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EFFECT OF BIO-AGENTS ON ANTHRACNOSE DISEASE [*COLLETOTRICHUM CAPSICI* (SYD.)BUTLER AND BISBY] OF CHILLI (*CAPSICUM ANNUUM* L.)

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ABSTRACT

Among spices, chilli is an economically important spice crop having outstanding nutritional and therapeutic value. India is a largest producer, consumer and exporter of chilli. This crop suffers from various diseases, among them, anthracnose disease caused by *Colletotrichum capsici* causes huge losses in India and abroad. Therefore, the present investigation on "Effect of bio-agents on anthracnose disease [*Colletotrichum capsici* (Syd.) Butler and Bisby] of chilli (*Capsicum annuum* L.)" was carried out in *Kharif* 2023 at Central Research field, Department of Plant Pathology, SHUATS, Prayagraj. The effect of treatmentson disease intensity (%) and yield (t/ha) of chilli was evaluated under field conditions. The combination of seedling dip treatment with *Trichoderma viride* @ 0.5% + *Pseudomonas fluorescens* @ 0.5% + *Bacillus subtilis* @ 0.5% conc. were recorded significantly reducing disease intensity at 60 DAT (10.50%), 75 DAT (21.47%) and 90 DAT (28.10%), highest yield (8.07t/ha) followed by *Trichoderma viride* @ 0.5% + *Pseudomonas fluorescens* @ 0.5% conc. at 60 DAT (12.28%), 75 DAT (23.53%) and 90 DAT (31.17%), yield (7.21t/ha) and least disease intensity was recorded in seedling dip treatment with *Bacillus subtilis* @ 0.5% conc. at 60 DAT (17.36%), 75 DAT (28.87%) and 90 DAT (36.37%), least yield (5.19t/ha) as compared to control at 60 DAT (23.18%), 75 DAT (33.24%) and 90 DAT (48.95%), lowest yield (4.68t/ha).

Keywords: *Bacillus subtilis,* bioagents, bioagents combination, chilli, *Colletotrichum capsici*, *Pseudomonas fluorescens, Trichoderma viride*

Introduction

Chilli (*Capsicum annuum* L.) is an important spice-cum-vegetable crop, often referred to as capsicum, hot pepper, sweet pepper or paparika. India stands 3rd in production of chilli (Saxena *et al.*, 2016) and *Capsicum annuum* is the widely cultivated species. Green chilli provides vitamin-C while, the red chilli provides vitamin-A (Martin *et al.,* 2004) in addition to iron, potassium and magnesium. India is the largest producer of chilli in the world accounting for over 45% of the total area under cultivation. AP, MH, KA, OR and TN account for about 75% of the total area as well as production of India. In India 2023, the production was around two million metric tonnes from an area of 6.84 lakh hectares. In Uttar Pradesh, Production share is leading in vegetable producing state was total production of 14.92 thousand MT. In 2023, Uttar Pradesh has 13th place in leading green chilli producing states of India (Satista, 2023).

Chilli (*Capsicum annuum* L.) suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also by abiotic stresses. Among fungal diseases, anthracnose/die-back/fruit rot caused by *Colletotrichum capsici* (Syd.) Butler and Bisby has become a most serious problem for chilli cultivation in India (Akhtar and Singh, 2007) and (Singh *et al.,* 2007). Anthracnose was reported first time in India from Coimbatore (Madras) (Sydow, 1913). The disease caused by *C. capsici* is one of the most economically important disease reducing marketable yield estimated

upto 50% in different parts of India (Pakdeevaraporn *et al*., 2005). The symptoms can occur on leaves, stems and both pre and post-harvest fruits. Typical symptoms on chilli fruit include sunken necrotic tissues, with concentric rings of acervuli (Agrios, 2005). The pathogen is both seed-borne and air-borne and affects seed germination and vigour to a greater extent (Gopinath *et al.,* 2006). *C. capsici* spread by watersplashes in the form of conidia and ascospores in the air (Nicholson and Moraes, 1980) and (Asalmol *et al.*, 2001).

Although different chemical fungicides are being recommended and used to combat the disease, there is a pressing need to explore biocontrol-based strategy for the management of crop diseases in the present arena of organic agriculture.

Bio-control is an effective alternate that provides disease management ability, while being relatively harmless to humans, selective in mode of action, difficult for pathogens to develop resistance (Singh *et al.,* 2012). There is not much research work that is being done on seed borne aspects of anthracnose of chilli. There is very little information available on management strategies like use of effective bio-agents (Gowda *et al.,* 2022). So far, biological control methods for chilli fruit rot/anthracnose disease have not received much attention (Raghunandan *et al.,* 2019). The potential for biological control of Colletotrichum species had been suggested as early as in 1970s (Lenné and Parbery, 1976). The use of bio-control agents individually and combinations are the best alternative for management of chilli anthracnose. Hence, the present investigation was undertaken to find the environment friendly management of anthracnose of chilli caused by *C. capsici* under field conditions.

Materials and Methods

The present research work was conducted during *Kharif* -2023 in the Central Research Field of the Department of Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh. The experiment was conducted in Randomized Block Design with seven treatments and three replications. Seven treatments were randomly arranged in each replication divided into twenty-one plots and each plot size was 2 x 1 m.

Table 1: Details of treatments

Sr. No.	Treatment No.	Treatment Details
1.	T_0	Control
2.	T_1	S.D. with *Trichoderma viride* ($1x10^8$ cfu/ml) @ 0.5 % conc.
3.	T_2	S.D. with *Pseudomonas fluorescens* ($1x10^8$ cfu/ml) @ 0.5% conc.
4.	T_3	S.D. with *Bacillus subtilis* ($1x10^8$ CFU/ml) @ 0.5% conc.
5.	T_4	S.D. with *T. viride* ($1x10^8$ cfu/ml) @ 0.5% conc. + *P. fluorescens* ($1x10^8$ cfu/ml) @ 0.5% conc.
6.	T_5	S.D. with *T. viride* ($1x10^8$ cfu/ml) @ 0.5% conc. + *B. subtilis* ($1x10^8$ cfu/ml) @ 0.5% conc.
7.	T_6	S.D. with *T. viride* ($1x10^8$ cfu/ml) @ 0.5% conc. + *P. fluorescens* ($1x10^8$ cfu/ml) @ 0.5% conc + *B. subtilis* ($1x10^8$ cfu/ml) @ 0.5% conc.

S.D. – Seedling Dip*

Methodology

Collection of diseased samples

Chilli leaves showing characteristic symptoms of anthracnose disease were collected from the Central Research Field (CRF), Naini Agricultural Institute, SHUATS, Prayagraj and used them for isolation of the test fungus.

Isolation of Pathogen

The infected chilli leaves, which displayed typical symptoms of anthracnose disease, were cleaned thoroughly by washing in running tap water for 4-5 minutes to remove any dirt from its surface. The infected leaf tissue was cut into small pieces up to 4-5 mm in diameter and surface sterilized by using one percent sodium hypochlorite (NaOCl) solution for one minute. After sterilization, the tissue was washed three times with distilled water to remove any remaining traces of the NaOCl solution. The pieces were then air-dried on blotting paper. Finally, the pieces were transferred aseptically to 90 mm petri plates containing 20 ml of PDA medium. The petri plates that had been inoculated were placed in a BOD incubator and kept at a temperature of 25±2°C for a period of three days. On the fourth day, hyphal tip isolation of *Colletotrichum capsici* was done.

Purification and Maintenance of culture

The hyphal tip method was utilized to purify the culture of *Colletotrichum capsici*. This procedure involved carefully selecting a single, actively growing

hyphal tip from a well- established fungal colony. The selected hyphal tip was then transferred to a fresh culture medium, such as potato dextrose agar (PDA), to promote growth. This technique is effective in isolating a pure culture, as it minimizes the risk of contamination. Following the transfer, the cultures were incubated at 25±2°C, allowing for optimal growth and maintenance of the fungal strain for further studies.

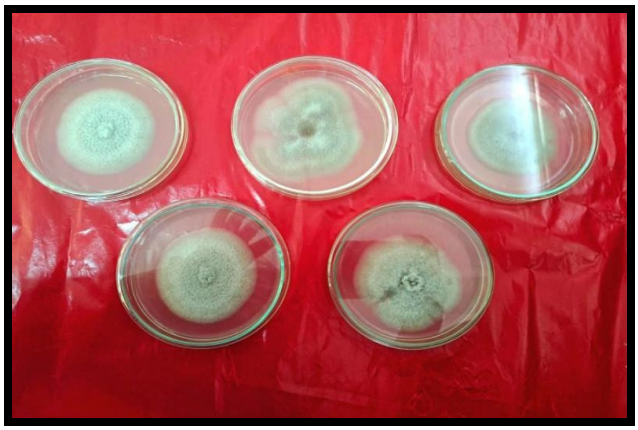

Plate 1: Pure culture plates of *Colletotrichum capsici*

Identification of Pathogen

From the pure culture, a small piece of *Colletotrichum capsici* culture was taken and uniformly spread on a clean glass slide with the help of needle. A drop of lactophenol followed by cotton blue were added, spread evenly over the slide with a needle, and then covered with a cover slip. The identification of *Colletotrichum capsici* was based on morphological characters such as size and shape of conidia of acervuli and existence with setae and cultural characters such as colony colour, growth rate and texture (Smith and Black, 1990). *Colletotrichum capsici* produce grey white scattered falcate conidia with black acervuli and non-uniform shape of mycelium (Gunnel and Gubler, 1992). A reference to standard literature and monographs were used to confirm observations regarding the conidia, conidiophore, setae and acervuli (Alexopoulos *et al.,* 1996).

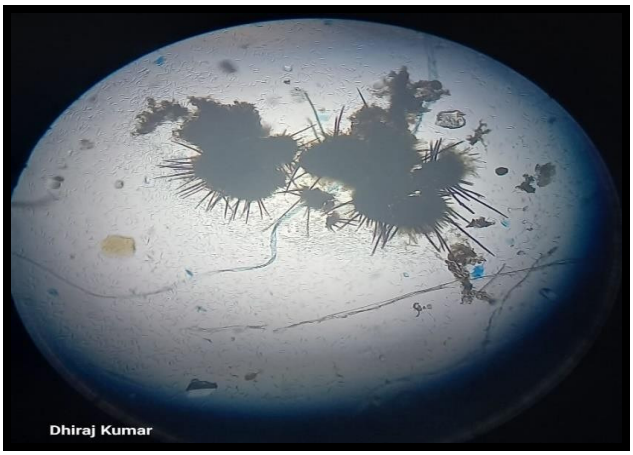

Plate 2: Microscopic view of *Colletotrichum capsici*

Preparation of treatments suspension

Each selected bio-agent purchased from market and the suspension was prepared separately by mixing 5 ml of the bio- agent in 1000 ml of distilled water. The roots of the seedlings were then dipped in the prepared suspension for five hours and allowed to air dry in shade for 15 minutes.

Table 2: Details of bio-agents

Sr. No.	Commercial name	Bio-agents	Treatment details
1.	NISARGA	*T. viride* ($1X10^8$ cfu/ml) (2% A.S.)	Multiplex Biotech Pvt. Ltd.
2.	SPARSHA	*P. fluorescens* ($1X10^8$ cfu/ml) (2% A.S.)	Multiplex Biotech Pvt. Ltd.
3.	MILDOWN	*B. subtilis* ($1X10^8$ cfu/ml) (2% A.S.)	IPL Biologicals

Transplanting of seedling

The experimental plot was laid out according to the statistical design, with chilli seedlings transplanted at a spacing of 60 cm between rows and 45 cm between plants to ensure optimal growth conditions. This arrangement helps in providing adequate space for each plant, facilitating proper development and easier management of the crop.

Preparation and application of conidial spray

The mycelial growth of *Colletotrichum capsici* was scraped from the culture test tube using a sterilized glass rod after adding 10 ml of distilled water. This mixture was then filtered through double-layered muslin cloth to eliminate mycelial fragments and cultural debris, resulting in the collection of conidial spores. The concentration of the conidial suspension was adjusted to 3×10^4 spores per milliliter by adding sterile distilled water, and the concentration was verified using a hemocytometer. The suspension was sprayed onto the plants 40 days after transplanting, utilizing a hand sprayer (Yadav *et al.,* 2017).

Effect of bio-agents on disease intensity (%) of anthracnose disease of chilli

Disease symptoms first time were observed on the plants 45 days after transplanting. The percentage of disease intensity was then recorded at intervals of 60,

75 and 90 days after transplanting (DAT). This systematic monitoring allowed for the assessment of disease progression and its impact on the chilli plants over time.

Plate 3 : Appearance of disease symptoms on leaves

Disease intensity (%)

Disease intensity was recorded in five randomly selected plants tagged in each plot at 60, 75 and 90 days after transplanting, disease intensity was observed as per the scale (Mayee and Datar, 1986) represented in Table 3 Disease intensity (%) formula used which was given by Wheeler (1969).

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all numerical rating}}{\text{No. of leaves observed} \times \text{maximum disease rating}} \times 100$$

Table 3: Disease intensity scale of anthracnose of chilli (Mayee and Datar, 1986)

Grade	Leaf area covered
0	No symptoms of disease on leaf
1	Small, irregular brown spots covering 1 percent or less area ofthe leaf
3	Brown, dirty, pin headed spots covering 1-10 percent area on theLeaf
5	Dark brown, dirty black spots with blackish margin covering 11-25 percent of the area of leaf
7	Dark brown, circular or irregular spots with blackish covering26-50 percent area of leaf
9	Dark brown, circular or irregular spots with blackish covering 51percent and above area of leaf

Fruit yield (t/ha)

The weight of chilli fruit obtained from all harvests was pooled together to obtain a cumulative weight for each plot. This aggregated weight provided a comprehensive measure of the total yield for each plot across the entire harvesting period.

Results and Discussion

Effect of treatments on disease intensity of anthracnose disease of chilli

In the present finding, the data of disease intensity (%) at 60 DAT shown in the Table 4 and Figure 1 reveals that minimum disease intensity (%) recorded in T_6 (seedling dip treatment with *Trichoderma viride* @ 0.5% + *Pseudomonas fluorescens* @ 0.5% + *Bacillus subtilis* @ 0.5% conc.) with 10.50 % followed by T_4 (seedling dip treatment with *Trichoderma viride* @ 0.5% + *Pseudomonas fluorescens* @ 0.5% conc.) with 12.28 % and maximum was observed in T_3 (seedling dip treatment with *Bacillus subtilis* @ 0.5% conc.) with 17.36 % as compared to control (23.18 %).

All the treatments were statistically significant over control. Among the treatments (T_1, T_3, T_5 and T_6) were significant to each other, however, (T_2 and T_3) and (T_4 and T_5) were non-significant to each other.

At 75 DAT the minimum disease intensity (%) was recorded in T_6 (seedling dip treatment with *Trichoderma viride* @ 0.5% + *Pseudomonas fluorescens* @ 0.5% + *Bacillus subtilis* @ 0.5% conc.) with 21.47 % followed by T_4 (seedling dip treatment with *Trichoderma viride* @ 0.5% + *Pseudomonas fluorescens* @ 0.5% conc.) with 23.53 % and maximum was observed in T_3 (seedling dip treatment with *Bacillus subtilis* @ 0.5% conc.) with 28.87 % as compared to control (33.24 %).

All the treatments were statistically significant over control. Among the treatments (T_1, T_3, T_5 and T_6) were significant to each other, however, (T_2 and T_3) and (T_4 and T_5) were non-significant to each other.

At 90 DAT the minimum disease intensity (%) was recorded in T_6 (seedling dip treatment with *Trichoderma viride* @ 0.5% + *Pseudomonas fluorescens* @ 0.5% + *Bacillus subtilis* @ 0.5% conc.) with 28.10 % followed by T_4 (seedling dip treatment with *Trichoderma viride* @ 0.5%+ *Pseudomonas fluorescens* @ 0.5% conc.) with 31.17 % and maximum was observed in T_3 (seedling dip treatment with *Bacillus subtilis* @ 0.5% conc.) with 36.37 % as compared to control (48.95 %).

All the treatments were that statistically significant over control. Among the treatments (T_2, T_3, T_5 and T_6) were significant to each other, however, (T_1 and T_2) and (T_4 and T_5) were non-significant to each other.

Table 4: Effect of treatments on disease intensity (%) of anthracnose disease of chilli (*Capsicum annuum* L.) at different time intervals of DAT

		Disease intensity (%)		
Tr. No.	**Treatments**	**60 DAT***	**75 DAT***	**90 DAT***
T0	Control	23.18	33.24	48.95
T1	*Trichoderma viride*	14.89	26.04	34.75[a]
T2	*Pseudomonas fluorescens*	16.30[a]	27.77[a]	35.45[a]
T3	*Bacillus subtilis*	17.36[a]	28.87[a]	36.37
T4	*T. viride* + *P. fluorescens*	12.28[b]	23.53[b]	31.17[b]
T5	*T. viride* + *B. subtilis*	13.27[b]	24.72[b]	31.71[b]
T6	*T. viride* + *P. fluorescens* + *B. subtilis*	10.50	21.47	28.10
	S.Em (±)	0.37	0.42	0.25
	C.D. (5%)	1.13	1.30	0.77

*Average of three replications
*Data followed by same alphabets in a column are non-significant to each other at 5% level.

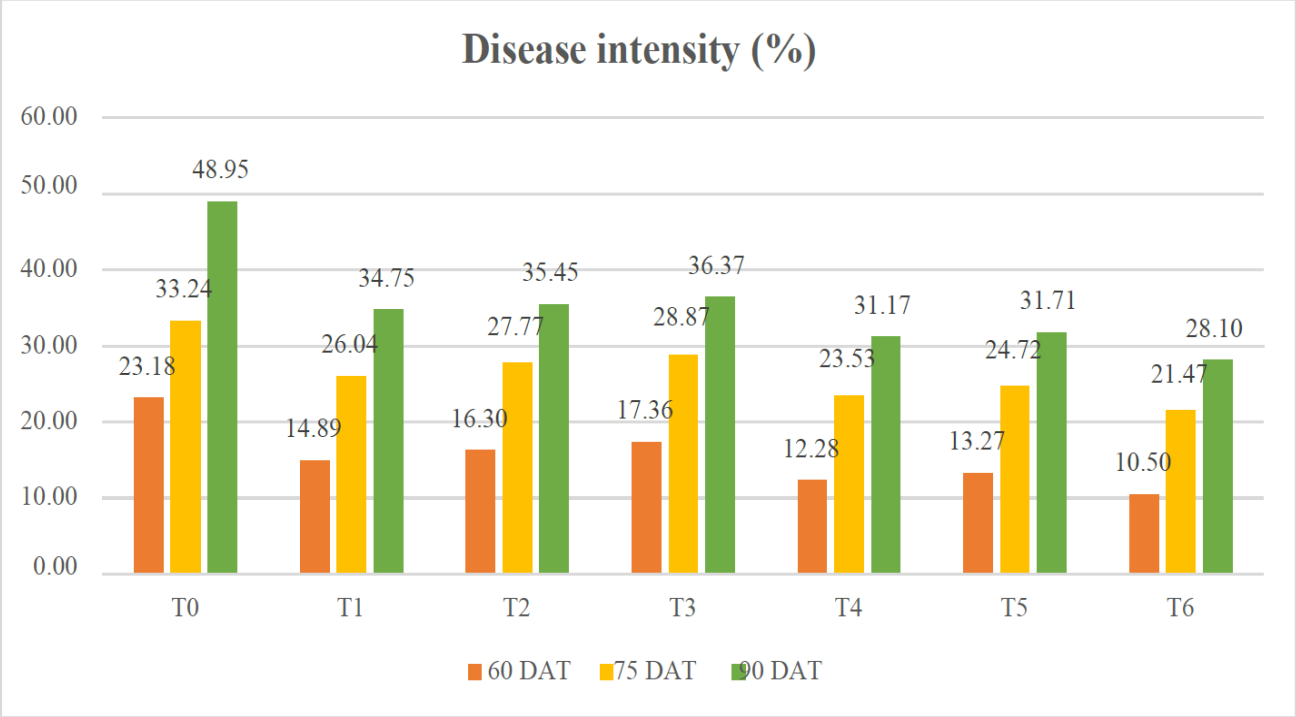

Fig. 1: Effect of treatments on disease intensity (%) of anthracnose disease of chilli (*Capsicum annuum* L.) at different time intervals of DAT

Effect of treatments on yield against anthracnose disease of chilli

In the present finding, the data of yield shown in the Table 5 and Figure 2 reveals that maximum yield was recorded in T_6 (seedling dip treatment with *Trichoderma viride* @ 0.5% + *Pseudomonas fluorescens* @ 0.5% + *Bacillus subtilis* @ 0.5% conc.) with 8.07 t/ha followed by T_4 (seedling dip treatment with *Trichoderma viride* @ 0.5% + *Pseudomonas fluorescens* @ 0.5% conc.) with 7.21 t/ha and least was observed in T_3 (seedling dip treatment with *Bacillus subtilis* @ 0.5% conc.) with 5.19 t/ha as compared to control (4.68 t/ha).

All the treatments were statistically significant over control. Among the treatments (T_1, T_3, T_4, T_5 and T_6) were significant to each other, however, (T_1 and T_2) were non-significant to each other.

Table 5: Effect of treatments on yield (t/ha)

Tr. No.	Treatments	Yield (t/ha)			Mean*
		R1	R2	R3	
T0	Control	4.72	4.67	4.62	4.68
T1	*Trichoderma viride*	6.12	6.22	6.2	6.18[a]
T2	*Pseudomonas fluorescens*	5.8	5.7	5.77	5.76[a]
T3	*Bacillus subtilis*	5.1	5.17	5.3	5.19
T4	*T. viride* + *P. fluorescens*	7.15	7.12	7.35	7.21
T5	*T. viride* + *B. subtilis*	6.75	6.67	6.7	6.71
T6	*T. viride* + *P. fluorescens* + *B.subtilis*	7.57	8.33	8.3	8.07
	S.Em (±)				0.15
	C.D. (5%)				0.45

*Average of three replications

*Data followed by same alphabets in a column are non-significant to each other at 5% level.

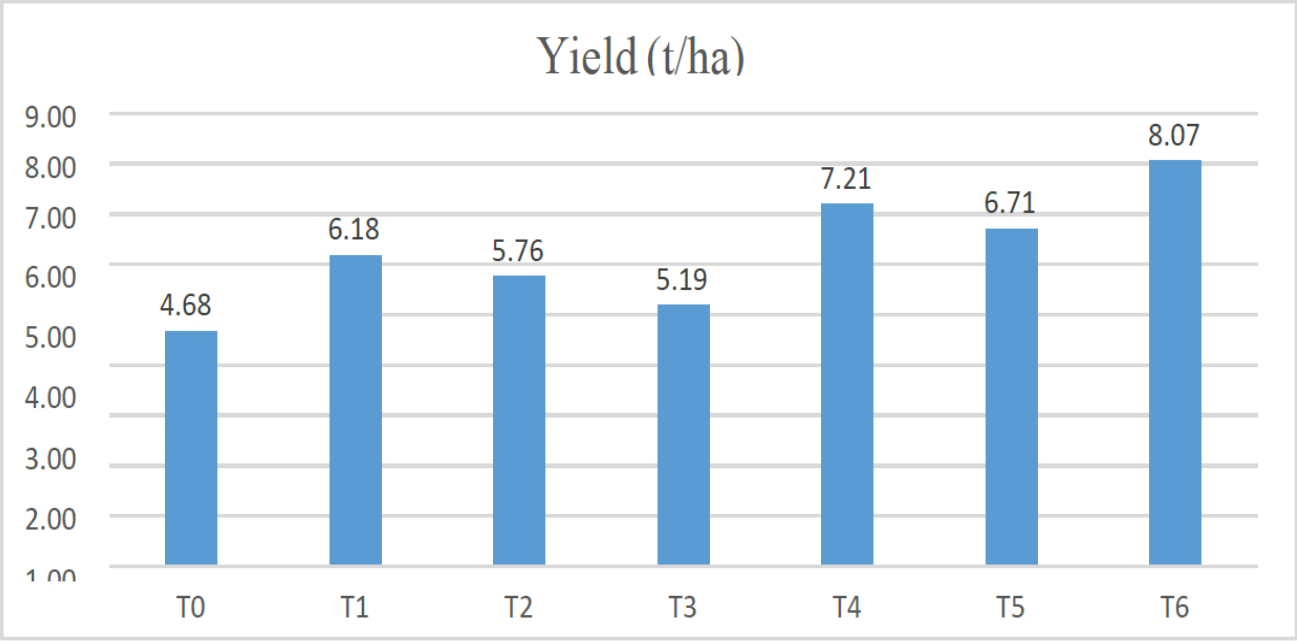

Fig. 2: Effect of treatments on yield (t/ha)

The results of present study, recorded among the treatments, disease intensity significantly decreased at 60, 75 and 90 days after transplanting (DAT) and yield significantly increased with this combination in treatment *T. viride* @ 0.5% + *P. fluorescens* @ 0.5% + B. subtilis @ 0.5% conc.. The results are in agreement with the similar findings of (Lakshmi *et al.*, 2023; Sindu *et al.*, 2022; Pooja and Simon, 2019). The probable reason for these findings may be due to the fungal and bacterial antagonists such as *T. viride*, *P. fluorescens* and *B. subtilis* producing plant promoting substances, antagonistic and antifungal activities of these agents. *Trichoderma viride* can produce harzianic acid, peptaibols, alamethicins, antibiotics, tricholin, 6-penthyl-α-pyrone, massoilactone and viridin which can increase growth of plants and induce resistance to disease (Di Pietro *et al.*, 1993; Lee *et al.*, 1995; Chet *et al.*, 1997; Vey *et al.,* 2001; Intana, 2003).

Pseudomonas fluorescens suppress the pathogens by various modes of actions namely competition for nutrients and space, antibiosis by production various antibiotics, siderophores and lytic enzymes. Other mechanisms include of production of hydrogen cyanide (Defago *et al.,* 1990) and degeneration of toxins (Borowitz *et al.,* 1992).

Bacillus subtilis acts as an effective biocontrol agent against anthracnose in chilli through antibiosis, competition, induced systemic resistance, mycoparasitism and biofilm formation, there by reducing disease severity and enhancing plant growth and yield (Pandey and Gupta, 2015; Saxena *et al.,* 2016; Lokhande *et al.,* 2019; Yadav *et al.,* 2021).

Conclusion

The result allows to conclude that the improvement knowledge about the use of bio-agents can propose new alternatives of disease management. The use of BCAs reduces the chemical load, which is unsustainable to the agriculture field. The plant treated with combine seedling dip treatment *T. viride* + *P. fluorescens* + *B. subtilis* has resulted in significantly reduction of disease and significantly increased the yield of chilli due to enormous potential to induce systemic resistance (ISR) against *C. capsici*. These BCAs not only induce the defence system but also suppress the pathogen establishment and ultimately reduce the anthracnose disease's development in chilli. As the human population is heading towards the organically produced and organically managed agriculture products, so it is concluded that the above findings will be useful for safe and environment friendly future management strategies. The findings of the present experiment found to be significantly effective under Prayagraj agro-climatic conditions, therefore for the validations of the results more such trials should be carried out in future under field conditions for further recommendations.

Therefore, the farmers may be advised to take combine bio-agents, which may raise a profitable production without polluting the environment.

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