

Seed biopriming with different bioagents to minimize collar rot disease of groundnut (*Arachis hypogea* L.)

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Summary

The present studies investigate the effectiveness of various bioagents, namely *Trichoderma viride*, *Pseudomonas fluorescens*, *Trichoderma harzenium* and *Bacillus subtilis* in promoting the growth, productivity and minimize the collar rot disease incidence in groundnut (*Arachis hypogea* L.) under *in vitro* and controlled pot conditions (upto 60 per cent disease control). Groundnut is a globally important oilseed crop known for its nutritional value and oil content. However, the sustainable production of groundnuts faces increasing challenges due to biotic and abiotic stress factors. Among the biotic diseases, collar rot disease of groundnut caused by *Aspergillus niger* is more extensive in *kharif* season and causes more damage in sandy loam soils. Bioagents like *Trichoderma harzenium*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* are very helpful in increasing plant growth and controlling collar rot disease in groundnut. This study aims to elucidate the physiological mechanism underlying these interactions and provide practical insights into the application of this environmentally friendly approach for sustainable groundnut cultivation. Under pot condition treatment integration of seed treatment with *Trichoderma viride* and enriched vermicompost significantly shows minimum per cent disease incidence 6% and maximum plant vigour (2000) obtained from the treatment integration of Seed treatment with *Trichoderma harzianum* and enriched Vermicompost. Our finding reveals that the application of integration of seed treatment with *Trichoderma viride* and enriched vermicompost significantly shows 57.14% disease control under pot conditions, outperformed the other bioagents in sustainable management of collar rot disease of groundnut and *Pseudomonas fluorescens* outperformed other bioagents in enhancement of growth of plant due to their PGPR activity.

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Introduction

Peanuts are a globally significant oilseed crop because of their nutritional value, and culinary diversity says that Groundnut (*Arachis hypogea* L.) are also known as peanuts, jack nut, manila nuts, and monkey nuts. Groundnut is from the family *Fabaceae*, and it fixes atmospheric nitrogen in the soil. The 3rd most crucial oil seed after Soya bean and Cotton with a production of 432 million tonnes; China (17.30 million tons) is the largest producer of peanut, accounting for 37% and India (6.70 million tons,) accounting for 14% of total world production respectively in 2018-19 amounting to a total 46.01 million tonnes (Anon. 2020). India is the world's leading producer of groundnuts, with 101 lakh tons produced at a productivity of 1816 kg per hectare in 2020–2021, having planted 55.6 lakh hectares of the crop. (Groundnut Outlook Report, January-May 2021)

In some states, groundnuts are grown as rainfed

crops in Kharif season and as irrigated crops in Rabi (Varghese 2011). However, peanuts' sustainable productivity has been challenged by biotic and abiotic stress factors (Patel et al. 2019). Environmental concerns regarding chemical interventions call for change from the traditional reliance on them. Seed biopriming is an environmentally friendly strategy which has gained importance (Sharma & Bhatnagar-Mathur 2021). Peanuts are grown in various cropping systems, including mixed, trapping and sequential cropping (Yashoda et al. 2016) and are an ideal candidate for exploring seed biopriming approaches. The bio-priming of seeds is whereby beneficial microorganisms are introduced to the seeds, hence promoting symbiotic relationships (Jeffries et al. 2017; Sinha et al. 2023). *Trichoderma*, *Pseudomonas*, and *Bacillus* genera, among other categories of these microorganisms, have attracted much attention as they help in enhancing plant growth, disease suppression as well as abiotic stress resistance mechanisms (Hermosa et al. 2019). An effective biocontrol agent is regarded as *Trichoderma viride* with exceptional antagonistic properties over a range of phytopathogens (Druzhinina et al. 2018) since they play an essential role in nutrient uptake, root

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development stimulation and production of secondary metabolites leading to better plant growth overall. In addition, *Pseudomonas fluorescens* produce large amounts of antimicrobial compounds that stimulate plant defence mechanisms as well as nutrient release. They can also mitigate abiotic stresses such as drought and salinity through the production of stress-responsive metabolites. Furthermore, they easily colonize the rhizosphere due to their efficiency in colonizing the rhizosphere and robust spore-forming ability.

Trichoderma species have been reported to be involved in plant growth improvement and reduction of phytopathogens through several diverse mechanisms. *Trichoderma* mycoparasitism on root-infecting fungi combined with its ability to induce plant defence responses support its potential as a biocontrol agent (Harman et al. 2004; Shores et al. 2010). *Pseudomonas* is a known metabolically versatile genus that has been acknowledged for suppressing soil-borne pathogens by production of antimicrobial compounds and induction of systemic resistance mechanisms in plants (Siddiqui et al. 2006). There is a need to explore further the possibilities of using *Pseudomonas* as a groundnut pathogen bio-control agent. *Bacillus* species have also shown an ability to promote plant growth and control plant diseases. This is because they produce antibiotics, lytic enzymes, and volatile organic compounds that are effective against microorganisms (Whipps 2001). This practice goes hand in hand with sustainable agriculture principles aimed at reducing chemical inputs. On the other hand, these beneficial organisms are less hazardous, eco-friendly and safer than synthetic pesticides.

Material and Methods

Trichoderma viride, *Bacillus subtilis* and *Pseudomonas fluorescens* were used as biological agents for biopriming genetically pure Peanut seeds. A laboratory experiment in a completely randomized design (CRD) was conducted to standardize appropriate biopriming treatments, assessing germination percentage, shoot length, root length, plant vigour and disease incidence. In addition, pot experiments using complete block design were done to evaluate the efficacy of bioagents on seed germination, root–shoot length and plant vigour. For the preparatory treatment, unsterilized seeds and externally sterilized seeds are used with 0.1% mercuric chloride solution for 1-2 minutes, and then washed with sterilized distilled water. Hydropriming was done by soaking the seeds in equal volume of water, while the control consisted of unprimed seeds. The biopriming treatments involved soaking the seeds in equal volumes of *Trichoderma viride* concentration, *Bacillus subtilis* concentration, and *Pseudomonas fluorescens* concentration for 8 hours, respectively. The dried seeds, after priming, were shaded back to their original moisture content level. Laboratory tests comprised four replicates of Petri dishes with seeds also under pot condition, each from an individual treatment, for determination of germination percentages, shoot lengths, and other parameters listed before. The pots were washed with water and disinfected with 4.0% formaldehyde (40 EC).

The soil was collected from AICRP's field at Ummedganj, Kota, mixed with vermicompost and then sterilized in a soil sterilizer. The sterilized soil pots were inoculated with sand maize inoculum and left for two days to establish the inoculum.

The pot experiment was conducted in the complete block design with four replications in the Kharif season of 2023 at Biopesticide *Trichoderma* laboratory, College of Agriculture, Ummedganj, Kota situated 25.133291° Latitude and 75.939639 Longitude in Kota district of Rajasthan.

Efficacy on Seed germination, root and shoot length and plant vigour

The germination of peanut seeds was examined by treatment with spore suspension of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* and transferred to appropriate pots with sterile soil. Untreated seeds served as controls. After eight days, the number of germinated seedlings in each replicate was counted, and germination was calculated and expressed using the following formula:

Germination percentage (%) = $\frac{\text{germinated seeds}}{\text{total seeds}} \times 100$.

The root length (The root length was measured from the collar region to the tip of the primary root, and The mean root length was expressed in centimetres) and shoot length (The shoot length was measured from the collar region to the tip of the primary leaf, The mean shoot length was worked out and expressed in centimetres) were observed and based on the root and shoot length with germination percentage; the vigour index was calculated using the formula of (Abdul Baki & Anderson 1973):

Vigour index = $\frac{(\text{mean shoot length} + \text{mean root length}) \times \text{germination percentage}}{2}$

In Vitro Experiments:

1. Arranging the Experiments: Each peanut seed was inoculated with one of 4 bioagents (*Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis*) in the Petri plates control group without a bioagent.
2. Measures: Seed germination percentage rate, Radicle and Plumule length and seedling vigour were recorded over a specified period.

The *in vitro* treatment schedule details are as follows:

- T₁: Seed treatment with *Trichoderma harzianum*
- T₂: Seed treatment with *Trichoderma viride*
- T₃: Seed treatment with *Pseudomonas fluorescens*
- T₄: Seed treatment with *Bacillus subtilis*
- T₅: Control (without seed treatment)

Pot Experiments:

1. Setting up the Experiment: Groundnut seeds were sown in pots, each treated with one of the bioagents described below or left untreated as a control group.
2. Measures: Seed germination, Root and Shoot length, Plant vigour and disease incidence were measured at regular intervals.

Pot experiment treatment schedule details are as follows

- T₁: Seed biopriming with *Trichoderma harzianum*
- T₂: Seed biopriming with *Trichoderma viride*
- T₃: Seed biopriming with *Pseudomonas fluorescens*

T₄: Seed biopriming with *Bacillus subtilis*

T₅: Seed treatment + *Trichoderma harzianum* with Vermicompost

T₆: Seed treatment + *Trichoderma viride* with Vermicompost

T₇: Vermicompost

T₈: Control

Seed germination, root length, and shoot length were monitored from the first day and plant vigour was monitored on a weekly basis by observation of symptoms and disease incidence was calculated by using the following formula:

$$\text{Disease Incidence (\%)} = \frac{\text{(Number of infected plants)}}{\text{(Total no. of plants)}} \times 100$$

Statistical analysis

Table 1. Per cent germination, plumule length, radicle length and seedling vigour index after seed biopriming of chickpea with different bioagents in vitro conditions

S. No.	Groundnut crop	Per cent germination	Plumule length	Radicle length	Seedling vigour index
	Treatments	Mean	Mean	Mean	Mean
1	T ₁	90	3.03333	2.4	490
2	T ₂	90	3.5	5.93333	842.33333
3	T ₃	85	6.36667	7.63333	1188.1667
4	T ₄	80	2.8	3.8	519.66667
5	T ₅	70	2.33333	2	300.5
	Sem (±)	3.651	1.320	1.647	146.64
	CD (0.05)	NS	-	-	NS

The normality of data was checked, and it found that data are treatment-wise normally distributed. All the treatments were replicated four times in a completely randomized design.

Result and Discussion

In the *in vitro* experiments, *Trichoderma viride* exhibited superior performance with a germination rate of 90%, radicle length of 5.9 cm, and Plumule length of 3.5 cm. *Pseudomonas fluorescens* shows a maximum Plumule length of 10.6 and a 6.3 cm radicle length. Along with *Bacillus subtilis*, it shows Plumule and radicle lengths of 2.8 and 3.8, respectively. At the same time, the control group lagged in all parameters.

In vitro experiment

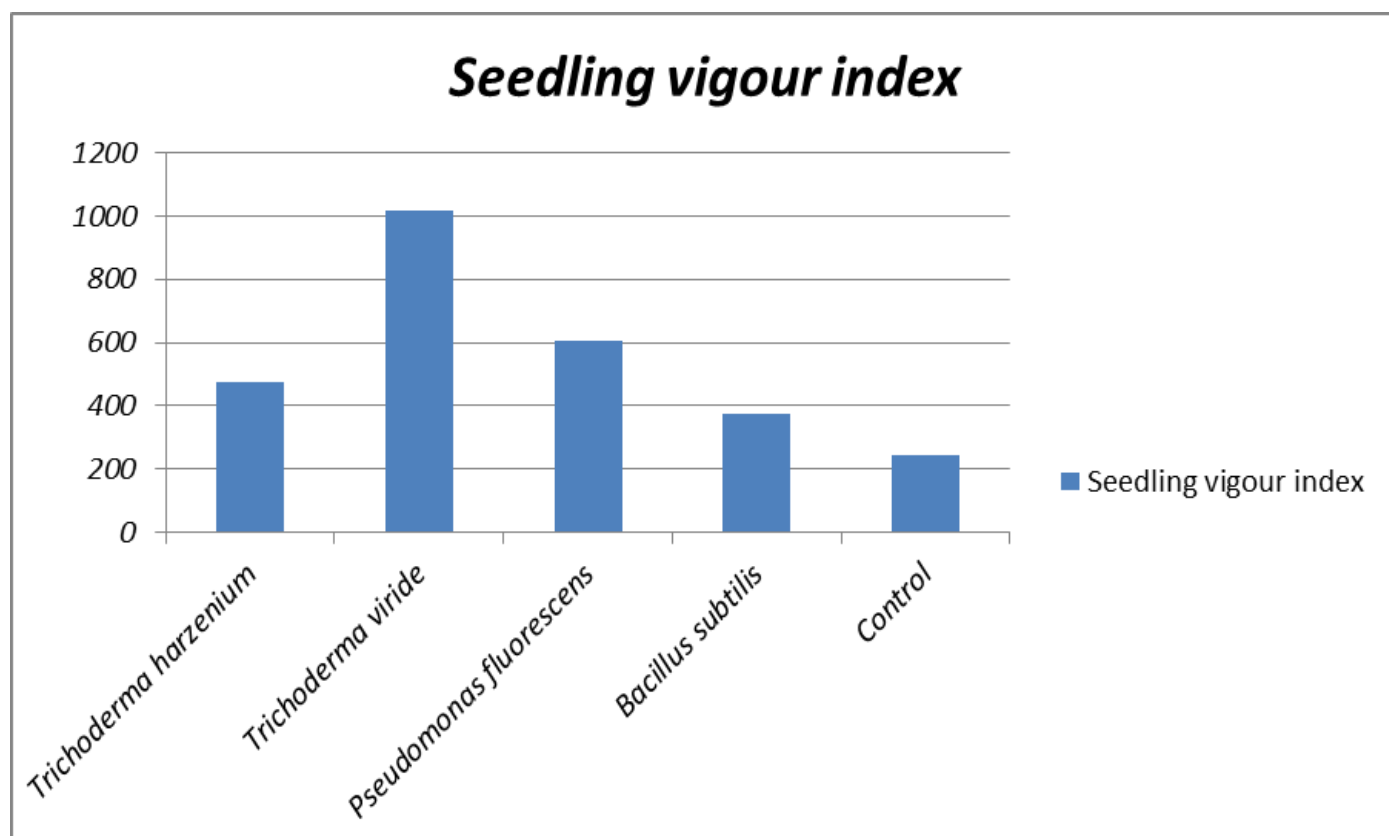
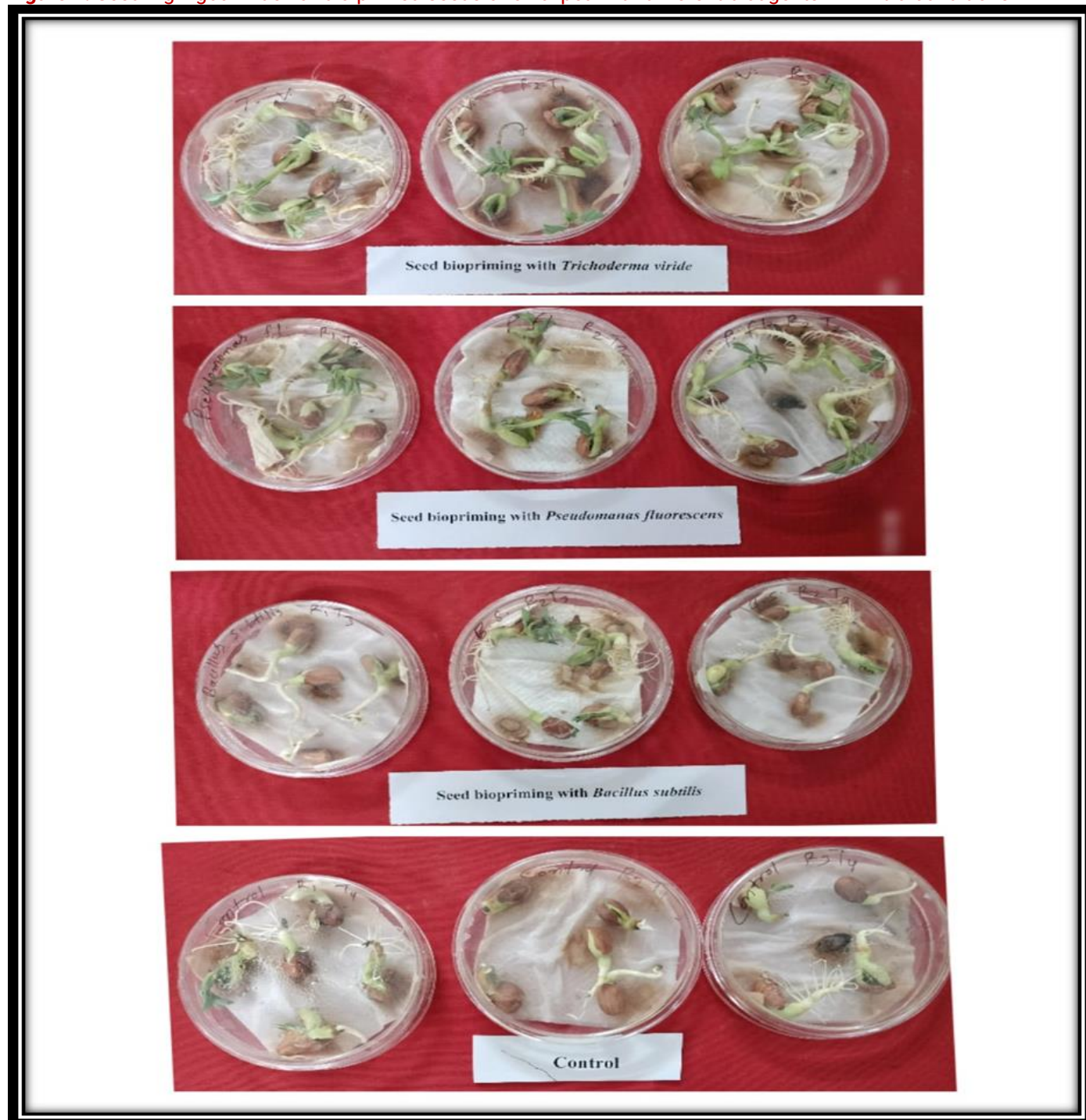


Figure 1. Seedling vigour index of bio-primed seeds of chickpea with different bioagents in *in vitro* conditions**Plate 1.** Seed biopriming of chickpea seeds with different bioagents**Pot experiment**

Pot experiments were conducted to evaluate the efficacy of *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis* and Vermicompost on seed germination, root and shoot length, plant vigour and disease incidence in Groundnut for the year 2023 Kharif season.

Seed treatment with *Trichoderma viride* significantly shows enhanced seed germination, increased shoot and root length and reduced stem rot disease incidence. However, treatment with *Pseudomonas fluorescens* shows excellent increment in shoot length, and *Bacillus subtilis* shows moderate increment in root, shoot length and reduced stem rot disease incidence.

Table 2. Germination %, shoot length, root length, plant vigour and % disease incidence of chickpea on different bioagents in pot conditions

	Treatments	% germination	Shoot length (cm)	Root length (cm)	Plant Vigour	PDI
1	T ₁	75	17.2	7	1815	8

2	T ₂	80		17		7.1		1928		7.5
3	T ₃	75		17.9		7.4		1897		8.5
4	T ₄	70		17.4		7		1709		9
5	T ₅	80		17.8		7.2		2000		6.5
6	T ₆	80		17.5		7.3		1985		6
7	T ₇	70		14		7		1470		12
8	T ₈	70		13.5		6.7		1414		14
	S Em (±)	2.88		0.07		0.05		7		0.11
	CD (0.05)	NS		0.2		0.17		211		0.33

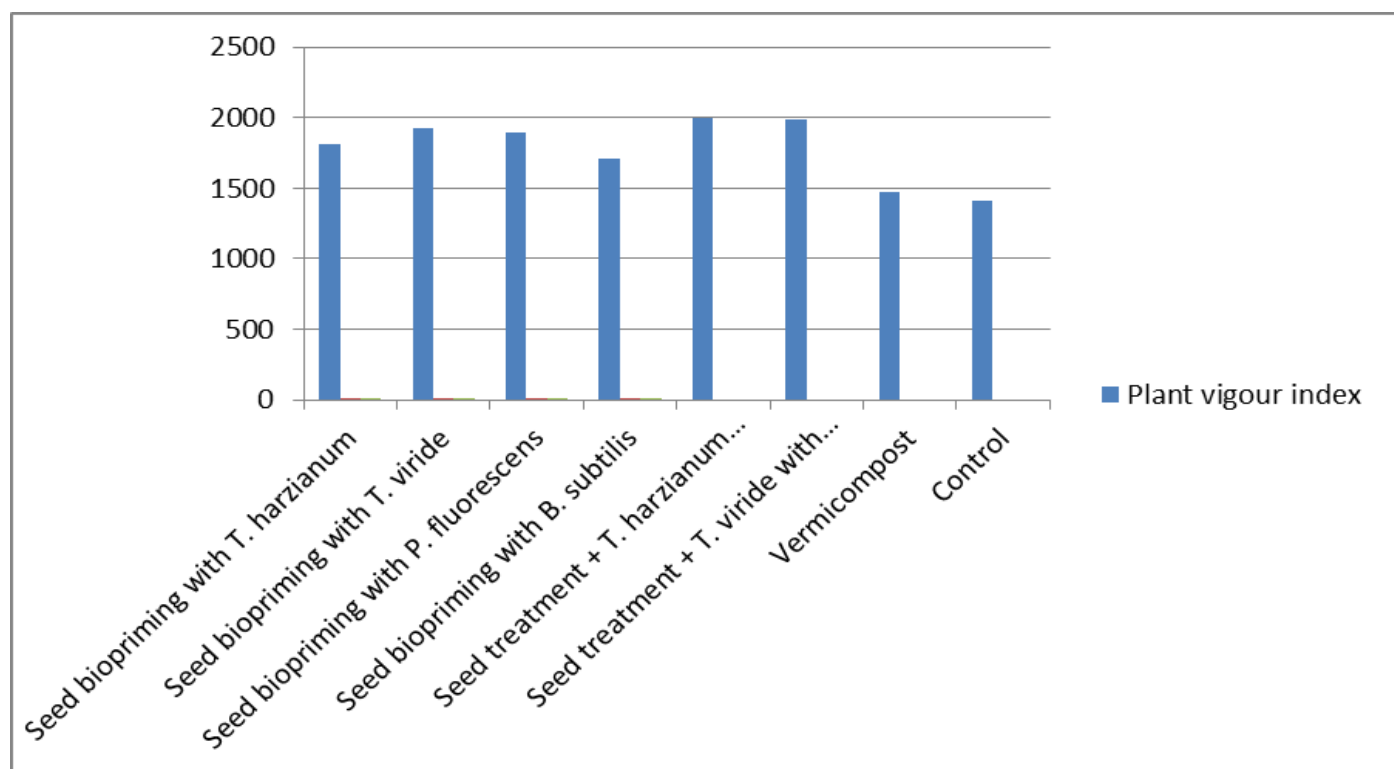
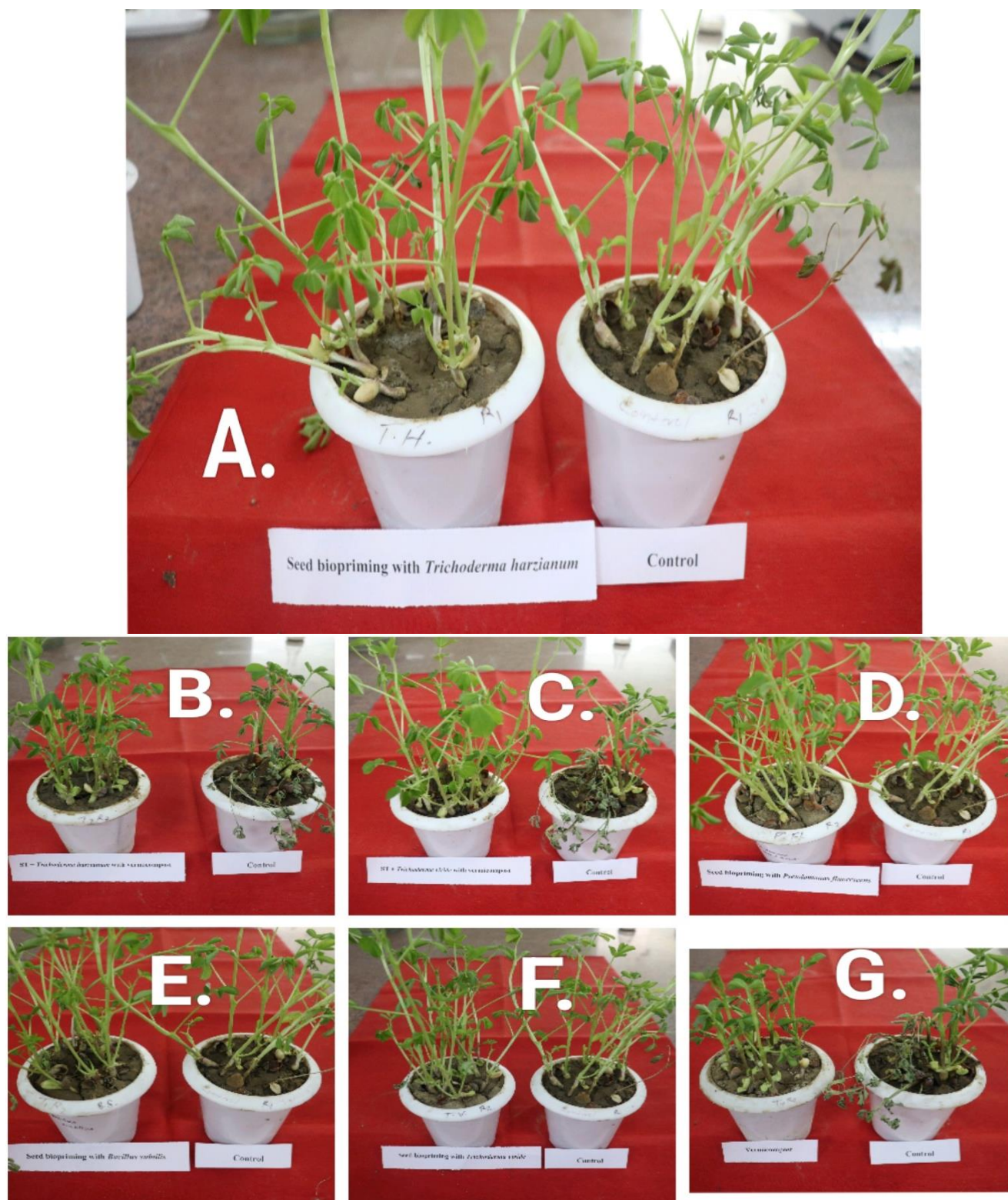


Figure 2. Plant Vigour Index Variation with different Biocontrol Agents in pot conditions



Images of Chickpea pot experiment with different bioagents: A. Seed biopriming with *Trichoderma harzianum* (B.) Seed biopriming with *Trichoderma viride* (C.) Seed biopriming with *Pseudomonas fluorescens* (D.) Seed biopriming with *Bacillus subtilis* (E.) Seed treatment + *Trichoderma harzianum* with Vermicompost (F.) Seed treatment + *Trichoderma viride* with Vermicompost (G.) Vermicompost

Saharan & Rathi (2016) also evaluated three bioagents, *T. viride*, *T. harzianum*, and *P. fluorescens*, for inhibition of mycelial growth of *A. niger* *in vitro*, where *T. viride* inhibited more incredible mycelial growth (78.32%) followed by *T. harzianum* (72.50%), while the bacterial agent *P. fluorescens* inhibited 23.80% mycelial growth. The highest shoot length observed in treatment seed

biopriming with *P. fluorescens* was 17.4 cm. Sharma et al. (2021) also evaluated 15 growth characteristics of groundnut that were influenced positively by the seed treatment of two species of *Trichoderma* and *Pseudomonas fluorescens*. The highest results were obtained in the seed treatment with *Trichoderma* species.

Mayekar et al. (2016) also evaluated six bioagents under *in vitro* against *A. niger*, out of which *Pseudomonas fluorescens* recorded significantly highest mycelial growth inhibition (61.66%) of the *Aspergillus niger*, followed by *T. longibrachiatum* (58.33%), *T. harzianum* (57.41%), *T. koningii* (53.52%), *Bacillus subtilis* (53.15%) and *Penicillium funiculosum* (50.00%).

Conclusion

This comprehensive study highlights the potential of seed biopriming with *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* as a sustainable strategy for enhancing groundnut productivity. The findings emphasize that *Trichoderma viride* and *P. fluorescens* show outstanding performance, offering practical insights for the environmentally friendly application of seed biopriming in sustainable groundnut cultivation.

Declaration of Interests

The authors have no conflict of interest to declare.

Data Sharing

All relevant data are within the manuscript.

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