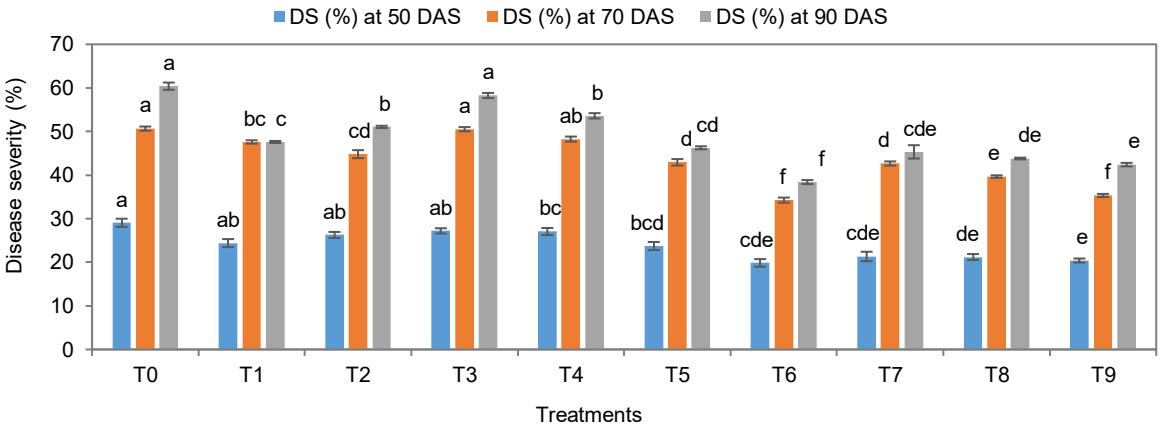

Figure 5. Disease severity (%) of Stemphylium blight of garlic upon inoculation of different treatments at 50, 70 and 90 DAS. T_0 = Control, T_1 = *Pseudomonas fluorescens* strain Psf3 @ 10 mL /5 plant, T_2 = *Bacillus subtilis* strain Bacs4 @ 10 mL /5 plant, T_3 = Salicylic Acid @ 36 *ppm,* T_4 = Chitosan @ 500 ppm, T_5 = Rovral @ 0.2%, T_6 = Rovral @ 0.2% + *Pseudomonas fluorescens* strain Psf3 @ 10 mL /5 plant, T_7 = Rovral @ 0.2% + Salicylic Acid @ 36 ppm and T_8 = Rovral @ 0.2% + Chitosan @ 500 ppm, T_9 = Rovral @ 0.2% + *Bacillus subtilis* strain Bacs4 @ 10 mL per 5 plants

3.4. Effect of treatments on vegetative growth parameters of garlic under field condition

Effect of treatments on plant height, no. of leaves per plant, leaf length, leaf sheath length and root length were recorded at 30, 50 and 70 days after sowing (DAS) and the findings are presented in Table 2, 3 and 4. At early growth stage (30 DAS), no significant differences observed among the treatments (all values marked "a"). At 50 DAS, T_6 recorded the highest plant height (42.27 cm), no. of leaves per plant (6.67), leaf length (33.43 cm), leaf sheath length (5.44 cm), and root length (9.93 cm), followed by T_9 and T_8. Treatments T_0 -T_4 consistently showed lower values across all parameters. Statistical differences became more prominent at 70 DAS. At this stage, T_6 exhibited superiority across all parameters, including plant height (55.03 cm), number of leaves/ plant (9.47), leaf length (45.77 cm), leaf sheath length (8.43 cm), and root length (14.03 cm). T_9 and T_8 also showed significantly higher growth than the control. Control plants (T_0) recorded the lowest plant height (38.30 cm), number of leaves/ plant (6.50), leaf length (30.77 cm), leaf sheath length (5.76 cm), and root length (7.10 cm).

3.5. Effect of treatments on biomass accumulation of garlic grown in field condition

Effect of treatments on fresh weight of whole plant (FWWP), dry weight of whole plant (DWWP), fresh weight of bulb (FWB) and dry weight of bulb (DWB) were recorded at 30, 50 and 70 DAS and the findings are presented in (Table 5). At 30 DAS, biomass accumulation is still low and uniform across the treatments. Mostly there

has no significance differences among the treatments. At second stage of data recording, T_6 showed the highest biomass accumulation with FWWP (9.86 g), DWWP (1.22 g), FWB (3.83 g), and DWB (0.42 g), indicating superior growth vigor and biomass production over the others treatments.

At 70 DAS, the highest FWWP (35.14 g), DWWP (7.98 g), FWB (19.75 g), and DWB (4.55 g) were found in T_6 which was followed by T_9 and T_8. T_0 (Control) consistently showed the lowest biomass values across all stages. It indicated that treatments positively influence plant growth and biomass accumulation. Finally, T_6 produced the highest bulb yield (6.23 ton/ha), which was significantly superior to all other treatments. On other hand, control treatment showed the lowest yield (2.51 ton/ha) (Figure 6).

4. Discussion

This study focuses on the integrated management of stemphylium blight of garlic through the use of some selected BCA (Biological control agents) and plant resistance inducer with traditionally used fungicide. In our study among different *Pseudomonas fluorescens* and *Bacillus subtilis* strains, *Pseudomonas fluorescens* strain Psf3 and *Bacillus subtilis* strain Bacs4 offered highest mycelial growth suppression (57.04 % and 54.44 %) respectively *in vitro*. Additionally, these two BCA when combined with chemical fungicide (Rovral @ 0.2 %) offered lowest disease incidence (47.88 % and 36.62 % lower than control) and severity (36.42 % and 24.95 % lower than control) respectively, among all treatments, including individual and combined application of BCA, inducer and fungicide. Similar findings were reported by Bachhav et al. (2024) where, more than 50% growth reduction of *Stemphylium vesicarium* was observed against both *Pseudomonas fluorescens* and *Bacillus subtilis* in onion. Park et al. (2024) found among five different groups of fungicides, Iprodione (Rovral) had the highest efficacy for suppressing the growth of *Stemphylium vesicarium.* A study by Shahnaz et al. (2012) also reports similar findings where combination of mancozeb (0.25 %) and *Trichoderma harzianum* showed maximum suppression of foliar blight of onion (20.24 % disease intensity) whereas highest disease intensity was recorded in untreated control (25.49 % in control) at 10 standard weeks.

Pseudomonas fluorescens is reported to synthesize different antibiotics like phenazines, dialkylresorcinols, pyoluteorin and pyrrolnitrin which have antifungal properties and cell wall degrading enzyme like chitinase, cellulases, β 1-3 glucanase (Bonaterra et al. 2022). They can also overwhelm pathogens in competition for space and nutrients due to synthesis of siderophore. These mechanisms along with activation of Induced systemic resistance through production of CLP (Cyclic peptides) makes them a strong opponent against plant pathogenic fungi (Bonaterra et al. 2022). Similarly, *Bacillus* sp as an antagonistic bacterium is also capable of cellulases, β 1-3 glucanase, protease, lipase, chitinases for breaking down pathogenic cell wall. Enhance enzymatic activity like PAL, POD for inducing defense response in host plants and iron sequestration buy siderophore production makes *Bacillus* sp suitable for controlling pathogens (Xia et al. 2023).

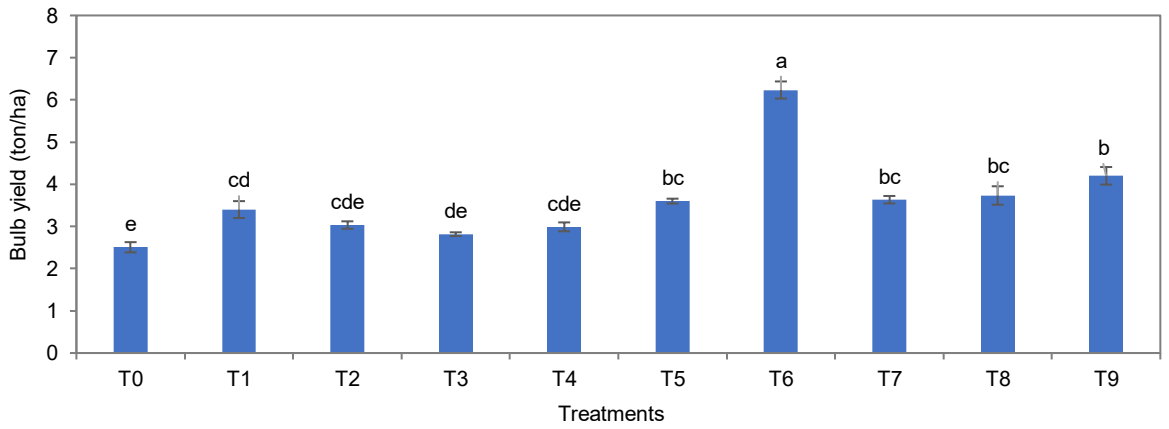

Figure 6. Effect of different treatments on bulb yield (ton/ha) of garlic. T_0 = Control, T_1 = *Pseudomonas fluorescens* strain Psf3 @ 10 mL /5 plant, T_2 = *Bacillus subtilis* strain Bacs4 @ 10 mL /5 plant, T_3 = Salicylic Acid @ 36 *ppm,* T_4 = Chitosan @ 500 ppm, T_5 = Rovral @ 0.2%, T_6 = Rovral @ 0.2% + *Pseudomonas fluorescens* strain Psf3 @ 10 mL /5 plant, T_7 = Rovral @ 0.2% + Salicylic Acid @ 36 ppm and T_8 = Rovral @ 0.2% + Chitosan @ 500 ppm, T_9 = Rovral @ 0.2% + *Bacillus subtilis* strain Bacs4 @ 10 mL per 5 plants

Furthermore, beside suppression of pathogens T_6 (Rovral @ 0.2 % + *Pseudomonas fluorescens* strain psf3 @ 10 mL /5 plant) offered highest growth of vegetative parameters as well as maximum yield followed by T_6 (Rovral @ 0.2 % + *Bacillus subtilis* strain Bacs4 @ 10 mL /5 plant) where T_6 produced 148.21 % higher bulb yield compared to untreated control, T_0. Our findings share similarity with the study of Pandey et al. (2023b) where application of bioagent AMC (Arka Microbial Consortia) together with fungicide against stemphylium blight disease resulted in highest marketable yield of onion (194.83 q/ha) which was statistically superior to the yield in untreated control (125 q/ha). The BCA utilizes several mechanisms for promoting plant growth. Direct approaches include

phytohormone production, solubilization of nutrients, helping plants in up taking higher amounts of iron by siderophopre production along with indirect approaches like antibiosis, parasitism, and competition against pathogens and thus reducing biotic stress on plants (Elnahal et al. 2022).

Also plant growth promoting bacteria can colonize and increase the surface of plants to provide for higher anchoring and nutrient uptake efficiency for enhanced growth (El-Saadony et al. 2022).

We suggest that a synergism between the positive effect of BCAs with superior antifungal activities of chemical fungicide, Rovral may be the reason for the better efficacy of combination treatment compared to their individual performance. Wang et al. (2024) speculated that a synergism may exist with BCA and fungicide, also the BCAs create a opening on the surface of pathogen cell membrane which serves as a entry point for fungicide, consequently fungicides demonstrate better efficiency in the disease. This may be responsible for their combined application more fruitful rather than single application. Protective biofilm production by the BCAs can be a reasonable explanation for their synergism with the fungicide (Liu et al. 2018). Our findings showed similarity with the result of Pandey et al. (2023a), where combined effect of biopesticide and fungicide performed better for controlling Stemphylium blight compared to their individual application.

Although Integrated approaches for disease management is getting attention of the researchers, it's application is still very low. Several reasons like institutional constraints, sociological and economical constraints, addiction of farmers to subsidies offered with fungicides and lack of availability of bioagents are some primary reasons (Pandey et al., 2016). The concerned authority should take these issues into consideration and promote IDM practices for crops, environment and health benefits.

5. Conclusion

This study evaluated the efficacy of co-application of biological control agents, inducers and commonly used fungicide, Rovral against a devastating disease of garlic, Stemphylium blight. Our findings demonstrated that compared to the individual application of different disease control agents, combined application not only demonstrated maximum suppression the disease, but also offered maximum production. As per our knowledge, much work regarding control of Stemphylium blight disease of garlic with mixture of bioagent and fungicide is not reported globally. These integrated disease management fulfills the requirement of MRL programme of European Union and also a much safer option for environment compared to traditional application of fungicide.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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