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Effect of seed treatment with biocontrol agents, organic amendments and fungicide on seedling emergence, pre and post emergence mortality and growth parameters of Safflower

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ABSTRACT

The effect of seed treatment with bioagents, organic amendments, fungicide and their combinations on seedling emergence, pre-emergence, post-emergence mortality and growth parameters of safflower conducted in glass house conditions at Department of Plant Pathology, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad. The dominant pathogen, which causes Fusarium wilt of safflower, was isolated and identified as Fusarium oxysporum f. sp. carthami commercial isolates of Trichoderma harzianum and Pseudomonas fluorescens were used in this study and isolates supplied by Bio fertilizer unit, PJTSAU, Hyderabad. Under in vitro conditions, the results revealed that among all the treatments tested, T_{11}-combination treatment of (T_1 + T_2 + T_3 + T_4 (seed inoculation with test pathogen followed by seed treatment with Trichoderma harzianum (10 g kg^{-1} seed) + T_2 (seed treatment with Pseudomonas fluorescens (10 g kg^{-1} seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1} seed) + T_4(soil application with neem cake (10 g kg^{-1} soil) was found superior in all the parameters discussed. When tested for growth parameters, shoot length was recorded highest in T_{11} (59.2 cm), root length was recorded highest in T_6 (10.0 cm) and total length was recorded highest in T_{11} (68.2 cm). Highest fresh weight (272.10 g) and dry weight (105.35 g) was also recorded from the same combination treatment.

Keywords: *Trichoderma harzianum, Pseudomonas fluorescens, carbendazim, neem cake, in vitro and safflower.*

Introduction

Despite the rapid spread of the crop, a disheartening trend is that productivity has reduced in recent years. In India, safflower production and productivity was 44 MT and 843 kg/ha during 2019-2020 (INDIASTAT, 2019-20). Several diseases are known to cause yield loss in safflower and many of these diseases are seed-borne *viz.*, Alternaria leaf blight (*Alternaria carthami*), Rust (*Puccinia carthami*) and Fusarium wilt (*Fusarium oxysporum* f. sp. *carthami*). Wilt of safflower caused by *Fusarium oxysporum* f. sp. *carthami* Klisiewicz and Houston, 1963 (*Foc*) has been assumed to increase economic importance in recent years with high incidence of the disease being reported in India than the other areas under the crop in the world. In oilseed crops the seed mycoflora is known to affect the quality and quantity of oil (Vidyasekaran *et al.,* 1972). Safflower seed dressing with fungicides significantly increases germination per cent and reduces pre and post-emergence mortality. Fungicides are effective against the Fusarium *in vitro* but they are less effective under *in vivo* conditions. For obtaining effective results of fungicides under *in vitro,* an increase in dose is not advocated because excessive use of chemicals is not eco-friendly and excess use of fungicides may develop fungicide resistant strain. Therefore, more environmentally benign friendly solutions are advocated in combination with bio-agents against the Fusarium wilt. The effect of antagonists on reduction of wilt disease in safflower has been reported, However, consistency in reduction of wilt in field is not seen. Therefore, it is necessary to identify an effective naturally occurring antagonist for sustainable management of the disease.

Prasad (2003) studied the efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *carthami*, the incitant of safflower wilt under glass house conditions and found that *T. viride* as soil application recorded less disease incidence (26.6 %) compared to seed treatment (46.6%), while seed treatment with Carbendazim recorded 80 per cent disease incidence as compared to 93.3 per cent in control.

Khan *et al*. (2004) reported that chickpea seed treatment (cv. BG-256) with commercial formulation (2g/ kg seed) of *T. harzianum* and *Pseudomonas fluorescence*, singly and in combination, significantly reduced the wilt incidence and *T. harzianum* was reported as most effective biocontrol agent. Waghmare *et al*. (2008) studied the antagonistic effect of Trichoderma spp. *viz., T. harzianum, T. viride, T. lignorum, T. konigii* and *T. hamatum* against *Fusarium oxysporum* f. sp. *carthami.* They reported that *T. harzianum* gave maximum control of most of the isolates followed by *T. hamatum, T. viride* and *T. lignorum.* Zote *et al*. (2007) reported that seed treatment with Carbendazim (0.2%) or Captan (0.2%) or *T. viride* @ 4g/kg) or soil application of Neem cake (@ l0g/kg soil) reduced the wilt incidence and increased germination.

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Material and Methods

The experiment was conducted in glass house conditions. Healthy seeds of safflower were surface sterilized and artificially inoculated with the test pathogen by rolling the seeds in 10 days old sporulating culture grown on PDA. The inoculated seeds were kept for 8 h in Petri plates having moistened blotter papers. After incubation, inoculated seeds were treated separately by coating with bio control agents, and organic amendments and were then sown in pots (25 cm diameter) filled with sterilized soil. Observations on pre-emergence mortality, post-emergence mortality, per cent seedling emergence and plant growth parameters were recorded.

Treatment Details

Design: CRD Replications: 4

Treatments: 12 Variety- PBNS-1

Table-3.3 Effect of seed treatment with biocontrol agents, organic amendments and fungicide on per cent seedling emergence, pre and post emergence mortality and growth parameters of Safflower

Treatments	Treatment Particulars
T_1	Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)
T_2	Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)
T_3	Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed)
T_4	Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)
T_5	T_1 + T_2 (T_1(Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)
T_6	T_1 + T_3 (T_1 (Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_3 (Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed)
T_7	T_2 + T_3 (T_2 (Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed + T3 (Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed)
T_8	T_3 + T_4 (T_3 (Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed) + T_4 (Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)
T_9	T_1 + T_4 (T_1 (Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) +T_4 (Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)
T_{10}	T_2 + T_4 (T_2(Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) + T_4 (Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)
T_{11}	T_1 + T_2 +T_3 + T_4 (Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) +T_2 (Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) + T_3 (Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed) + T_4 (Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)
T_{12}	Inoculated control

Results and Discussion

Glass house Experiment

Effect of seed treatment with biocontrol agents, organic amendments and fungicide on per cent seedling emergence, pre-emergence mortality and post-emergence mortality, Root and Shoot Length, fresh and dry weight of Safflower cv. PBNS-12 was studied in pot culture on safflower cv. PBNS-12 under glass house conditions.

1. Effect of seed treatment of bioagents, organic amendments and carbendazim on seedling emergence of safflower cv. PBNS-12 under glass house conditions

The results of experiments presented in (table 1) revealed that seedling emergence was significantly improved in all the treatments compared to the control (55.0 %). Seed treatment with combined treatment of T_{11} (T_1 + T_2 + T_3 + T_4 (T_1 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg^{-1}seed)) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) + T_4 (soil application with neem cake (10 g kg^{-1} soil) gave maximum seedling emergence (100%) followed by T_6 (T_1 + T_3 (T_1 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg^{-1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) recorded (95.0 %), T_5 (T_1+ T_2(T_1 (seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed), T_7 (T_2 + T_3(T_2 (seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg^{-1}seed) + T3 (seed treatment with carbendazim (1 g kg^{-1}seed) and T_3 (seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg^{-1}seed) recorded (80.0%) when compared to 55.0 per cent recorded in control.

2. Effect of seed treatment of bioagents, organic amendments and carbendazim on pre-emergence mortality of safflower cv. PBNS-12 under glass house conditions

The effect of seed treatment with biocontrol agents, organic

amendments and fungicide on pre-emergence mortality of safflower cv. PBNS-12 in *Fusarium oxysporum* f. sp. *carthami* was studied in pots and results indicated that all the seed treatments were significantly superior in reducing the pre-emergence mortality when compared to control (65.0%) (table 2). However seed treatment with T_{11} (T_1 + T_2 + T_3 + T_4 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg^{-1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg^{-1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) + T_4(soil application with neem cake (10 g kg^{-1}soil) minimum per cent of pre emergence mortality (10%) followed by T_6 (T_1 + T_3 (T_1 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) and T_5 (T_1 + T_2 (T_1(seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) recorded (20.0%).

3. Effect of seed treatment of bioagents, organic amendments and carbendazim on post-emergence mortality of safflower cv. PBNS-12 under glass house conditions

The effect of seed treatment with bio-control agents, organic amendments and fungicide on post-emergence mortality of safflower cv. PBNS-12 in *Fusarium oxysporum* f. sp. *carthami* was studied in pots and results indicated that all the seed treatments were significantly superior in reducing the post-emergence mortality when compared to control (75.0 %) table 3. However seed treatment with T_{11} (T_1 +T_2 +T_3 + T_4 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg^{-1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg^{-1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) + T_4(soil application with neem cake (10 g kg^{-1}soil) and T_6 (T_1 + T_3 (T_1 (Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) minimum per cent of post emergence mortality (15 %) followed by T_5 (T_1 + T_2 (T_1(seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) recorded (25.0 %).

4. Effect of Biocontrol Agents, Organic Amendments and Fungicide on Shoot Length of Safflower cv. PBNS-12 Under Glass House Conditions

It is obvious from table 4 revealed that all the treatments were found to be effective in increasing the shoot length of safflower. The increase in plant biometrics (shoot length) ranges from 50.0 to 59.2 cm. Combined seed treatment of T_{11} (T_1 + T_2 + T_3 + T_4 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg^{-1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg^{-1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) + T_4 (soil application with neem cake (10 g kg^{-1}soil) recorded the maximum shoot length of 59.25 cm and followed by seed treatment with T_5 (T_1 + T_2 (T_1 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)recorded (55.5 cm) and T_1 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) were recorded (54.5 cm).

While the treatment T_4 (seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil) recorded the minimum shoot length of 48.5 cm.

5. Effect of Biocontrol Agents, Organic Amendments and Fungicide on Root Length of Safflower cv. PBNS-12 Under Glass House Conditions

The data presented in table 4 revealed that all the treatments were found effective in increasing the root length of safflower. The increase in plant biometrics (root length) ranges from 6.5 to 10.0 cm. Combined seed treatment of T_6 (T_1 + T_3 (T_1 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) recorded (10.0 cm) followed by seed treatment with T_{11} (T_1 + T_2 + T_3 + T_4 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg^{-1}seed) +T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg^{-1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) + T_4(soil application with neem cake (10 g kg^{-1}soil)recorded (9.0 cm) and T_9 (T_1 + T_4 (T_1 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_4 (soil application with neem cake (10 g kg-^{1}soil) recorded (8.7 cm). While the treatment T_2 (seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) and T_3 (seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed) recorded the minimum root length of 6.5 cm.

6. Effect of Biocontrol agents, Organic Amendments and Fungicides on Root and Shoot Length of Safflower cv. PBNS-12 Under Glass House Conditions

The total length also increased significantly over the control (table 4). The treatment T_{11} (T_1 + T_2 + T_3 + T_4 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg^{-1}seed) +T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg^{-1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) + T_4(soil application with neem cake (10 g kg^{-1}soil) recorded with 68.25 cm followed by T_5 (T_1+ T_2 (T_1(seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) recorded 64.0 cm respectively when compared to control (51.5 cm). While the treatment T_4 (seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil) recorded the minimum total length of 55.7 cm.

7. Effect of Biocontrol Agents, Organic Amendments and Fungicide on Fresh Weight of Safflower cv. PBNS-12 Under Glass House Conditions

The data presented in (table 5) revealed that all the treatments were found to be effective in increasing the fresh weight of safflower. The increase in plant biometrics (fresh weight) ranges from 176.20 to 272.10 g plant-^{1}. Combined seed treatment of T_{11} (T_1 + T_2 + T_3 + T_4 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg^{-1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg^{-1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) + T_4(soil application with neem cake (10 g kg^{-1}soil) recorded (272.10 g plant-^{1}) followed by seed treatment with T_6 (T_1 + T_3 (T_1 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed)) recorded (267.65 g plant-^{1}).

While the treatment T_4 (seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil) recorded the minimum fresh weight of 220.75 g plant-^{1}.

7.a. Effect of Biocontrol agents, Organic Amendments and Fungicide on Dry Weight of Safflower cv. PBNS-12 Under Glass House Conditions

The data presented in table 5 revealed that all the treatments were found to be effective in increasing the dry weight of safflower. The increase in plant biometrics (dry weight) ranges from 75.83 to 105.35 g plant-^{1}. Combined seed treatment of T_{11} (T_1+ T_2+ T_3+ T_4 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg^{-1}seed) + T_2 (seed treatment with *Pseudomonas fluorescens* (10 g kg^{-1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed) + T_4 (soil application with neem cake (10 g kg^{-1}soil) maximum dry weight recorded (105.35 g plant-^{1}) followed by seed treatment with T_6 (T_1 + T_3 (T_1 (seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_3 (seed treatment with carbendazim (1 g kg^{-1}seed)) recorded (98.65 g plant-^{1}). While the treatment T_2 (seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) recorded the minimum dry weight of 75.83 g plant-^{1}.

Increased seedling emergence with the integration of *Trichoderma* sp., *P. fluorescens* botanical formulations and fungicides was also reported by earlier workers. Manjula *et al*. (2004) reported that combined treatment with *P. fluorescens* and *T.viride* improved seedling emergence and decreased the seedling mortality of ground nut.

The beneficial effect of seed treatments with bioagents and fungicides in minimising the pre-emergence mortality is following Chakrabarti and Rao (1992) in maize and Govindappa *et al*. (2011) in safflower.

Similar findings reported Raju *et al*. (2003) studied the effect of biocontrol agents against *Fusarium oxysporum* f. sp. *carthami* causing wilt of safflower and reported that seed treatment with thiram + *T. harzianum* + *T. viride* completely inhibited the disease which was on par with seed treatment with *T. viride*, *T. harzianum* and carbendazim.

Similarly Prameela *et al.* (2005) reported maximum mycelial inhibition of *Fusarium oxysporum* f. sp. *carthami* to the tune of 62, 39 and 36 per cent was recorded by bioagents *T. viride, T. harzianum* and *P. fluorescens,* respectively.

Similar observations were reported by Rajeswari *et al*. (2012) studied seed mycoflora associated with safflower seed and evaluated the efficacy of seed treatments with bioagents and fungicides. Results indicated that seed treatments with bioagents (6 g/kg), fungicides and botanicals (10 ml/kg) enhanced seedling quality and were found to be effective in the reduction of total seed mycoflora and seedling mortality.

Combined seed treatment with *T. viride* and *B. subtilis* resulted in increased fresh and dry weight of shoots, roots and nodules of broad bean apart from controlling infection by *F. solani* (Yehia *et al*. 1982).While the results of treatments T_6 (combined seed treatment of ThM_1+ *P. fluorescens*) is in agreement with Jensen *et al*. (2002) who reported that *B. subtilis* in combination with *T. harzianum* when given as a seed treatment, resulted in increased biomass of dry apart from decreasing the severity of *Fusarium oxysporum* f. sp. *phaseoli* infection.

Among the combined seed treatment, the results obtained in treatment T_{10} where *P. fluorescens* was applied to soil are in agreement with Hoflich *et al,* (1994) who reported that *P. fluorescence* increased root length, lateral root development, shoot and root dry matter and seed yield of winter wheat against soil-borne root pathogen *i.e. Fusarium solani*. Application of *P. aeruginosa* to soil resulted in growth promotion of chilli seedlings in addition to suppressing the root infection by *F. solani, R. solani* (Siddiqui and Ehteshamul Haque, (2001).

The results obtained in the treatment T_{11} were in agreement with Jayaraj and Radha Krishanan (2003) who also reported that seed treatment with carbendazim followed by *T. harzianum* resulted in increased plant biomass apart from reducing the infection by *R. solani.*

Summary and conclusions

Susceptible variety PBNS-12 was used to find the effect of seed treatment with bioagents, organic amendments and carbendazim and their combinations on percent seedling emergence, pre-emergence and post emergence mortality in glass house conditions. Among all the treatments tested, T_{11}-combination treatment of *Trichoderma harzianum* + *Pseudomonas fluorescens* + Carbendazim was found superior for all the parameters discussed *i.e.* 100 per cent seedling emergence, 10 per cent pre emergence mortality and 15 per cent post emergence mortality. When tested for growth parameters, highest shoot length (59.25cm), root length (9.0 cm), total length (68.25cm), fresh weight (272.10 g) and dry weight (105.35) was also recorded from the same combination treatment.

Table 1. Effect of seed treatment of bioagents, organic amendments and carbendazim on seedling emergence of safflower cv. PBNS-12 under glass house conditions

Treatments	Particulars	Seedling emergence (%)
T_1	Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)	70.0 (57.0)
T_2	Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	60.0 (50.7)
T_3	Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed)	80.0 (63.4)
T_4	Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)	60.0 (50.7)
T_5	T1+T_2 (T_1(Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	80.0 (63.4)
T_6	T1+T_3 (T_1 (Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)) + T_3 (Seed treatment with carbendazim (1 g kg-1seed)	95.0 (83.3)

T_7	T_2+T_3 (T_2 (Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed + T_3 (Seed treatment with carbendazim (1 g kg-1seed)	80.0 (63.4)
T_8	T_3+T_4 (T_3 (Seed inoculation with test pathogen followed by Seed treatment with carbendazim (1 g kg-^{1}seed) + T4 (Soil application with neem cake (10 g kg-^{1}soil)	70.0 (57.0)
T_9	T_1+T_4 (T_1 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) +T_4 (Soil application with neem cake (10 g kg-^{1}soil)	75.0 (60.2)
T_{10}	T_2+T_4 (T_2(Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) + T_4 (Soil application with neem cake (10 g kg-^{1}soil)	70.0 (57.0)
T_{11}	T_1+T_2+T_3+T_4 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-1seed) + T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed) + T_3 (Seed treatment with carbendazim (1 g kg-1seed) + T_4 (Soil application with neem cake (10 g kg-1soil)	100.0 (90.0)
T_{12}	Inoculated control	55.0 (47.8)
SE(m)±		2.92
C.D 5%		8.42

**Mean of four replications*
*** Figures in parenthesis are angular transformed value*

Table 2. Effect of seed treatment of bioagents, organic amendments and carbendazim on pre-emergence mortality of safflower cv. PBNS-12 under glass house conditions

Treatments	Particulars	Pre emergence Mortality (%)
T_1	Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)	30.0 (32.8)
T_2	Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	40.0 (37.7)
T_3	Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed)	30.0 (32.8)
T_4	Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)	35.0 (34.5)
T_5	T_1+T_2 (T_1(Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	20.0 (38.4)
T_6	T1+T_3 (T_1 (Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)) + T_3 (Seed treatment with carbendazim (1 g kg-1seed)	20.0 (23.0)
T_7	T_2+T_3 (T_2 (Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed + T_3 (Seed treatment with carbendazim (1 g kg-1seed)	25.0 (19.9)
T_8	T_3+T_4 (T_3 (Seed inoculation with test pathogen followed by Seed treatment with carbendazim (1 g kg-^{1}seed) + T4 (Soil application with neem cake (10 g kg-^{1}soil)	30.0 (32.8)
T_9	T_1+T_4 (T_1 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) +T_4 (Soil application with neem cake (10 g kg-^{1}soil)	35.0 (27.4)
T_{10}	T_2+T_4 (T_2(Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) + T_4 (Soil application with neem cake (10 g kg-^{1}soil)	30.0 (34.5)
T_{11}	T_1+T_2+T_3+T_4 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-1seed) + T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed) + T_3 (Seed treatment with carbendazim (1 g kg-1seed) + T_4 (Soil application with neem cake (10 g kg-1soil)	10.0 (36.0)
T_{12}	Inoculated control	65.0 (38.4)
SE(m)±		3.92
C.D 5%		11.3

**Mean of four replications*
*** Figures in parenthesis are angular transformed values*

Table 3. Effect of seed treatment of bioagents, organic amendments and carbendazim on post-emergence mortality of safflower cv. PBNS-12 under glass house conditions

Treatments	Particulars	Post emergence mortality (%)
T_1	Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)	35.0 (36.0)
T_2	Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	40.0 (39.2)
T_3	Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed)	30.0 (32.8)
T_4	Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)	45.0 (42.1)
T_5	T_1+T_2 (T_1(Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)+ T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	25.0 (29.7)
T_6	T_1+T_3 (T_1 (Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)) + T_3 (Seed treatment with carbendazim (1 g kg-1seed)	15.0 (19.9)
T_7	T_2+T_3 (T_2 (Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed + T_3 (Seed treatment with carbendazim (1 g kg-1seed)	35.0 (36.0)
T_8	T_3+T_4 (T_3 (Seed inoculation with test pathogen followed by Seed treatment with carbendazim (1 g kg-^{1}seed) + T4 (Soil application with neem cake (10 g kg-^{1}soil)	30.0 (32.8)
T_9	T_1+T_4 (T_1 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) +T_4 (Soil application with neem cake (10 g kg-^{1}soil)	40.0 (39.2)
T_{10}	T_2+T_4 (T_2(Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) + T_4 (Soil application with neem cake (10 g kg-^{1}soil)	45.0 (41.8)
T_{11}	T_1+T_2+T_3+T_4 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-1seed) + T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed) + T_3 (Seed treatment with carbendazim (1 g kg-1seed) + T_4 (Soil application with neem cake (10 g kg-1soil)	15.0 (19.9)
T_{12}	Inoculated control	75.0 (60.0)
SE(m)±		3.98
C.D 5%		11.4

**Mean of four replications*
*** Figures in parenthesis are angular transformed values*

Table 4 Effect of seed treatment of bioagents, organic amendments and carbendazim on growth parameters of safflower cv. PBNS-12 under glass house conditions

Treatments	Particulars	Growth parameters		
		Shoot length (cm)	Root length (cm)	Root and shoot length (cm)
T_1	Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)	54.5	6.7	61.5
T_2	Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	50.0	6.5	56.5
T_3	Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed)	53.5	6.5	60.0
T_4	Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)	48.5	7.2	55.7
T_5	T_1+T_2 (T_1(Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	55.5	8.5	64.0

T_6	T_1+T_3 (T_1 (Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)) + T_3 (Seed treatment with carbendazim (1 g kg-1seed)	52.0	10.0	62.0
T_7	T_2+T_3 (T_2 (Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed + T3 (Seed treatment with carbendazim (1 g kg-1seed)	52.5	7.5	60.0
T_8	T_3+T_4 (T_3 (Seed inoculation with test pathogen followed by Seed treatment with carbendazim (1 g kg-^{1}seed) + T4 (Soil application with neem cake (10 g kg-^{1}soil)	50.0	7.0	57.0
T_9	T_1+T_4 (T_1 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) +T_4 (Soil application with neem cake (10 g kg-^{1}soil)	50.5	8.7	59.2
T_{10}	T_2+T_4 (T_2(Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) + T_4 (Soil application with neem cake (10 g kg-^{1}soil)	50.75	7.5	56.7
T_{11}	T_1+T_2+T_3+T_4 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-1seed) + T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed) + T_3 (Seed treatment with carbendazim (1 g kg-1seed) + T_4 (Soil application with neem cake (10 g kg-1soil)	59.25	9.0	68.2
T_{12}	Inoculated control	43.5	6.25	51.5
SE(m) ±		1.0	0.26	
C.D 5%		2.8	0.75	

Table 5. Effect of seed treatment of bioagents, organic amendments and carbendazim on growth parameters of safflower cv. PBNS-12 under glass house conditions

Treatments	**Particulars**	**Growth parameters**	
		Fresh weight (g plant^{-1})	**Dry weight (g plant^{-1})**
T_1	Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)	236.23	83.73
T_2	Seed inoculation with test pathogen followed by seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	225.95	75.83
T_3	Seed inoculation with test pathogen followed by seed treatment with carbendazim (1 g kg-^{1}seed)	236.43	86.70
T_4	Seed inoculation with test pathogen followed by soil application with neem cake (10 g kg-^{1}soil)	220.75	77.25
T_5	T1+T_2 (T_1(Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) + T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed)	253.83	93.88
T_6	T1+T_3 (T_1 (Seed inoculation with test pathogen followed by seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed)) + T_3 (Seed treatment with carbendazim (1 g kg-1seed)	267.65	98.65
T_7	T_2+T_3 (T_2 (Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed + T_3 (Seed treatment with carbendazim (1 g kg-1seed)	244.05	85.50
T_8	T_3+T_4 (T_3 (Seed inoculation with test pathogen followed by Seed treatment with carbendazim (1 g kg-^{1}seed) + T4 (Soil application with neem cake (10 g kg-^{1}soil)	244.20	96.25
T_9	T_1+T_4 (T_1 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-^{1}seed) +T_4 (Soil application with neem cake (10 g kg-^{1}soil)	234.23	87.88
T_{10}	T_2+T_4 (T_2(Seed inoculation with test pathogen followed by Seed treatment with *Pseudomonas fluorescens* (10 g kg-^{1}seed) + T_4 (Soil application with neem cake (10 g kg-^{1}soil)	242.73	81.98

T_{11}	T_1+T_2+T_3+T_4 (Seed inoculation with test pathogen followed by Seed treatment with *Trichoderma harzianum* (10 g kg-1seed) + T_2 (Seed treatment with *Pseudomonas fluorescens* (10 g kg-1seed) + T_3 (Seed treatment with carbendazim (1 g kg-1seed) + T_4 (Soil application with neem cake (10 g kg-1soil)	272.10	105.35
T_{12}	Inoculated control	176.20	70.18
SE(m) ±		1.74	0.99
C.D 5%		5.02	2.85

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Conflict of interest: No Conflict of interest

The future scope of the study: Management of biocontrol agents and organic amendments against safflower wilt caused by *Fusarium oxysporum* f.sp *carthami* under field conditions.

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