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Bio-efficacy of *Bacillus subtilis* isolates against soil borne pathogens

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Abstract
Biological control using beneficial microorganisms, particularly *Bacillus subtilis*, offers an eco-friendly strategy for managing soil-borne plant pathogens. The present study evaluated five *B. subtilis* isolates (BS1-BS5) against major soil pathogens (*Fusarium oxysporum* f. sp. *capsici*, *F. oxysporum* f. sp. *ciceri*, *Sclerotium rolfsii* and *Rhizoctonia bataticola*) using the dual culture technique. Significant differences were observed among the isolates in their antagonistic potential. Against *F. oxysporum* f. sp. *capsici*, isolate T_4 exhibited the highest inhibition (76.60%), followed by T_3 and T_1. In the case of *F. oxysporum* f. sp. *ciceri*, isolates T_2 and T_4 showed maximum inhibition (82.13%). For *S. rolfsii*, isolates T_3 and T_4 recorded the strongest antagonism, with inhibition exceeding 80%. Similarly, against *R. bataticola*, isolates T_5 and T_4 demonstrated the highest suppression, with inhibition up to 88.25%. Overall, all *B. subtilis* isolates significantly reduced pathogen growth compared to the control, with T_2, T_3, T_4 and T_5 showing strong and consistent antagonistic effects. These findings corroborate earlier reports highlighting the efficacy of *B. subtilis* as a biocontrol agent through mechanisms such as production of antifungal metabolites, competition and rhizosphere colonization. The results confirm the promise of *B. subtilis* isolates, particularly T_4 and T_5 as effective candidates for the biological management of soil-borne diseases such as wilt, collar rot and dry root rot in crops.

Keywords: *Bacillus subtilis*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia bataticola*, dual culture, biocontrol

1. Introduction
Biological control using beneficial microorganisms particularly *Bacillus subtilis* has emerged as a promising strategy for managing soil borne pathogens (Kloepper *et al.*, 2004; Compant *et al.*, 2005) [1, 2]. *Bacillus subtilis* is a Gram-positive, rod-shaped, aerobic and motile bacterium that belongs to the family Bacillaceae and the order Bacillales (Claus & Berkeley, 1986) [3]. This saprophytic bacterium is widely distributed in nature and can be isolated from soil, water, air and decaying plant materials (Earl *et al.*, 2008; Chen *et al.*, 2009) [4]. Its ability to produce a wide range of antimicrobial metabolites and promote plant growth makes it one of the most effective and well-studied biocontrol agents.

2.Materials and Methods
The present investigations entitled "Bio-efficacy of *Bacillus subtilis* isolates for management of soil borne pathogens" was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2024-2025. Five isolates of *Bacillus subtilis* were obtained from different districts of Maharashtra (Amravati, Akola, Nagpur, Bhandara, and Buldhana) using the serial dilution method and confirmed by morphological and biochemical tests. Dual culture assays were conducted to evaluate the antagonistic potential of these *Bacillus subtilis* isolates against four soil-borne pathogens (*Fusarium oxysporum* f. sp. *capsici*, *F. oxysporum* f. sp. *ciceri*, *Sclerotium rolfsii* and *Rhizoctonia bataticola*).

2.1. Dual culture of *Bacillus subtilis* against four soil borne pathogens
The pathogens was inoculated at the center of a Petri plate containing a combined medium of Potato Dextrose Agar (PDA) and Nutrient Agar (NA). *Bacillus* isolates were streaked in a

square shape around the centrally placed pathogen. The plates were then incubated at 28 °C for three to seven days. A control plate, inoculated with the pathogen alone in the absence of any antagonistic bacteria was also maintained. All treatments, including the control, were performed in triplicates. After the incubation period, the radial growth of the fungal mycelium on each plate was measured. The percent inhibition of radial growth of the pathogen by *Bacillus* isolates, in comparison to the control was calculated using the formula described by Vincent (1927) [7].

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where;
I = Percent inhibition of mycelium
C = Growth of fungal mycelium in control.
T = Growth of fungal mycelium in treatment.

3. Results and Discussions

An experiment was conducted to identify efficient isolates of *Bacillus subtilis*, namely BS1 (T_1), BS2 (T_2), BS3 (T_3), BS4 (T_4) and BS5 (T_5), against soil-borne plant pathogens using combined media (CM) plates through the dual culture technique.

3.1. Dual culture of *Bacillus subtilis* against *F. oxysporum* f. sp. *capsici*

The data presented in Table 1, Plate1 and Fig. 1 revealed that there was a significant difference among the five *Bacillus subtilis* isolates in their ability to inhibit the growth of *Fusarium oxysporum* f. sp. *capsici*. At 3 days after inoculation (DAI), the minimum mycelial growth of the pathogen was recorded in treatment T_3 (8.25 mm), followed by T_4 (8.75 mm) and T_1 (11.50 mm). At 5 DAI, the lowest mycelial growth was again observed in T_3 and T_4 (12.25 mm each), followed by T_1 (14.75 mm). By 7 DAI, treatment T_4 exhibited the minimum mycelial growth (19.25 mm), followed by T_3 (21.50 mm) and T_2 (22.50 mm). In contrast, the maximum growth of *F. oxysporum* f. sp. *capsici* was consistently recorded in the control treatment. With respect to percent inhibition over control, isolate T_4 exhibited the highest inhibition at 7 DAI (76.60%), closely followed by T_3 (73.87%) and T_1 (72.33%). Similar antagonistic potential of *Bacillus* strains against *Fusarium* wilt of sweet pepper was also reported by Abada and Ahmed (2014) [6].

Table 1: Antagonistic effect of *Bacillus subtilis* isolates against *F. oxysporum* f. sp. *capsici* by dual culture method

Treatments	Antagonistic effect of *Bacillus subtilis* isolates against *F. oxysporum* f. sp. *capsici*					
	3 DAI		5 DAI		7 DAI	
	Mycelial growth of *F. oxysporum* f. sp. *capsici* (mm)	Percent inhibition over control (%)	Mycelial growth of *F. Oxysporum* f. sp. *capsici* (mm)	Percent inhibition over control (%)	Mycelial growth of *F. oxysporum* f. sp. *capsici* (mm)	Percent inhibition over control (%)
T_1	11.50	53.54	14.75	68.45	22.75	72.33
T_2	11.75	52.53	15.00	67.91	22.50	72.64
T_3	8.25	66.67	12.25	73.80	21.50	73.87
T_4	8.75	64.65	12.25	73.80	19.25	76.60
T_5	13.50	45.45	15.25	67.38	25.50	68.99
T_6 - Control	24.75	-	46.75	-	82.25	-
F test	Sig	-	Sig	-	Sig	-
SE (m ±)	0.36	-	0.53	-	0.33	-
CD (P = 0.01)	1.44	-	2.12	-	1.32	-

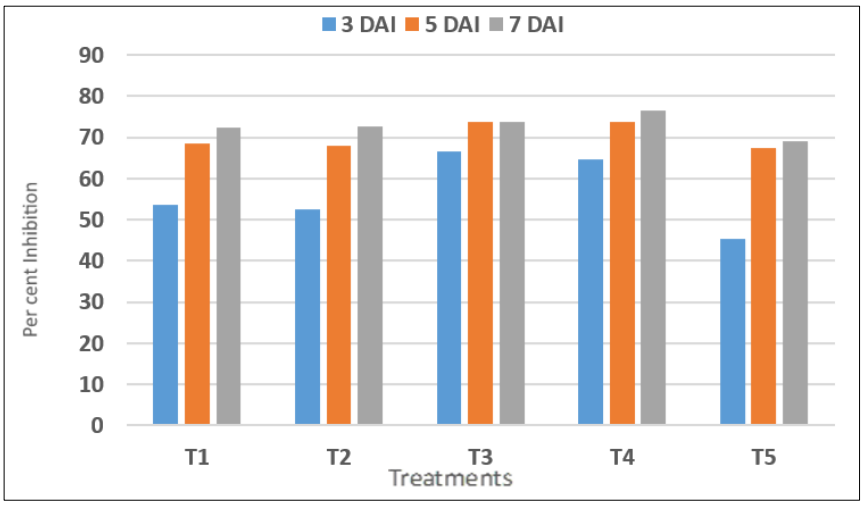

Fig 1: Per cent growth inhibition of *F. oxysporum* f. sp. *capsici* by isolates of *Bacillus subtilis*

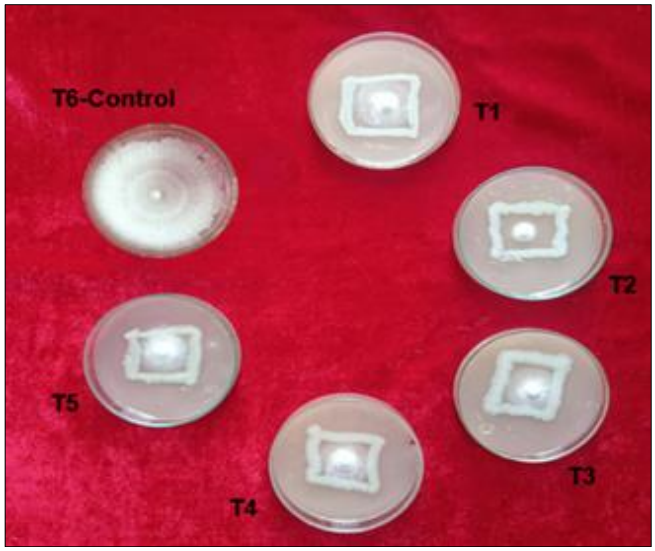

Plate 1: Efficacy of isolates of *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *capsici*

3.2. Dual culture of *Bacillus subtilis* against *F. oxysporum* f. sp. *ciceri*

The data presented in Table 2, Plate 2 and Fig. 2 indicated that there was a significant difference among the five *Bacillus subtilis* isolates in inhibiting the growth of *Fusarium oxysporum* f. sp. *ciceri*. At 3 days after inoculation (DAI), the minimum mycelial growth of the pathogen was recorded in treatments T_2 and T_3 (5.50 mm), followed by T_4 (6.00 mm). At 5 DAI, treatments T_3 and T_4 again showed the lowest mycelial growth (8.75 mm for T_3 and 10.50 mm for T_1), with T_3 exhibiting the highest percent inhibition (77.27%). At 7 DAI, the lowest mycelial growth was observed in T_2 and T_4 (10.50 mm), while the highest percent inhibition was also noted in T_2 and T_4 (82.13%). In contrast, the maximum mycelial growth of *F. oxysporum* f. sp. *ciceri* was consistently observed in the control treatment. All *B. subtilis* isolates significantly inhibited pathogen growth, with T_2 and T_4 showing the strongest antagonism. Similar reports highlight the efficacy of *B. subtilis* against *Fusarium spp*. through antibiotics, lipopeptides and secondary metabolites (Kumar *et al*., 2012) [8].

Table 2: Antagonistic effect of *Bacillus subtilis* isolates against *F. oxysporum* f. sp. *ciceri* by dual culture method

Treatments	Antagonistic effect of *Bacillus subtilis* isolates against *F. oxysporum* f. sp.*ciceri*					
	3 DAI		5 DAI		7 DAI	
	Mycelial growth of *F. oxysporum* f. sp. *ciceri* (mm)	Percent inhibition over control (%)	Mycelial growth of *F. oxysporum* f. sp. *ciceri* (mm)	Percent inhibition over control (%)	Mycelial growth of *F. oxysporum* f. sp. *ciceri* (mm)	Percent inhibition over control (%)
T_1	7.50	59.46	10.50	72.73	13.50	77.02
T_2	5.50	70.27	9.50	75.32	10.50	82.13
T_3	5.50	70.27	8.75	77.27	11.50	80.43
T_4	6.00	67.57	8.75	77.27	10.50	82.13
T_5	8.50	54.05	11.00	71.43	13.50	77.02
T_6 - Control	18.50	-	38.50	-	58.75	-
F test	Sig	-	Sig	-	Sig	-
SE (m ±)	0.28	-	0.31	-	0.30	-
CD (P = 0.01)	1.15	-	1.27	-	1.21	-

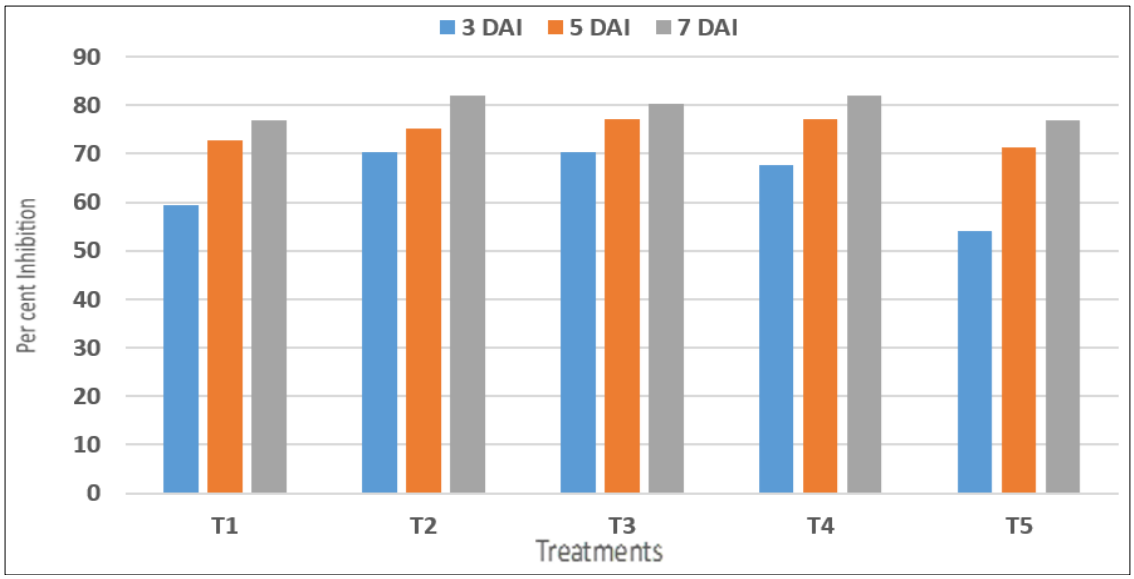

Fig 2: Per cent growth inhibition of *F. oxysporum* f. sp. *ciceri* by isolates of *Bacillus subtilis*

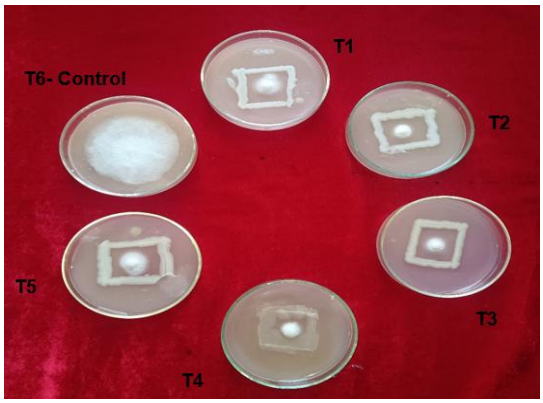

Plate 2: Efficacy of isolates of *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *ciceri*

3.3. Dual culture of *Bacillus subtilis* against *Sclerotium rolfsii*

The data presented in Table 3 and Fig 3 indicated that there was a significant difference among the five *Bacillus subtilis* isolates in inhibiting the growth of *Sclerotium rolfsii*. At 3 days after inoculation (DAI), the minimum mycelial growth of the pathogen was recorded in T_3 (10.50 mm), followed by T_4 (11.50 mm) and T_2 (13.25 mm). At 5 DAI, treatment T_3 again exhibited the lowest mycelial growth (13.75 mm), followed by T_4 (14.25 mm) and T_5 (17.50 mm). By 7 DAI, T_3 showed the minimum mycelial growth (17.50 mm), followed by T_4 (18.00 mm) and T_2 (18.50 mm). In contrast, the maximum mycelial growth of *S. rolfsii* was consistently recorded in the control treatment throughout all intervals. With respect to percent inhibition over control, treatment T_3 recorded the highest inhibition at 7 DAI (79.94%), followed closely by T_4 (80.52%) and T_5 (76.50%). All B. subtilis isolates significantly inhibited the mycelial growth of S. rolfsii, with T3 and T4 being most effective. These results agree with earlier findings (Gopalakrishnan *et al*., 2014) [9] highlighting the antagonistic potential of *B. subtilis* through antifungal metabolites, competition and mycoparasitism, confirming its promise as a biocontrol agent against collar rot.

Table 3: Antagonistic effect of *Bacillus subtilis* isolates against *Sclerotium rolfsii* by dual culture method

Treatments	Antagonistic effect of *Bacillus subtilis* isolates against *Sclerotium rolfsii*					
	3 DAI		5 DAI		7 DAI	
	Mycelial growth of *Sclerotium rolfsii* (mm)	Percent inhibition over control (%)	Mycelial growth *Sclerotium rolfsii.* (mm)	Percent inhibition over control (%)	Mycelial growth of *Sclerotium rolfsii* (mm)	Percent inhibition over control (%)
T_1	15.00	46.43	18.50	60.64	22.00	74.79
T_2	13.25	52.68	15.00	68.09	18.50	78.80
T_3	10.50	62.50	13.75	70.74	17.50	79.94
T_4	11.50	58.93	14.25	69.68	17.00	80.52
T_5	14.50	48.21	17.50	62.77	20.50	76.50
T_6 - Control	28.00	-	47.00	-	87.25	-
F test	Sig	-	Sig	-	Sig	-
SE (m ±)	0.34	-	0.33	-	0.37	-
CD (P = 0.01)	1.36	-	1.34	-	1.49	-

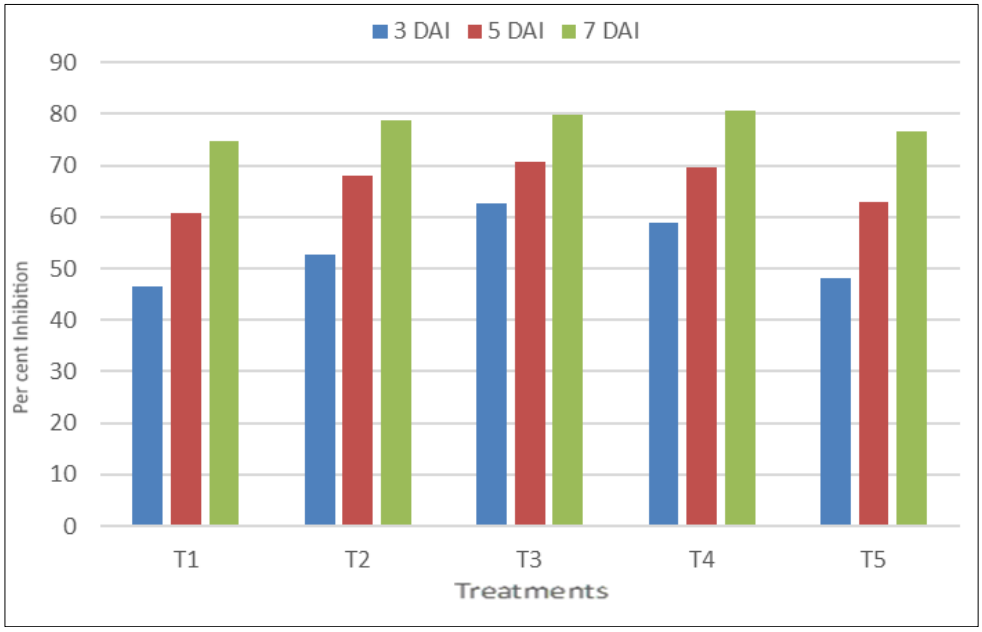

Fig 3: Per cent growth inhibition of *Sclerotium rolfsii* by isolates of *Bacillus subtilis*

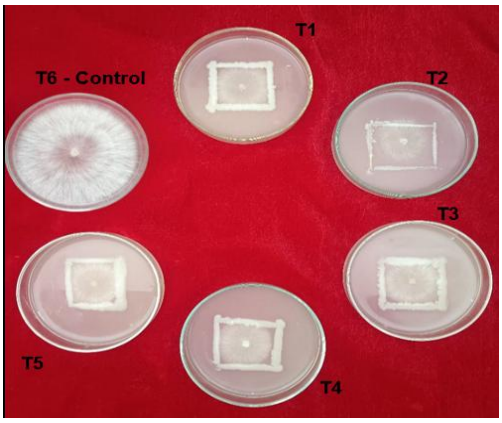

Plate 3: Efficacy of isolates of *Bacillus subtilis* against *Sclerotium rolfsii*

3.4. Dual culture of *Bacillus subtilis* against *Rhizoctonia bataticola*

The data presented in Table 4 and Fig 4 indicated that there was a significant difference among the five *Bacillus subtilis* isolates in inhibiting the growth of *Rhizoctonia bataticola*. At 3 days after inoculation (DAI), the minimum mycelial growth of the pathogen was recorded in T_4 and T_5 (5.00 mm each), followed by T_3 (8.50 mm). At 5 DAI, the lowest mycelial growth was observed in T_5 (7.50 mm), followed by T_4 (8.50 mm) and T_3 (10.50 mm). By 7 DAI, T_5 exhibited the minimum mycelial growth (10.50 mm), followed by T_4 (11.00 mm) and T_3 (11.50 mm). In contrast, the maximum mycelial growth of *R. bataticola* was consistently observed in the control treatment across all observation periods. With respect to percent inhibition over control, treatment T_5 demonstrated the highest inhibition at 7 DAI (88.25%), followed closely by T_4 (87.69%) and T_3 (86.84%). All *B. subtilis* isolates significantly inhibited the mycelial growth of *R. bataticola*, with T_5 and T_4 showing the strongest antagonism. These results are consistent with earlier reports (Meena *et al*., 2015) [10] highlighting the role of *B. subtilis* in suppressing dry root rot through rhizosphere colonization and production of antifungal metabolites.

Table 4: Antagonistic effect of *Bacillus subtilis* isolates against *Rhizoctonia bataticola* by dual culture method

Treatments	Antagonistic effect of *Bacillus subtilis* isolates against *Rhizoctonia bataticola*					
	3 DAI		5 DAI		7 DAI	
	Mycelial growth of *Rhizoctonia bataticola* (mm)	Percent inhibition over control (%)	Mycelial growth of *Rhizoctonia bataticola* (mm)	Percent inhibition over control (%)	Mycelial growth of *Rhizoctonia bataticola* (mm)	Percent inhibition over control (%)
T_1	23.75	15.18	37.50	16.67	76.75	12.04
T_2	25.00	10.71	40.00	11.11	78.50	10.03
T_3	8.50	69.64	10.50	76.67	11.50	86.84
T_4	5.00	82.14	8.50	81.11	10.75	87.69
T_5	5.00	82.14	7.50	83.33	10.25	88.25
T_6 - Control	28.00	-	45.00	-	87.25	-
F test	Sig	-	Sig	-	Sig	-
SE (m ±)	0.40	-	0.37	-	0.42	-
CD (P = 0.01)	1.62	-	1.51	-	1.69	-

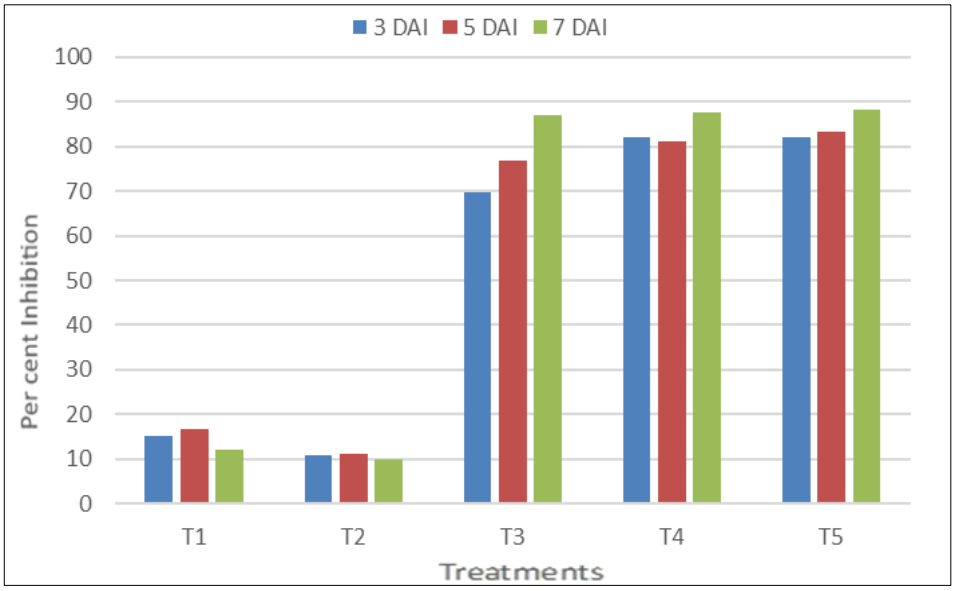

Fig 4: Per cent growth inhibition of *Rhizoctonia bataticola* by isolates of *Bacillus subtilis*

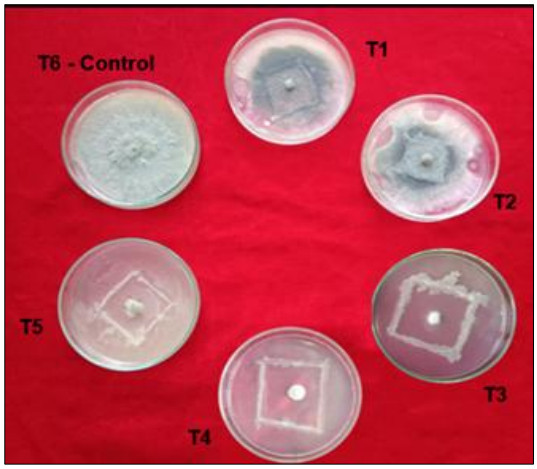

Plate 4; Efficacy of isolates of *Bacillus subtilis* against *Rhizoctonia bataticola*

Conclusion

All *Bacillus subtilis* isolates exhibited significant antagonism against major soil-borne pathogens, with specific isolates showing superior activity. T_4 and T_3 against *F. oxysporum* f. sp. *capsici*, T_2 and T_4 against *F. oxysporum* f. sp. *ciceri*, T_3 and T_4 against *S. rolfsii*, and T_5 and T_4 against *R. bataticola*. The consistent inhibition across pathogens confirms the strong biocontrol potential of *B. subtilis*, mediated through mechanisms such as antifungal metabolite production, competition, and rhizosphere colonization.

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