

Original Research Article

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Impacts of Indigenous *Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens* on Microbial Population in Soil, Plant Growth Promoting and Disease Control Potential in Soybean

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ABSTRACT

Keywords

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Soybean (*Glycine max* L. Merrill) is an important leguminous crop contain huge amount of protein and oil. Among the fungal diseases of soybean, collar rot caused by *Sclerotium rolfsii* and charcoal rot caused by *Corynespora cassicola* are the economically important diseases which attack on root, stem and foliate parts of the plants. An experiment was conducted on efficacy of indigenous *Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens* on microbial population in soil, plant growth promoting and disease control potential in soybean and results indicated that the highest impact of *Pseudomonas fluorescens* was recorded on microbial population in soil which showed 26.61% increase in fungi population and 25.97 % increase in bacteria population followed by *T. viride* (18.20 and 18.07%) and *T. harzianum* (7.92 and 7.90%). Minimum mortality (1.97%) was recorded with the application of T₁₀ – Seed treatment with T₁₈ @ 20% followed two foliar sprays followed by T₉ – Seed treatment with T₁₈ @ 15% followed two foliar sprays (2.36%). In case target leaf spot minimum incidence (73.54%), PDI (10.93%) was recorded with the application of T₁₄ – Seed treatment with *Pseudomonas fluorescens* @ 15% followed T₁₅ – Seed treatment with *Pseudomonas fluorescens* @ 20% followed two foliar sprays (9.81%). Maximum no. of pods per plant (73.30), yield per plot (4.274 kg) and yield per ha (27.40 q) was obtained from treatment T₁₅ – Seed treatment with *Pseudomonas fluorescens* @ 20% followed two foliar sprays followed by T₁₀ – Seed treatment with T₁₈ @ 20% followed two foliar sprays.

Introduction

Soybean (*Glycine max* L. Merrill) is an important leguminous crop contain huge amount of protein and oil. In India, soybean is

grown in rainfed area of Madhya Pradesh, Maharashtra, Rajasthan, Chhattisgarh, Gujarat, Karnataka, Telangana, Uttar Pradesh and Uttarakhand states. Soybean has been sown on an area of 109.34 lakh in India

during 2017-18 and total production of soybean was obtained 12.22 million tonnes during 2017-18 (Anonymous, 2018). In Chhattisgarh, soybean has grown in 1.320 lakh ha area which gives 0.863 lakh tonnes production during 2017-18 (Anonymous 2017; Sharma and Patel 2017). Both area and production of soybean has been decreased during 2017-18 as compared to 2016-17 due to the low productivity during previous years. The production and productivity of soybean are extremely influence by time and pattern of rainfall in Chhattisgarh state resulting cultivation of soybean has been discouraged in Chhattisgarh. Main reasons of low productivity are less rainfall during sowing and crop period, high rainfall at the maturity to harvesting time, attack of several diseases during flowering to maturity stage. In Chhattisgarh farmers are diverted to cultivate other crops such as pigeonpea, urdbean, mungbean etc due to the low productivity and also received very less profit from soybean crop. In Chhattisgarh, significant yield losses have been observed in soybean crop due to occurrence of major diseases such as rust (*Phakopsora pachyrhizi*), yellow mosaic disease (MYMV), bacterial pustule (*Xanthomonas campestris* pv. *glycines*), collar rot (*Sclerotium rolfsii*), target leaf spot (*Corynespora cassiicola*), charcoal rot (*Macrophomina phaseolina*) and leaf spot (*Myrothecium roridum*). Among them, all fungal diseases are soil borne except rust. Collar rot and charcoal rot are the economically important diseases which attack the root, stem and foliate parts of the plants. The target leaf spot disease of soybean caused by *Corynespora cassiicola* was first reported in 1945 (Olive *et al.*, 1945). *C. cassiicola* is found on or within 530 plant species from 380 genera including dicot, monocot, fern and cycad hosts and acts as a pathogen, saprophyte or endophyte (Smith, 2008). *C. cassiicola* have been reported on 68 different plant species causing infection on leaves,

stem, and roots of plant (Dixon *et al.*, 2009). Target leaf spot has become a major problem in all most of soybean growing states including Chhattisgarh (Patel, 2005).

Collar rot disease caused by *Sclerotium rolfsii* is one of the most destructive soil borne disease of soybean which causes 30-40% yield losses in India (Debbarma *et al.*, 2017). *Sclerotium rolfsii* is a polyphagous fungus which has wide range of hosts. Collar rot disease can only be controlled with the seed and soil treatment with fungicides and biocontrol agents whereas, target leaf spot can be controlled by chemical fungicides and biocontrol agents applied as seed, soil and foliage treatments. Several finding concluded that both the diseases can be controlled with the seed and foliar application of bioagents. The application of microorganisms as agent for biocontrol of plant diseases in agriculture is now considered an important alternative to the use of chemical fungicides. *Pseudomonas fluorescens* have capability to effectively control fungal pathogens such as *Fusarium oxysporium*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* (Ganesan and Gnanamanickam, 1987). All root and foliar diseases can also be minimized by the seed treatment with commercial formulation of *Trichoderma harzianum* or *T. viride* or *Pseudomonas fluorescens* (Kumar *et al.*, 2015) and foliar application of *Trichoderma harzianum* or *T. viride* or *Pseudomonas fluorescens* are capable reduced the infection of *Sclerotium rolfsii* and *C. cassiicola* from the foliage in the field without using of recommended chemical fungicides such as Thiram or Carbendazim or Mancozeb or Tebuconazole. Besides, biocontrol measures solve the ecological and economical problem of disease and chemical fungicides in Indian agriculture and also give several eco-friendly innovative approaches for control of diseases. Inherent hazardous effects involved in conventional chemicals management coupled

with the inclination of farmers towards organic farming (Parmar *et al.*, 2018). Collar rot caused by *Sclerotium rolfsii* and target leaf spot caused by *C. cassiicola* are considered as economically important diseases of Soybean. Hence, a study was carried out on “efficacy of indigenous *Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens* on microbial population in soil, plant growth promoting and disease control potential in soybean”.

Materials and Methods

An experiment was conducted to find out the growth promoting activity and disease control efficiency of *Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens* in Soybean at S.K. College of Agriculture and Research Station (IGKV), Kawardha (Kabirdham), Chhattisgarh. Two diseases viz., collar rot and target leaf spot which are more prevalent in this region were targeted to minimize the diseases. Layout was made in Randomized Block Design (RBD) with 16 treatments viz., T₁ – Seed treatment with *Trichoderma harzianum* @ 1% followed two foliar sprays, T₂ – Seed treatment with *T. harzianum* @ 5% followed two foliar sprays, T₃ – Seed treatment with *T. harzianum* @ 10% followed two foliar sprays, T₄ – Seed treatment with *T. harzianum* @ 15% followed two foliar sprays, T₅ – Seed treatment with *T. harzianum* @ 20% followed two foliar sprays, T₆ – Seed treatment with *Trichoderma viride* @ 1% followed two foliar sprays, T₇ – Seed treatment with *T. viride* @ 5% followed two foliar sprays, T₈ – Seed treatment with *T. viride* @ 10% followed two foliar sprays, T₉ – Seed treatment with *T. viride* @ 15% followed two foliar sprays, T₁₀ – Seed treatment with *T. viride* @ 20% followed two foliar sprays, T₁₁ – Seed treatment with *Pseudomonas fluorescens* @ 1% followed two foliar sprays, T₁₂ – Seed treatment with *P. fluorescens* @ 5% followed two foliar sprays,

T₁₃ – Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays, T₁₄ – Seed treatment with *P. fluorescens* @ 15% followed two foliar sprays, T₁₅ – Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays and T₁₆ – Control (Untreated) and three replication. Seeds of variety JS-335 were taken and treated with different formulation of bio-control agents @ 10g/kg seeds one day before sowing as per the treatment details. Treated seeds were sown in plots have net plot size of 4.0M X 3.9 M by maintaining the row to row distance of 30cm and plant to plant 10cm. All the recommended agronomic practices were adopted to maintain the good canopy of the crop. In case of foliar spray, solution of bio-control agents @ 10g/L of water was prepared as per treatments details. First spray was given at 30 days after sowing and second sprays at 45 days after sowing.

Soil samples were collected from each replication of all the treatment before sowing and after harvesting for measurement of microbial populations load in the experimental field. Microbial population was measured using serial dilution and spread method techniques on Potato Dextrose Agar Media. Three Petri dishes were prepared for each sample. Number of bacterial and fungal colony was counted using colony counter at 24, 48, 72 hours after incubation in each replication of all the treatments. Observations were also recorded on plant height (cm), root length (cm), number of branches per plant, number of pods per plant, grain yield. Number of nodules per plant was recorded at the time of flowering. Collar rot incidence was recorded at weekly intervals from seedling to maturity. The observation on occurrence of target leaf spot on soybean leaf was also recorded in all the treatments. The observation of disease severity was recorded on randomly selected five plant of each replication of each treatment. The severity of

Target Leaf spot was recorded on soybean foliage using 0-9 scale described as 0 = No lesions, 1= 0.1 - 1% leaf area covered with lesion, limited only lower canopy, 2=1.1–10% leaf area cover with lesion, limited only lower canopy, 3 =10.1–20% leaf area cover with lesion, limited only lower canopy. 4=20.1–30% of the leaf area covered, spread up to middle canopy, 5=30.1–40 % of the leaf area covered, spread up to middle canopy, no defoliation, 6=40.1–50 % leaf area covered, spread up to middle canopy, few leaves drops, 7=50.1–60 % leaf area covered with lesion, spread up to upper canopy, few leaf drop,, plant damage up to 30%, 8= 60.1–70 % leaf area covered with lesion, spread up to upper canopy, some leaf drop, plant damage 30 to 50%, 9 = More than 70% leaf area covered with lesion, lesion very common on whole foliage of plant, defoliation common, death of plant common, plant damage more than 70%. Percent disease index (PDI) for each treatment was calculated as follows:

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of individual disease rating}}{\text{Total no. of plant examined} \times \text{Maximum no. of disease rating}} \times 100$$

Results and Discussion

Impact on Microbial population

Microbial population in soil have been presented in table 1 reveal that maximum bacterial population ($4.96\text{cfu} \times 10^4 \text{g}^{-1}$) was recorded after harvesting in treatment T_{15} – Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays followed by T_{14} – Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays ($4.81\text{cfu} \times 10^4 \text{g}^{-1}$), T_{10} – Seed treatment with *T. viride* @ 20% followed two foliar sprays ($4.63\text{cfu} \times 10^4 \text{g}^{-1}$), T_{13} – Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays ($4.56\text{cfu} \times 10^4 \text{g}^{-1}$), T_9 – Seed treatment with *T. viride* @ 15% followed two foliar sprays ($4.47\text{cfu} \times 10^4 \text{g}^{-1}$) whereas, bacterial population was $3.62\text{cfu} \times 10^4 \text{g}^{-1}$ before

sowing. In untreated plots fungi and bacteria population was recorded 2.50 and $3.69\text{cfu} \times 10^4 \text{g}^{-1}$, respectively after the harvesting of crop. Highest impact of *Pseudomonas fluorescens* was recorded microbial population in soil which showed 26.61% increase in fungi and 25.97 % increase in bacteria population over before treatment followed by *T. viride* (18.20 and 18.07%) and *T. harzianum* (7.92 and 7.90%) whereas, in control plot increase in fungi population was recorded only 2.40% and increase in bacteria population was 1.93 % (Figure 1).

Impacts on Collar rotdisease

Data pertaining to incidence of collar rot indicated that the minimum collar rot incidence (1.97%) was recorded in treatment T_{10} =Seed treatment with *T. viride* @ 20% followed two foliar sprays which was at par with T_9 =Seed treatment with *T. viride* @ 15% followed two foliar sprays (2.36%), T_5 =Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (2.76%), T_8 =Seed treatment with *T. viride* @ 10% followed two foliar sprays (2.76%) and significantly lower over T_1 =Seed treatment with *T. harzianum* @ 1% followed two foliar sprays (5.12%), T_2 =Seed treatment with *T. harzianum* @ 5% followed two foliar sprays (4.72%), T_3 =Seed treatment with *T. harzianum* @ 10% followed two foliar sprays (4.33%), T_4 =Seed treatment with *T. harzianum* @ 15% followed two foliar sprays (3.74%), T_6 =Seed treatment with *T. viride* @ 1% followed two foliar sprays (3.74%), T_7 =Seed treatment with *T. viride* @ 5% followed two foliar sprays (3.35%), T_{11} =Seed treatment with *P. fluorescens* @ 1% followed two foliar sprays (6.10 %), T_{12} =Seed treatment with *P. fluorescens* @ 5% followed two foliar sprays (5.31%), T_{13} =Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays (5.12%), T_{14} =Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays (4.53%), T_{15} =Seed

treatment with *P. fluorescens* @ 20% followed two foliar sprays (3.74%). In control plot, collar rot incidence was recorded highest (6.89%).

Impacts on Target Leaf spot disease

Minimum target leaf spot disease incidence (68.73%) in treatment T₁₅=Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays which was at par with T₁₄=Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays (73.54%), T₁₃=Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays (77.66%), T₁₀=Seed treatment with *T. viride* @ 20% followed two foliar sprays (71.82%), T₉=Seed treatment with *T. viride* @ 15% followed two foliar sprays (74.57%), T₈=Seed treatment with *T. viride* @ 10% followed two foliar sprays (77.66%), T₅=Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (73.54%) and significantly lower over rest of the treatments (Table 2). In case of PDI, minimum PDI (9.81%) was recorded in treatment T₁₅=Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays followed by T₁₀=Seed treatment with *T. viride* @ 20% followed two foliar sprays (10.00%), T₁₄=Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays (10.93%), T₉=Seed treatment with *T. viride* @ 15% followed two foliar sprays (11.48%) and T₁₃=Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays (12.22%). Treatment T₁₅=Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays showed significantly lower PDI over T₁=Seed treatment with *T. harzianum* @ 1% followed two foliar sprays (17.96%), T₂=Seed treatment with *T. harzianum* @ 5% followed two foliar sprays (16.11%), T₃=Seed treatment with *T. harzianum* @ 10% followed two foliar sprays (15.00%), T₄=Seed treatment with *T. harzianum* @ 15% followed two foliar sprays (13.70%), T₅=Seed treatment with *T.*

harzianum @ 20% followed two foliar sprays (12.78%), T₆=Seed treatment with *T. viride* @ 1% followed two foliar sprays (15.19%), T₇=Seed treatment with *T. viride* @ 5% followed two foliar sprays (14.26%), T₈=Seed treatment with *T. viride* @ 10% followed two foliar sprays (12.96%), T₁₁=Seed treatment with *P. fluorescens* @ 1% followed two foliar sprays (14.63%) and T₁₂=Seed treatment with *P. fluorescens* @ 5% followed two foliar sprays (13.15%) (Table 2).

Impacts on Plant growth parameters

Maximum plant height (81.47CM) was observed in treatment T₁₀=Seed treatment with *T. viride* @ 20% followed two foliar sprays. It was at par with T₃=Seed treatment with *T. harzianum* @ 10% followed two foliar sprays (76.00CM), T₄=Seed treatment with *T. harzianum* @ 15% followed two foliar sprays (77.00CM), T₅=Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (78.86CM), T₈=Seed treatment with *T. viride* @ 10% followed two foliar sprays (75.87CM), T₉=Seed treatment with *T. viride* @ 15% followed two foliar sprays (77.60CM), T₁₃=Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays (76.87CM), T₁₄=Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays (78.40CM), T₁₅=Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays (80.80CM) and significantly superior over rest of the treatment and minimum plant height (72.87CM) was recorded in untreated plot (Table 3).

In case of root length, highest root length (26.60CM) was recorded in treatment T₁₀=Seed treatment with *T. viride* @ 20% followed two foliar sprays followed by T₁₅=Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays (25.53CM),

T₉=Seed treatment with *T. viride* @ 15% followed two foliar sprays (25.40CM), T₅=Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (25.00CM), T₈=Seed treatment with *T. viride* @ 10% followed two foliar sprays (24.47CM) and T₄=Seed treatment with *T. harzianum* @ 15% followed two foliar sprays (24.27CM) whereas, minimum root length (25.53CM) was observed in untreated plot (Table 3). Data pertaining to No. of primary branches per plant have been presented in table 3 reveal that the non-significant difference was recorded among the all treatments. Highest number of primary branches recorded was 5.20 per plant in T₁₀=Seed treatment with *T. viride* @ 20% followed two foliar sprays followed by T₉=Seed treatment with *T. viride* @ 15% followed two foliar sprays (5.07/plant) and least number of primary branches (4.41/plant) was recorded in untreated plot.

Impacts on nodulation

Data pertaining to nodulation was recorded at the time of flowering and presented in table 3. Highest number of active nodules (43.63/plant) was recorded in treatment T₁₀=Seed treatment with *T. viride* @ 20% followed two foliar sprays. It was at par with T₅=Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (40.17/plant), T₈=Seed treatment with *T. viride* @ 10% followed two foliar sprays (39.67/plant), T₉=Seed treatment with *T. viride* @ 15% followed two foliar sprays (40.13/plant), T₁₅=Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays (41.83/plant) and statistically significant over rest of the treatments.

Impacts on Number of pods

Maximum number of pods per plant (75.27) was recorded in treatment T₁₀=Seed treatment

with *T. viride* @ 20% followed two foliar sprays followed by T₁₅=Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays (73.30/plant), T₉=Seed treatment with *T. viride* @ 15% followed two foliar sprays (71.67/plant), T₁₄=Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays (71.08/plant), T₅=Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (71.01/plant), T₈=Seed treatment with *T. viride* @ 10% followed two foliar sprays (70.69/plant), T₄=Seed treatment with *T. harzianum* @ 15% followed two foliar sprays (70.42/plant), T₁₃=Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays (70.36/plant), T₇=Seed treatment with *T. viride* @ 5% followed two foliar sprays (69.51/plant), T₃=Seed treatment with *T. harzianum* @ 10% followed two foliar sprays (69.38/plant), T₁₂=Seed treatment with *P. fluorescens* @ 5% followed two foliar sprays (68.72/plant), T₂=Seed treatment with *T. harzianum* @ 5% followed two foliar sprays (67.94/plant), T₆=Seed treatment with *T. viride* @ 1% followed two foliar sprays (67.54/plant), T₁₁=Seed treatment with *P. fluorescens* @ 1% followed two foliar sprays (66.76/plant), T₁=Seed treatment with *T. harzianum* @ 1% followed two foliar sprays (66.63/plant) whereas, minimum pods per plant (65.45) was recorded in untreated plants (Table 4).

Impacts on seed yield

Seed yield per plot and per hectare have been presented in table 4 indicated that the maximum seed yield per plot (4.388 kg) was obtained in treatment T₁₀=Seed treatment with *T. viride* @ 20% followed two foliar sprays which was at par with T₅=Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (4.140 kg/plot), T₈=Seed treatment with *T. viride* @ 10% followed two foliar sprays (4.121 kg/plot), T₉=Seed treatment with *T. viride* @ 15% followed two foliar

sprays (4.178 kg/ plot), T₁₄=Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays (4.144 kg/ plot), T₁₅=Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays (4.274 kg/ plot) and significantly superior over rest all the treatments. In case of seed yield per hectare, maximum seed yield (28.13 q/h) was obtained in treatment T₁₀=Seed treatment with *T. viride* @ 20% followed two foliar sprays. It was statistically at par with T₁₅=Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays (27.40 q/h), T₉=Seed treatment with *T. viride* @ 15%

followed two foliar sprays (26.78 q/h), T₁₄=Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays (26.56 q/h), T₅=Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (26.54 q/h), T₈=Seed treatment with *T. viride* @ 10% followed two foliar sprays (26.42 q/h), T₄=Seed treatment with *T. harzianum* @ 15% followed two foliar sprays (26.32 q/h), T₁₃=Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays (26.29 q/h) and significantly superior over rest of the treatments. Minimum seed yield per hectare (24.46q) was obtained in untreated plots.

Table.1 Effect of *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* on microbial population in soil

Treatment	Microbial population (cfu x10 ⁶ g ⁻¹)					
	Before Treatment (Before Sowing)		After Treatment (After harvesting)		Increased population over before treatment (%)	
	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria
T ₁ =Seed treatment with <i>T. harzianum</i> @ 1% followed two foliar sprays	2.45	3.62	2.52	3.73	2.86	3.04
T ₂ =Seed treatment with <i>T. harzianum</i> @ 5% followed two foliar sprays			2.57	3.80	4.90	4.97
T ₃ =Seed treatment with <i>T. harzianum</i> @ 10% followed two foliar sprays			2.64	3.90	7.76	7.73
T ₄ =Seed treatment with <i>T. harzianum</i> @ 15% followed two foliar sprays			2.70	3.98	10.20	9.94
T ₅ =eed treatment with <i>T. harzianum</i> @ 20% followed two foliar sprays			2.79	4.12	13.88	13.81
T ₆ =Seed treatment with <i>T. viride</i> @ 1% followed two foliar sprays			2.66	3.93	8.57	8.56
T ₇ =Seed treatment with <i>T. viride</i> @ 5% followed two foliar sprays			2.76	4.07	12.65	12.43
T ₈ =Seed treatment with <i>T. viride</i> @ 10% followed two foliar sprays			2.89	4.27	17.96	17.96
T ₉ =Seed treatment with <i>T. viride</i> @ 15% followed two foliar sprays			3.03	4.47	23.67	23.48
T ₁₀ =Seed treatment with <i>T. viride</i> @ 20% followed two foliar sprays			3.14	4.63	28.16	27.90
T ₁₁ =Seed treatment with <i>P. fluorescens</i> @ 1% followed two foliar sprays			2.82	4.16	15.10	14.92
T ₁₂ =Seed treatment with <i>P. fluorescens</i> @ 5% followed two foliar sprays			2.92	4.31	19.18	19.06
T ₁₃ =Seed treatment with <i>P. fluorescens</i> @ 10% followed two foliar sprays			3.09	4.56	26.12	25.97
T ₁₄ =Seed treatment with <i>Pseudomonas fluorescens</i> @ 15% followed two foliar sprays			3.26	4.81	33.06	32.87
T ₁₅ =Seed treatment with <i>P. fluorescens</i> @ 20% followed two foliar sprays			3.42	4.96	39.59	37.02
T ₁₆ =Control			2.50	3.69	2.04	1.93
SEm±			0.05	0.07	-	-
CD at 5%			0.14	0.19	-	-

Table.2 Efficacy of *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* against collar rot and target leaf spot of soybean

Treatment	Collar rot incidence (%)	Target Leaf spot	
		Incidence (%)	PDI (%)
T ₁ =Seed treatment with <i>T. harzianum</i> @ 1% followed two foliar sprays	5.12 (1308)	84.54 (66.85)	17.96 (25.07)
T ₂ =Seed treatment with <i>T. harzianum</i> @ 5% followed two foliar sprays	4.72 (12.55)	82.47 (65.25)	16.11 (23.66)
T ₃ =Seed treatment with <i>T. harzianum</i> @ 10% followed two foliar sprays	4.33 (12.01)	80.41 (63.73)	15.00 (22.79)
T ₄ =Seed treatment with <i>T. harzianum</i> @ 15% followed two foliar sprays	3.74 (11.15)	78.69 (62.51)	13.70 (21.72)
T ₅ =eed treatment with <i>T. harzianum</i> @ 20% followed two foliar sprays	2.76 (9.56)	73.54 (59.04)	12.78 (20.95)
T ₆ =Seed treatment with <i>T. viride</i> @ 1% followed two foliar sprays	3.74 (11.15)	83.51 (66.04)	15.19 (22.94)
T ₇ =Seed treatment with <i>T. viride</i> @ 5% followed two foliar sprays	3.35 (10.55)	80.41 (63.73)	14.26 (22.19)
T ₈ =Seed treatment with <i>T. viride</i> @ 10% followed two foliar sprays	2.76 (9.56)	77.66 (61.79)	12.96 (21.10)
T ₉ =Seed treatment with <i>T. viride</i> @ 15% followed two foliar sprays	2.36 (8.84)	74.57 (59.72)	11.48 (19.81)
T ₁₀ =Seed treatment with <i>T. viride</i> @ 20% followed two foliar sprays	1.97 (8.07)	71.82 (57.94)	10.00 (18.44)
T ₁₁ =Seed treatment with <i>P. fluorescens</i> @ 1% followed two foliar sprays	6.10 (14.30)	78.69 (62.51)	14.63 (22.49)
T ₁₂ =Seed treatment with <i>P. fluorescens</i> @ 5% followed two foliar sprays	5.31 (13.32)	81.44 (64.48)	13.15 (21.26)
T ₁₃ =Seed treatment with <i>P. fluorescens</i> @ 10% followed two foliar sprays	5.12 (13.08)	77.66 (61.79)	12.22 (20.46)
T ₁₄ =Seed treatment with <i>Pseudomonas fluorescens</i> @ 15% followed two foliar sprays	4.53 (12.29)	73.54 (59.04)	10.93 (19.31)
T ₁₅ =Seed treatment with <i>P. fluorescens</i> @ 20% followed two foliar sprays	3.74 (11.15)	68.73 (56.00)	9.81 (18.25)
T ₁₆ =Control	6.89 (15.22)	85.57 (67.67)	19.26 (26.03)
SEm±	0.77	2.15	0.88
CD at 5%	2.11	5.98	2.44

Table.3 Effect of *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* on plant growth and nodulation of soybean

Treatment	Plant height (cm)	Root length (cm)	No. of primary branches per plant	No. of nodules per plant
T ₁ =Seed treatment with <i>T. harzianum</i> @ 1% followed two foliar sprays	73.87	21.13	4.57	33.20
T ₂ =Seed treatment with <i>T. harzianum</i> @ 5% followed two foliar sprays	74.53	22.67	4.62	34.93
T ₃ =Seed treatment with <i>T. harzianum</i> @ 10% followed two foliar sprays	76.00	23.80	4.72	35.97
T ₄ =Seed treatment with <i>T. harzianum</i> @ 15% followed two foliar sprays	77.00	24.27	4.85	36.07
T ₅ =Seed treatment with <i>T. harzianum</i> @ 20% followed two foliar sprays	78.67	25.00	4.98	40.17
T ₆ =Seed treatment with <i>T. viride</i> @ 1% followed two foliar sprays	74.13	22.87	4.68	35.50
T ₇ =Seed treatment with <i>T. viride</i> @ 5% followed two foliar sprays	74.40	23.80	4.76	37.40
T ₈ =Seed treatment with <i>T. viride</i> @ 10% followed two foliar sprays	75.87	24.47	4.90	39.67
T ₉ =Seed treatment with <i>T. viride</i> @ 15% followed two foliar sprays	77.60	25.40	5.07	40.13
T ₁₀ =Seed treatment with <i>T. viride</i> @ 20% followed two foliar sprays	81.47	26.60	5.20	43.63
T ₁₁ =Seed treatment with <i>P. fluorescens</i> @ 1% followed two foliar sprays	73.47	22.73	4.53	34.73
T ₁₂ =Seed treatment with <i>P. fluorescens</i> @ 5% followed two foliar sprays	74.86	23.33	4.57	36.70
T ₁₃ =Seed treatment with <i>P. fluorescens</i> @ 10% followed two foliar sprays	76.87	23.80	4.62	38.53
T ₁₄ =Seed treatment with <i>Pseudomonas fluorescens</i> @ 15% followed two foliar sprays	78.40	23.93	4.68	39.25
T ₁₅ =Seed treatment with <i>P. fluorescens</i> @ 20% followed two foliar sprays	80.80	25.53	4.79	41.83
T ₁₆ =Control	72.87	21.33	4.41	32.57
SEm±	2.13	0.95	0.62	1.57
CD at 5%	5.93	2.64	NS	4.37

Table.4 Effect of *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* on no. of pods and seed yield of soybean

Treatment	No. of pods per plant	Grain yield	
		Kg/plot	Quintal per ha.
T₁=Seed treatment with <i>T. harzianum</i> @ 1% followed two foliar sprays	66.63	3.884	24.90
T₂ =Seed treatment with <i>T. harzianum</i> @ 5% followed two foliar sprays	67.94	3.961	25.39
T₃=Seed treatment with <i>T. harzianum</i> @ 10% followed two foliar sprays	69.38	4.045	25.93
T₄=Seed treatment with <i>T. harzianum</i> @ 15% followed two foliar sprays	70.42	4.106	26.32
T₅=Seed treatment with <i>T. harzianum</i> @ 20% followed two foliar sprays	71.01	4.140	26.54
T₆=Seed treatment with <i>T. viride</i> @ 1% followed two foliar sprays	67.54	3.938	25.24
T₇=Seed treatment with <i>T. viride</i> @ 5% followed two foliar sprays	69.51	4.052	25.98
T₈=Seed treatment with <i>T. viride</i> @ 10% followed two foliar sprays	70.69	4.121	26.42
T₉=Seed treatment with <i>T. viride</i> @ 15% followed two foliar sprays	71.67	4.178	26.78
T₁₀=Seed treatment with <i>T. viride</i> @ 20% followed two foliar sprays	75.27	4.388	28.13
T₁₁=Seed treatment with <i>P. fluorescens</i> @ 1% followed two foliar sprays	66.76	3.892	24.95
T₁₂=Seed treatment with <i>P. fluorescens</i> @ 5% followed two foliar sprays	68.72	4.007	25.68
T₁₃=Seed treatment with <i>P. fluorescens</i> @ 10% followed two foliar sprays	70.36	4.102	26.29
T₁₄=Seed treatment with <i>Pseudomonas fluorescens</i> @ 15% followed two foliar sprays	71.08	4.144	26.56
T₁₅=Seed treatment with <i>P. fluorescens</i> @ 20% followed two foliar sprays	73.30	4.274	27.40
T₁₆=Control	65.45	3.816	24.46
SEm±	2.03	0.101	0.75
CD at 5%	5.64	0.281	2.09

Fig.1 Impact of *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* on fungi and bacteria population

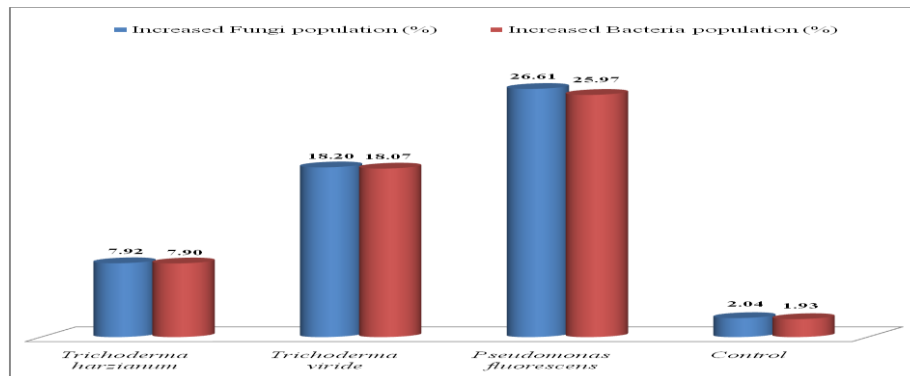


Plate.1 Impact of *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* on soybean

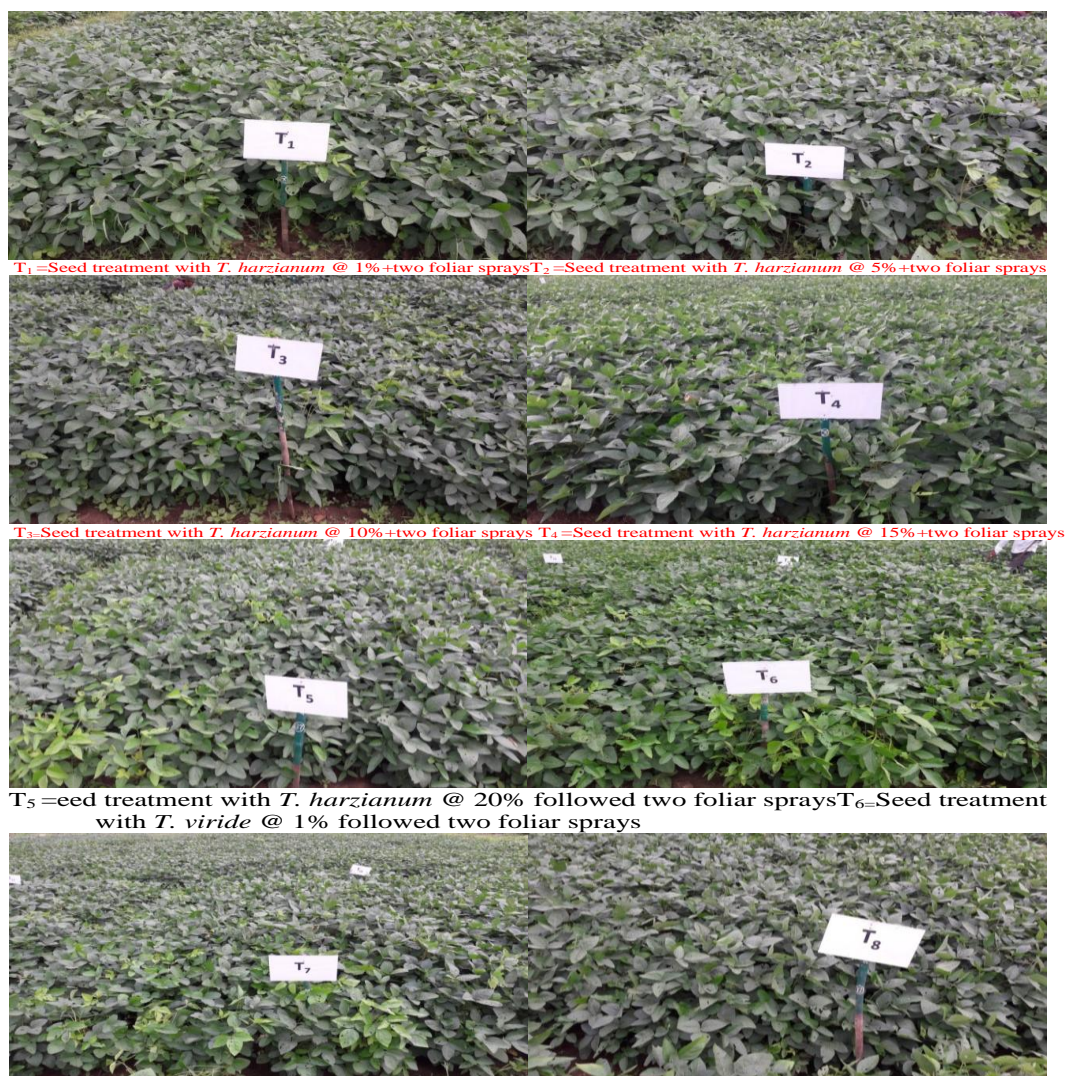
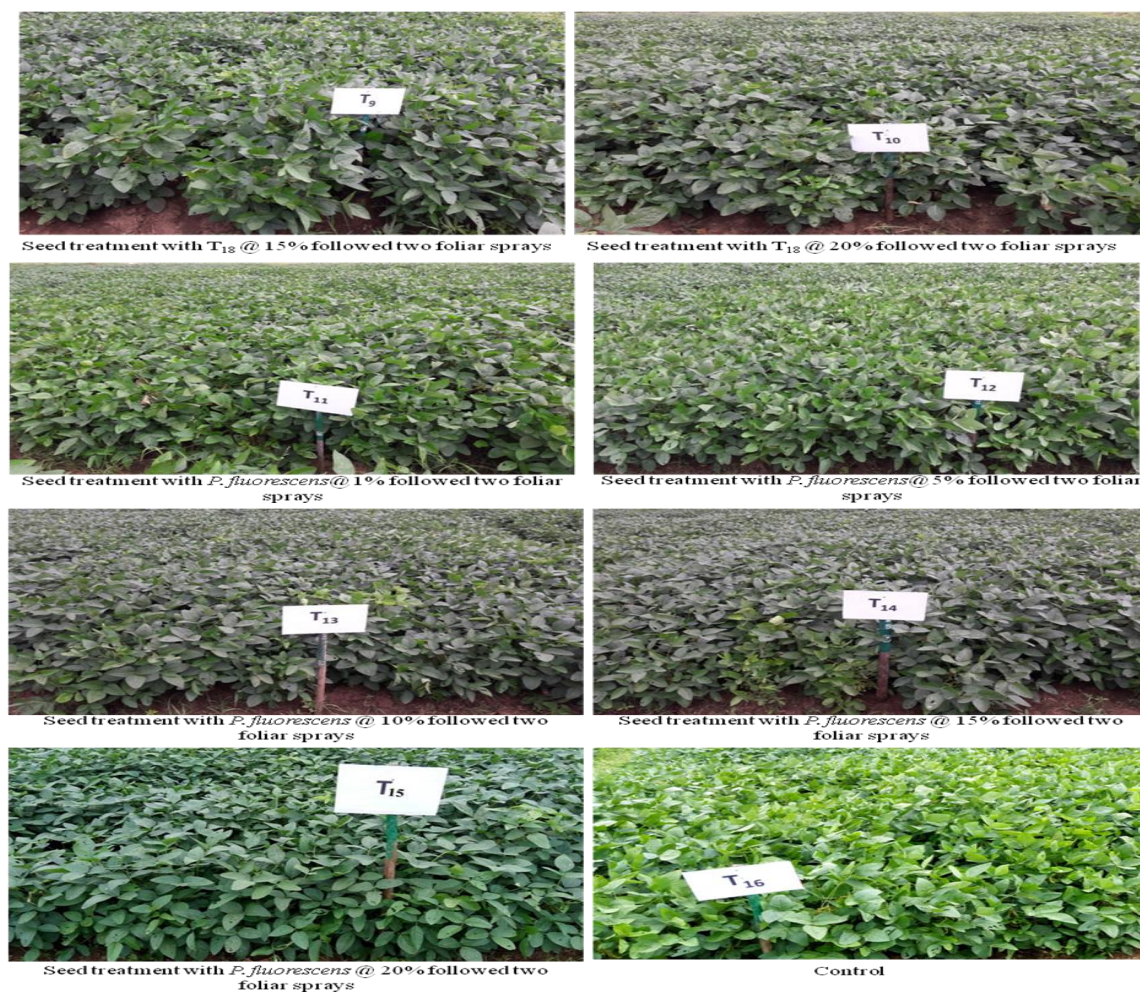


Plate.2 Impact of *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* on soybean



The present findings indicated that the seed treatment with *T. viride* or *T. harzianum* followed by foliar spray showed significant positive effects in reducing collar rot disease in soybean and also increased the plant height, root length, nodulation, number of pods and seed yield. These results corroborate with the findings of Meher *et al.*, (2018). They tested bio-efficacy of twenty native isolates of *Trichoderma* spp. against *Sclerotium rolfsii* and their effects on growth parameters of chickpea plant. Tr- 7 was found to be most effective with minimum seedling mortality of 6.67%. *Trichoderma* spp. has wide range of mechanisms for disease control i.e., Mycoparasitism and hyphallysis,

antibiosis, competition for nutrients and space. Several species of genus *Trichoderma* have been identified as growth promoting agents (Rudresh *et al.*, 2005; Jash *et al.*, 2007; Swathi *et al.*, 2015). *Trichoderma* are more capable to enhance the growth of plants and also increase the crop production in several crops (Balasubramanian 2003). Katwasra (2002) reported that *Pseudomonas fluorescens* was most effective in reducing the incidence of dry root rot with 71.8 % disease control. Belkar and Gade (2013) found efficacy of seed treatment with *Pseudomonas fluorescens* @ 10g / kg of seed was found effective against *Rhizoctonia*, *Sclerotium* pathogens. Konde *et al.*, (2017) revealed that

seed treatment with carbendazim + *Trichoderma viride* recorded significantly maximum seed germination (94.44%) while highest grain yield (1808 kg ha⁻¹) was observed with the soil application of *Trichoderma viride* + *Trichoderma harzianum*. Khodke and Raut (2011) found effectiveness of seed treatment and soil application of fungicides, bioagents and its combinations in increasing seed germination and reducing pre and post emergence mortality. Suryawanshi *et al.*, (2015) studied on collar rot caused by *Sclerotium rolfsii* Sacc. on brinjal and revealed significantly highest mycelial growth inhibition was recorded with *Bacillus megaterium* and *P. fluorescens*. Rajendraprasad *et al.*, (2017) evaluated twenty four isolates of *Trichoderma harzianum* and *Trichoderma viride* and twelve different *Bacillus subtilis* and *Pseudomonas fluorescence*. The combination of potential *Trichoderma harzianum*-1 and *Pseudomonas fluorescence* bacterial biocontrol agents also proved effective in increasing germination and to reduce pre and post emergence collar rot in the pots when inoculated with *Sclerotium rolfsii*. Seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1) seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3) was recorded 52.08 and 49.17 percent germination respectively when inoculated with *Sclerotium rolfsii*. The lowest incidence (47.92 percent) of pre emergence damping off was recorded in seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1). Gandhi *et al.*, (2017) reported that the *Pseudomonas fluorescens* as soil application gave maximum disease control (55.11 %) of collar rot of sunflower and also maximum seed yield. Ingle *et al.*, (2018) revealed that the *Pseudomonas fluorescens* and *Trichoderma viride* seed dressing with Carboxin 37.5% +

Thiram 37.5% (combi product) @ 2g/kg most effective regarding seed germination, incidence of root rot. Shyam and Tiwari (2018) evaluated efficacy of *Trichoderma harzianum* with integration of fungicides collar rot disease of chickpea caused by *Sclerotium rolfsii* in Chhattisgarh and reveal that the *Trichoderma harzianum* were found significantly effective for the prevention of mortality caused by *S. rolfsii* in chickpea. Singh *et al.*, (2017) evaluated integration of *Trichoderma*, *Pseudomonas* and fungicides for the control of Collar rot disease of Chickpea and reported *Trichoderma harzianum* @ 8q/ha-1 (Soil) + Hexaconazole @ 3ml/kg-1 seed and *Pseudomonas fluorescens* @ 8q/ha-1 (Soil) + Hexaconazole @ 3ml/kg-1 seed were significantly effective against collar rot disease.

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