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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A B S T R A C T

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.

Keywords
Collar rot, Target leaf spot, Pseudomonas fluorescens, Soybean, Trichoderma harzianum, Trichoderma viride

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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 floweringto 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), collarrot (Sclerotium rolfsi), 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 causes 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. cassicola 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 prevent 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 plat 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 covered 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, few leaves drops, 7=50.1–60 % leaf area covered with lesion, spread up to upper canopy, 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:

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\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 (4.96cfu X 10^4 g\(^{-1}\)) was recorded after harvesting in treatment T\(_{15}\) – Seed treatment with \textit{P. fluorescens} @ 20% followed two foliar sprays which was at par with T\(_{12}\) = Seed treatment with \textit{Pseudomonas fluorescens} @ 15% followed two foliar sprays (4.53%), T\(_{14}\) = Seed treatment with \textit{T. harzianum} @ 5% followed two foliar sprays (5.31%), T\(_{11}\) = Seed treatment with \textit{P. fluorescens} @ 1% followed two foliar sprays (6.10 %), T\(_{7}\) = Seed treatment with \textit{T. viride} @ 5% followed two foliar sprays (3.35%), T\(_{10}\) = Seed treatment with \textit{T. viride} @ 20% followed two foliar sprays (4.63cfu X 10^4 g\(^{-1}\)), T\(_{13}\) – Seed treatment with \textit{P. fluorescens} @ 10% followed two foliar sprays (4.56cfu X10^4 g\(^{-1}\)), T\(_{9}\) – Seed treatment with \textit{T. viride} @ 15% followed two foliar sprays (4.47cfu X 10^4 g\(^{-1}\)) whereas, bacterial population was 3.62cfu X10^4 g\(^{-1}\) before sowing. In untreated plots fungi and bacteria population was recorded 2.50 and 3.69cfu X 10^4 g\(^{-1}\), respectively after the harvesting of crop. Highest impact of \textit{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 \textit{T. viride} (18.20 and 18.07%) and \textit{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 rot disease

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 \textit{T. viride} @ 20% followed two foliar sprays which was at par with T\(_{9}\) = Seed treatment with \textit{T. viride} @ 15% followed two foliar sprays (2.36%), T\(_{5}\) = Seed treatment with \textit{T. harzianum} @ 20% followed two foliar sprays (2.76%), T\(_{8}\) = Seed treatment with \textit{T. viride} @ 10% followed two foliar sprays (2.76%) and significantly lower over T\(_{1}\) – Seed treatment with \textit{T. harzianum} @ 1% followed two foliar sprays (5.12%), T\(_{2}\) = Seed treatment with \textit{T. harzianum} @ 5% followed two foliar sprays (4.72%), T\(_{3}\) = Seed treatment with \textit{T. harzianum} @ 10% followed two foliar sprays (4.33%), T\(_{4}\) = Seed treatment with \textit{T. harzianum} @ 15% followed two foliar sprays (3.74%), T\(_{6}\) = Seed treatment with \textit{T. harzianum} @ 20% followed two foliar sprays (3.35%), T\(_{11}\) = Seed treatment with \textit{P. fluorescens} @ 1% followed two foliar sprays (6.10 %), T\(_{12}\) = Seed treatment with \textit{P. fluorescens} @ 5% followed two foliar sprays (5.31%), T\(_{13}\) = Seed treatment with \textit{P. fluorescens} @ 10% followed two foliar sprays (5.12 %), T\(_{14}\) = Seed treatment with \textit{Pseudomonas fluorescens} @ 10% followed two foliar sprays (4.53%), T\(_{15}\) = Seed treatment with \textit{P. fluorescens} @ 15% followed two foliar sprays (4.53%), T\(_{16}\) = Seed treatment with \textit{Pseudomonas fluorescens} @ 20% followed two foliar sprays (4.63cfu X 10^4 g\(^{-1}\)).
treatment with \textit{P. fluorescens} @ 20% followed two foliar sprays (3.74%). In control plot, collar rot incidence was recorded highest (6.89%).

\textbf{Impacts on Target Leaf spot disease}

Minimum target leaf spot disease incidence (68.73\%) in treatment T\textsubscript{15}=Seed treatment with \textit{P. fluorescens} @ 20\% followed two foliar sprays which was at par with T\textsubscript{14}=Seed treatment with \textit{Pseudomonas fluorescens} @ 15\% followed two foliar sprays (73.54\%), T\textsubscript{13}=Seed treatment with \textit{P. fluorescens} @ 10\% followed two foliar sprays (77.66\%), T\textsubscript{10}=Seed treatment with \textit{T. viride} @ 20\% followed two foliar sprays (71.82\%), T\textsubscript{9}=Seed treatment with \textit{T. viride} @ 15\% followed two foliar sprays (74.57\%) and significantly lower over rest of the treatments (Table 2).

In case of PDI, minimum PDI (9.81\%) was recorded in treatment T\textsubscript{15}=Seed treatment with \textit{P. fluorescens} @ 20\% followed two foliar sprays followed by T\textsubscript{10}=Seed treatment with \textit{T. viride} @ 20\% followed two foliar sprays (10.00\%), T\textsubscript{14}=Seed treatment with \textit{Pseudomonas fluorescens} @ 15\% followed two foliar sprays (10.93\%), T\textsubscript{9}=Seed treatment with \textit{T. viride} @ 15\% followed two foliar sprays (11.48\%) and T\textsubscript{13}=Seed treatment with \textit{P. fluorescens} @ 10\% followed two foliar sprays (12.22\%). Treatment T\textsubscript{15}=Seed treatment with \textit{P. fluorescens} @ 20\% followed two foliar sprays showed significantly lower PDI over T\textsubscript{1}=Seed treatment with \textit{T. harzianum} @ 1\% followed two foliar sprays (17.96\%), T\textsubscript{2}=Seed treatment with \textit{T. harzianum} @ 5\% followed two foliar sprays (16.11\%), T\textsubscript{3}=Seed treatment with \textit{T. harzianum} @ 10\% followed two foliar sprays (15.00), T\textsubscript{4}=Seed treatment with \textit{T. harzianum} @ 15\% followed two foliar sprays (13.70\%), T\textsubscript{5}=Seed treatment with \textit{T. harzianum} @ 20\% followed two foliar sprays (12.78\%), T\textsubscript{6}=Seed treatment with \textit{T. viride} @ 1\% followed two foliar sprays (15.19\%), T\textsubscript{7}=Seed treatment with \textit{T. viride} @ 5\% followed two foliar sprays (14.26\%), T\textsubscript{8}=Seed treatment with \textit{T. viride} @ 10\% followed two foliar sprays (12.96\%), T\textsubscript{11}=Seed treatment with \textit{P. fluorescens} @ 1\% followed two foliar sprays (14.63\%) and T\textsubscript{12}=Seed treatment with \textit{P. fluorescens} @ 5\% followed two foliar sprays (13.15\%) (Table 2).

\textbf{Impacts on Plant growth parameters}

Maximum plant height (81.47CM) was observed in treatment T\textsubscript{10}=Seed treatment with \textit{T. viride} @ 20\% followed two foliar sprays. It was at par with T\textsubscript{3}=Seed treatment with \textit{T. harzianum} @ 10\% followed two foliar sprays (76.00CM), T\textsubscript{4}=Seed treatment with \textit{T. harzianum} @ 15\% followed two foliar sprays (77.00CM), T\textsubscript{5}=Seed treatment with \textit{T. harzianum} @ 20\% followed two foliar sprays (78.86CM), T\textsubscript{8}=Seed treatment with \textit{T. viride} @ 10\% followed two foliar sprays (75.87CM), T\textsubscript{9}=Seed treatment with \textit{T. viride} @ 15\% followed two foliar sprays (77.60CM), T\textsubscript{13}=Seed treatment with \textit{P. fluorescens} @ 10\% followed two foliar sprays (76.87CM), T\textsubscript{14}=Seed treatment with \textit{Pseudomonas fluorescens} @ 15\% followed two foliar sprays (78.40CM), T\textsubscript{15}=Seed treatment with \textit{P. fluorescens} @ 20\% followed two foliar sprays (80.80CM) and significantly superior over rest of the treatments 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 T\textsubscript{10}=Seed treatment with \textit{T. viride} @ 20\% followed two foliar sprays followed by T\textsubscript{15}=Seed treatment with \textit{P. fluorescens} @ 20\% followed two foliar sprays (25.53CM), T\textsubscript{14}=Seed treatment with \textit{Pseudomonas fluorescens} @ 15\% followed two foliar sprays (24.10CM), T\textsubscript{9}=Seed treatment with \textit{T. viride} @ 15\% followed two foliar sprays (23.60CM), T\textsubscript{8}=Seed treatment with \textit{T. viride} @ 10\% followed two foliar sprays (22.60CM), T\textsubscript{7}=Seed treatment with \textit{T. harzianum} @ 20\% followed two foliar sprays (21.80CM), T\textsubscript{5}=Seed treatment with \textit{T. harzianum} @ 10\% followed two foliar sprays (20.60CM), T\textsubscript{4}=Seed treatment with \textit{T. harzianum} @ 15\% followed two foliar sprays (19.00CM), T\textsubscript{3}=Seed treatment with \textit{T. harzianum} @ 5\% followed two foliar sprays (18.00CM), T\textsubscript{2}=Seed treatment with \textit{T. harzianum} @ 1\% followed two foliar sprays (17.00CM) and significantly lower over rest of the treatments and minimum plant height (12.87CM) was recorded in untreated plot (Table 3).
T₀ = Seed treatment with *T. viride* @ 15% followed two foliar sprays (25.40 CM), T₅ = Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (25.00 CM), T₈ = Seed treatment with *T. viride* @ 10% followed two foliar sprays (24.47 CM) and T₄ = Seed treatment with *T. harzianum* @ 15% followed two foliar sprays (24.27 CM) whereas, minimum root length (25.53 CM) 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 (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 (40.17/plant), T₅ = Seed treatment with *T. harzianum* @ 20% followed two foliar sprays (40.13/plant), T₈ = Seed treatment with *T. viride* @ 10% followed two foliar sprays (39.67/plant), T₀ = Seed treatment with *T. viride* @ 15% followed two foliar sprays (39.67/plant) and least number of primary branches (4.41/plant) was recorded in untreated plot.

**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), T₀ = 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), T₂ = Seed treatment with *T. harzianum* @ 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), T₁ = Seed treatment with *T. harzianum* @ 1% followed two foliar sprays (67.54/plant), T₁₁ = Seed treatment with *P. fluorescens* @ 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), T₀ = Seed treatment with *T. viride* @ 15% followed two foliar sprays (4.121 kg/plot) whereas, minimum seeds per plant (65.45) was recorded in untreated plants (Table 4).
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), 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 x10⁶ g⁻¹) |
|-----------|-------------------------------------|
|           | Before Treatment (Before Sowing)    | After Treatment (After harvesting) | Increased population over before treatment (%) |
|           | Fungi | Bacteria | Fungi | Bacteria | Fungi | Bacteria |
| T₁= Seed treatment with T. harzianum @ 1% followed two foliar sprays | 2.45 | 3.62 | 2.52 | 3.73 | 2.86 | 3.04 |
| T₂= Seed treatment with T. harzianum @ 5% followed two foliar sprays | 2.57 | 3.80 | 4.90 | 4.97 |
| T₃= Seed treatment with T. harzianum @ 10% followed two foliar sprays | 2.64 | 3.90 | 7.76 | 7.73 |
| T₄= Seed treatment with T. harzianum @ 15% followed two foliar sprays | 2.70 | 3.98 | 10.20 | 9.94 |
| T₅= Seed treatment with T. harzianum @ 20% followed two foliar sprays | 2.79 | 4.12 | 13.88 | 13.81 |
| T₆= Seed treatment with T. viride @ 1% followed two foliar sprays | 2.66 | 3.93 | 8.57 | 8.56 |
| T₇= Seed treatment with T. viride @ 5% followed two foliar sprays | 2.76 | 4.07 | 12.65 | 12.43 |
| T₈= Seed treatment with T. viride @ 10% followed two foliar sprays | 2.89 | 4.27 | 17.96 | 17.96 |
| T₉= Seed treatment with T. viride @ 15% followed two foliar sprays | 3.03 | 4.47 | 23.67 | 23.48 |
| T₁₀= Seed treatment with T. viride @ 20% followed two foliar sprays | 3.14 | 4.63 | 28.16 | 27.90 |
| T₁₁= Seed treatment with P. fluorescens @ 1% followed two foliar sprays | 2.82 | 4.16 | 15.10 | 14.92 |
| T₁₂= Seed treatment with P. fluorescens @ 5% followed two foliar sprays | 2.92 | 4.31 | 19.18 | 19.06 |
| T₁₃= Seed treatment with P. fluorescens @ 10% followed two foliar sprays | 3.09 | 4.56 | 26.12 | 25.97 |
| T₁₄= Seed treatment with Pseudomonas fluorescens @ 15% followed two foliar sprays | 3.26 | 4.81 | 33.06 | 32.87 |
| T₁₅= Seed treatment with P. fluorescens @ 20% followed two foliar sprays | 3.42 | 4.96 | 39.59 | 37.02 |
| T₁₆= 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**<sub>1</sub> = Seed treatment with *T. harzianum* @ 1% followed two foliar sprays | 5.12 (1308)              | 84.54 (66.85)                 | 17.96 (25.07) |
| **T**<sub>2</sub> = Seed treatment with *T. harzianum* @ 5% followed two foliar sprays | 4.72 (12.55)             | 82.47 (65.25)                 | 16.11 (23.66) |
| **T**<sub>3</sub> = Seed treatment with *T. harzianum* @ 10% followed two foliar sprays | 4.33 (12.01)             | 80.41 (63.73)                 | 15.00 (22.79) |
| **T**<sub>4</sub> = Seed treatment with *T. harzianum* @ 15% followed two foliar sprays | 3.74 (11.15)             | 78.69 (62.51)                 | 13.70 (21.72) |
| **T**<sub>5</sub> = Seed treatment with *T. harzianum* @ 20% followed two foliar sprays | 2.76 (9.56)              | 73.54 (59.04)                 | 12.78 (20.95) |
| **T**<sub>6</sub> = Seed treatment with *T. viride* @ 1% followed two foliar sprays | 3.74 (11.15)             | 83.51 (66.04)                 | 15.19 (22.94) |
| **T**<sub>7</sub> = Seed treatment with *T. viride* @ 5% followed two foliar sprays | 3.35 (10.55)             | 80.41 (63.73)                 | 14.26 (22.19) |
| **T**<sub>8</sub> = Seed treatment with *T. viride* @ 10% followed two foliar sprays | 2.76 (9.56)              | 77.66 (61.79)                 | 12.96 (21.10) |
| **T**<sub>9</sub> = Seed treatment with *T. viride* @ 15% followed two foliar sprays | 2.36 (8.84)              | 74.57 (59.72)                 | 11.48 (19.81) |
| **T**<sub>10</sub> = Seed treatment with *T. viride* @ 20% followed two foliar sprays | 1.97 (8.07)              | 71.82 (57.94)                 | 10.00 (18.44) |
| **T**<sub>11</sub> = Seed treatment with *P. fluorescens* @ 1% followed two foliar sprays | 6.10 (14.30)             | 78.69 (62.51)                 | 14.63 (22.49) |
| **T**<sub>12</sub> = Seed treatment with *P. fluorescens* @ 5% followed two foliar sprays | 5.31 (13.32)             | 81.44 (64.48)                 | 13.15 (21.26) |
| **T**<sub>13</sub> = Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays | 5.12 (13.08)             | 77.66 (61.79)                 | 12.22 (20.46) |
| **T**<sub>14</sub> = Seed treatment with *P. fluorescens* @ 15% followed two foliar sprays | 4.53 (12.29)             | 73.54 (59.04)                 | 10.93 (19.31) |
| **T**<sub>15</sub> = Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays | 3.74 (11.15)             | 68.73 (56.00)                 | 9.81 (18.25)  |
| **T**<sub>16</sub> = 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 |
|--------------------------------|-------------------|------------------|----------------------------------|--------------------------|
| **T1** = Seed treatment with *T. harzianum* @ 1% followed two foliar sprays | 73.87             | 21.13            | 4.57                             | 33.20                    |
| **T2** = Seed treatment with *T. harzianum* @ 5% followed two foliar sprays | 74.53             | 22.67            | 4.62                             | 34.93                    |
| **T3** = Seed treatment with *T. harzianum* @ 10% followed two foliar sprays | 76.00             | 23.80            | 4.72                             | 35.97                    |
| **T4** = Seed treatment with *T. harzianum* @ 15% followed two foliar sprays | 77.00             | 24.27            | 4.85                             | 36.07                    |
| **T5** = Seed treatment with *T. harzianum* @ 20% followed two foliar sprays | 78.67             | 25.00            | 4.98                             | 40.17                    |
| **T6** = Seed treatment with *T. viride* @ 1% followed two foliar sprays | 74.13             | 22.87            | 4.68                             | 35.50                    |
| **T7** = Seed treatment with *T. viride* @ 5% followed two foliar sprays | 74.40             | 23.80            | 4.76                             | 37.40                    |
| **T8** = Seed treatment with *T. viride* @ 10% followed two foliar sprays | 75.87             | 24.47            | 4.90                             | 39.67                    |
| **T9** = Seed treatment with *T. viride* @ 15% followed two foliar sprays | 77.60             | 25.40            | 5.07                             | 40.13                    |
| **T10** = Seed treatment with *T. viride* @ 20% followed two foliar sprays | 81.47             | 26.60            | 5.20                             | 43.63                    |
| **T11** = Seed treatment with *P. fluorescens* @ 1% followed two foliar sprays | 73.47             | 22.73            | 4.53                             | 34.73                    |
| **T12** = Seed treatment with *P. fluorescens* @ 5% followed two foliar sprays | 74.86             | 23.33            | 4.57                             | 36.70                    |
| **T13** = Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays | 76.87             | 23.80            | 4.62                             | 38.53                    |
| **T14** = Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays | 78.40             | 23.93            | 4.68                             | 39.25                    |
| **T15** = Seed treatment with *P. fluorescens* @ 20% followed two foliar sprays | 80.80             | 25.53            | 4.79                             | 41.83                    |
| **T16** = 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 *T. harzianum* @ 1% followed two foliar sprays | 66.63 | 3.884 | 24.90 |
| T₂=Seed treatment with *T. harzianum* @ 5% followed two foliar sprays | 67.94 | 3.961 | 25.39 |
| T₃=Seed treatment with *T. harzianum* @ 10% followed two foliar sprays | 69.38 | 4.045 | 25.93 |
| T₄=Seed treatment with *T. harzianum* @ 15% followed two foliar sprays | 70.42 | 4.106 | 26.32 |
| T₅=Seed treatment with *T. harzianum* @ 20% followed two foliar sprays | 71.01 | 4.140 | 26.54 |
| T₆=Seed treatment with *T. viride* @ 1% followed two foliar sprays | 67.54 | 3.938 | 25.24 |
| T₇=Seed treatment with *T. viride* @ 5% followed two foliar sprays | 69.51 | 4.052 | 25.98 |
| T₈=Seed treatment with *T. viride* @ 10% followed two foliar sprays | 70.69 | 4.121 | 26.42 |
| T₉=Seed treatment with *T. viride* @ 15% followed two foliar sprays | 71.67 | 4.178 | 26.78 |
| T₁₀=Seed treatment with *T. viride* @ 20% followed two foliar sprays | 75.27 | 4.388 | 28.13 |
| T₁₁=Seed treatment with *P. fluorescens* @ 1% followed two foliar sprays | 66.76 | 3.892 | 24.95 |
| T₁₂=Seed treatment with *P. fluorescens* @ 5% followed two foliar sprays | 68.72 | 4.007 | 25.68 |
| T₁₃=Seed treatment with *P. fluorescens* @ 10% followed two foliar sprays | 70.36 | 4.102 | 26.29 |
| T₁₄=Seed treatment with *Pseudomonas fluorescens* @ 15% followed two foliar sprays | 71.08 | 4.144 | 26.56 |
| T₁₅=Seed treatment with *P. fluorescens* @ 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

- **T1**: Seed treatment with *T. harzianum* @ 1% + two foliar sprays
- **T2**: Seed treatment with *T. harzianum* @ 5% + two foliar sprays
- **T3**: Seed treatment with *T. harzianum* @ 10% + two foliar sprays
- **T4**: Seed treatment with *T. harzianum* @ 15% + two foliar sprays
- **T5**: Seed treatment with *T. harzianum* @ 20% + two foliar sprays
- **T6**: Seed treatment with *T. viride* @ 1% + two foliar sprays
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 sprays 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 hyphal lysis, antibiotics, 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 *Baccillus 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 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 
fluorescens* -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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