

Research Article

Management of Stem Rot of Groundnut Incited by *S. Rolfsii* through Important Bioagents

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Abstract

Groundnut is an important oilseed crop of India, grown extensively in various parts of the country. Stem rot caused by (*Sclerotium rolfsii* Sacc.) is the major disease, affecting the groundnut cultivation across the India and worldwide. The main aim of this study was to evaluate the antagonistic potentiality of *T. harzianum* (Th-BKN), *T. viride* (Tv-BKN), *P. fluorescens* (Pf-BKN) and *B. subtilis* (Bs-BKN) against stem rot of groundnut in field conditions. Among the treatment, the treatment module consisting with soil application with *T. harzianum* (Th-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹ gave highest root length, shoot length, fresh weight, dry weight, disease control, pod yield in kgha⁻¹ and lowest disease incidence (9.90 cm, 21.05 cm, 222.60 gm, 65.20 gm, 86.30%, 2173 kgha⁻¹ and 7.51 %) followed by in module consisting with treatment of soil application with *T. viride* (Tv-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹ (9.10 cm, 20.03 cm, 202 gm, 55.03 gm, 79.78%, 1933 kgha⁻¹ and 11.08%,) respectively.

Keywords: *Sclerotium rolfsii*, Fungal bioagents and Bacterial bioagents

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Introduction

Groundnut (*Arachis hypogea* L.) is commonly called peanut, goober pea, pindad, jack nut, manila nut, pygmy nut, pignut and monkey nut [1]. It is known as 'king of oil seeds [2]. It belongs to the family of leguminaceae and originated from South America. It was cultivated as early as 1000 B. C. [3]. It has wide range of cultivation in tropical and subtropical countries in the world. Groundnut is an important oilseed crop of India, grown extensively in various parts of the country in both *Kharif* and *Rabi* seasons. India occupies the first position, both with regard to area and production of groundnut in the world. Oilseed crops have a specific place in Indian agriculture because edible oil is the next to food grain in Indian diet. Groundnut is one of the important oil seed crops of the world and major source of edible oil. The kernel contains 40 to 50 per cent oil and 25 to 30 per cent protein. It also contains 18 per cent carbohydrates and minerals like Ca, Mg and Fe in higher levels in an available form, vitamins B₁, B₂ and niacin are present in a considerable level. It is used in hydrogenation and soap industries. After extraction of the residual oil, the cake contains 7 to 8 per cent nitrogen, which is used both in fertilizer and cattle feed. The groundnut haulms provide nutritive fodder during summer season in dry farming area. It helps in improving soil fertility. Groundnut is grown on 23.25 million hectares worldwide with a total production of 40.08 million metric tonne and productivity of 1676 kgha⁻¹ [4]. Developing countries constitute 97 per cent of the global area and 94 per cent of the global production of this crop. The country major groundnut producing state has brought India's estimated *kharif* groundnut output down 24 per cent [5]. In Rajasthan, it is an important oilseed crop of the semi-arid tropics, cultivated in about 5.16 lakh hectares with an annual production of 10.48 lakh tones and productivity of 2029 kgha⁻¹ [6].

More than 70 diseases due to fungi, bacteria, viruses, nematodes etc. have been reported and estimated yield loss to be up to 70 per cent [7, 8]. One of the most important factors contributing to the low yield is the diseases affecting the crop. The yield is declining due to several factors such as disease incidence, uncertain environmental conditions, low input applications and unavailability of high yielding as well as disease resistant varieties. Collar rot (*Aspergillus niger* Van Teighem), early leaf spot (*Cercospora arachidicola* Hori), late leaf spot (*Cercospora personatum* Berk and Curt), root rot (*Rhizoctonia solani* Kuhn), rust (*Puccinia arachidis* Speg.) and stem rot (*Sclerotium rolfsii* Sacc.) are the major diseases of groundnut prevalent in India [9]. Among the diseases the stem rot disease is quite wide spread across the states due to congenial weather conditions and causes considerable yield losses [10]. *Sclerotium rolfsii* distributed in tropical and subtropical regions of the world where high temperatures prevail. The fungus is seed and

soil borne in nature and has a wide host range of 500 species in about 100 families including groundnut, green bean, lima bean, onion, garden bean, pepper, potato, sweet potato, tomato and water melon [2]. Stem rot is more extensive in the *kharif* than the *rabi* and summer seasons and causes more damage in sandy loam and medium black soil. In India, stem rot incidence occurs in all groundnut growing states and more than 5 lakh/ha of groundnut area are affected by the pathogen. This disease causes severe damage during any stage of crop growth and yield losses over 25% have been reported by Mayee and Datar, (1988) [11] but it can reach over 80% in heavily infected fields [12].

The conventional method to control the diseases in groundnut crop is fungicide application [13-15]. The fungicides presently recommended to manage these diseases provide protection for limited period. The continuous use of traditional fungicides may cause bioaccumulation of the toxic residues besides giving rise to resistant strains. Increased public concern about pesticide utilization and the health hazards necessitates the exploitation of alternative methods of disease control like bioagents. Ramesh and Korikanthimath (2006) [16] reported that seed treatment with biocontrol agents significantly increased the germination percentage (11-23 % and 54-82 per cent over control during 2002 and 2003, respectively) and reduced disease incidence significantly (40-58 and 55-77 %) over control during 2002 and 2003, respectively. Treatment with *T. viride* and *P. fluorescens* increased pod yield by 14-35 % and 22- 33 % over control during 2002 and 2003, respectively. Disease reduction was at par with the carbendazim seed treatment and hence use of bio-control agents as seed treatment with and without soil application could be an effective strategy to reduce root rot disease and enhance yield of groundnut under rainfed conditions. These bioagents are less detrimental, eco-friendly and safer than synthetic pesticides [17]. Biological methods offer an excellent alternate strategy for effective control of various diseases and plant growth promotional activity. Hence, the present investigation was undertaken to find out the potentiality of various fungal and bacterial bioagents for management of stem rot diseases of groundnut under field conditions.

Materials and Methods

Field experiments were conducted in the cropping season of 2016 at the Instructional Research farm of college of Agricultural, Swami Keswananad Agricultural University (SKRAU), Bikaner. The soil of Bikaner is dominant in sandy loam, low to medium in available N, P, and low in K content. The experiments were carried out on fixed sites in under arid agro climatic zones of Rajasthan. The experiment was conducted to study the efficacy of selective fungal and bacterial bioagents as seed treatment, seed treatment along with soil application and soil application with Farm Yard Manure (FYM) for the management of *S. rolfisii* disease of groundnut. The experiment was conducted in randomized block design (RBD) with three replications using the susceptible cultivar 'HNG 10' in 5 x 3 m² plot size with row to row spacing at 30 cm and plant to plant spacing at 10 cm during *Kharif*. The recommended dose of fertilizers @ 20 kg N, 40 kg P and 250 kg Gypsum ha⁻¹ were applied. The crop was sown during the second week of June. Three irrigations were applied at different crop stages and weeding was done as and when required. The talc based formulations of native isolates of *T. viride* (2 x 10⁷cfu g⁻¹) and other fungal and bacterial bio-agents were taken from experiential learning lab mass production of bio-agents, Department of Plant Pathology, College of Agriculture, SKRAU, Bikaner, native isolate identified through ITCC, IARI, New Delhi, Accession number- 7424-09 for studies against the disease. Two drenching of chlorpyrifos @ 2.4 lha⁻¹ was applied for termite control. Different modules of treatments comprising fungal and bacterial antagonists are as follows: T₁-Seed treatment with *T. harzianum* (Th-BKN) @ 10 gkg⁻¹; T₂. Seed treatment with *T. viride* (Tv-BKN) @ 10 gkg⁻¹; T₃- Seed treatment with *P. fluorescens* (Pf-BKN) @ 10 gkg⁻¹; T₄- Seed treatment with *B. subtilis* (Bs-BKN) @ 10 gkg⁻¹; T₅- Seed treatment with *T. harzianum* (Th-BKN) @ 10 gkg⁻¹ + Soil application with *T. harzianum* (Th-BKN) @ 10 kgha⁻¹; T₆- Seed treatment with *T. viride* (Tv-BKN) @ 10 gkg⁻¹ + Soil application with *T. viride* (Tv-BKN) @ 10 kgha⁻¹; T₇- Seed treatment with *P. fluorescens* (Pf-BKN) @ 10 gkg⁻¹ + Soil application with *P. fluorescens* (Pf-BKN) @ 10 kgha⁻¹; T₈- Seed treatment with *B. subtilis* (Bs-BKN) @ 10 gkg⁻¹ + Soil application with *B. subtilis* (Bs-BKN) @ 10 kgha⁻¹; T₉- Soil application with *T. harzianum* (Th-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹; T₁₀- Soil application with *T. viride* (Tv-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹; T₁₁- Soil application with *P. fluorescens* (Pf-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹; T₁₂- Soil application with *B. subtilis* (Bs-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹; T₁₃. Control (Treatment with water). Each treatment was replicated thrice. An observation on per cent disease incidence of stem rot was recorded on plot basis up to 90 days after sowing. Per cent disease incidence and per cent disease control were calculated by using the following formula. DI = [Number of plants infected / Total number of plants observed] x 100 and PDC = [Plant died in control (%) - Plant died in treatment (%) / Plant died in control (%)] x 100. The yield obtained from each plot was recorded after threshing. Data were analysed using arc sin transformation by using software OPSTAT single factor analysis of variance (ANOVA).

Results and Discussion

The results presented in Table 1 and 2 revealed that all the treatment combinations of bioagents significantly reduced the stem rot disease incidence during 2016 and the yield was significantly higher as compared to untreated control. Besides, the disease control, potentiality of bio-agents either alone or with the combination of soil application and FYM on root, shoot fresh weight and dry weight were found superior. Effect of different bio-agents on root length, shoot length, fresh weight and dry weight of groundnut plants were observed. The results given in **Table 1** revealed that root length, shoot length, fresh weight and dry weight of groundnut plants significantly increased in all bio-agent treatments used either as seed treatment, combination with seed treatment and soil application or combination of soil application with FYM. In fungal bioagents, the root length, shoot length, fresh weight and dry weight were significantly higher (9.90 cm, 21.05 cm, 222.60 gm and 65.20 gm) in module consisting with treatment of T₉- SA with *T. harzianum* (Th-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹ followed by (9.10 cm, 20.03 cm, 202 gm and 55.03 gm) in module comprises with treatment of T₁₀- SA with *T. viride* (Tv-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹ respectively. Similarly, the effect of bacterial bio-agents on root and shoot length, fresh and dry weight was found maximum (8.65 cm, 18.07 cm, 193.20 gm and 46.70 gm) in module consisting with treatment T₁₂- SA with *B. subtilis* (Bs-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹; followed by (8.10 cm, 16.09 cm, 178.32 gm and 37.53 gm) in module comprises with treatment of T₁₁- SA with *P. fluorescens* (Pf-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹ as compared to untreated control (6.50 cm, 9.02 cm, 146.60 gm and 22.60 gm) respectively. The results also showed that soil application of bio-agents with FYM were relatively more effective in increasing root and shoot length as compared to individual treatments of seed treatment and combination with seed treatment and soil application.

Table 1 Efficacy of bio-agents on root, shoot length, fresh and dry weight of groundnut plants under field conditions

Treatment	Root length (cm)	Shoot length (cm)	Fresh weight (gm.)	Dry weight (gm.)
T ₁ -ST with <i>T. harzianum</i> (Th-BKN) @ 10 gkg ⁻¹	7.25	14.07	178.00	33.20
T ₂ - ST with <i>T. viride</i> (Tv-BKN) @ 10 gkg ⁻¹	7.10	12.02	166.00	28.60
T ₃ - ST with <i>P. fluorescens</i> (Pf-BKN) @ 10 gkg ⁻¹	6.80	10.01	153.30	25.40
T ₄ - ST with <i>B. subtilis</i> (Bs-BKN) @ 10 gkg ⁻¹	7.00	11.01	160.00	26.33
T ₅ - ST with <i>T. harzianum</i> (Th-BKN) @ 10 gkg ⁻¹ + SA with <i>T. harzianum</i> (Th-BKN) @ 10 kgha ⁻¹	8.21	17.02	186.00	44.33
T ₆ - ST with <i>T. viride</i> (Tv-BKN) @ 10 gkg ⁻¹ + SA with <i>T. viride</i> (Tv-BKN) @ 10 kgha ⁻¹	8.05	16.06	181.60	41.33
T ₇ - ST with <i>P. fluorescens</i> (Pf-BKN) @ 10 gkg ⁻¹ + SA with <i>P. fluorescens</i> (Pf-BKN) @ 10 kgha ⁻¹	7.15	13.08	174.00	31.00
T ₈ - ST with <i>B. subtilis</i> (Bs-BKN) @ 10 gkg ⁻¹ + SA with <i>B. subtilis</i> (Bs-BKN) @ 10 kgha ⁻¹	7.50	15.05	176.60	32.00
T ₉ - SA with <i>T. harzianum</i> (Th-BKN) @ 10 kgha ⁻¹ + FYM @ 10 tonn.ha ⁻¹	9.90	21.05	222.60	65.20
T ₁₀ - SA with <i>T. viride</i> (Tv-BKN) @ 10 kgha ⁻¹ + FYM @ 10 tonn.ha ⁻¹	9.10	20.03	202.00	55.03
T ₁₁ - SA with <i>P. fluorescens</i> (Pf-BKN) @ 10 kgha ⁻¹ + FYM @ 10 tonn.ha ⁻¹	8.10	16.09	178.32	37.53
T ₁₂ - SA with <i>B. subtilis</i> (Bs-BKN) @ 10 kgha ⁻¹ + FYM @ 10 tonn.ha ⁻¹	8.65	18.07	193.20	46.70
T ₁₃ . Control (Treatment with water)	6.50	9.02	146.60	22.60
SEm (±)	0.25	0.54	5.69	1.59
CD (P=0.05)	0.78	1.66	17.54	4.88
CV (%)	5.64	6.30	7.03	7.29

ST- Seed treatment; SA- Soil application

There are several evidences regarding positive influence of bio-agents like *Trichoderma* spp., *P. fluorescens* and *B. subtilis*, etc. on root and shoot length and vigour of plants. During the present studies, observations on root and shoot length of groundnut plants grown in fields were recorded at the time of harvest. The bio-agent treatments positively influenced the root and shoot length of groundnut plants. The results indicated that the treatments of bio-agents used as soil application combined with FYM were relatively more effective in increasing root and shoot length compared to individual treatment and combination with seed treatment and soil application. The effectiveness of these

isolate in enhancing the growth parameters *i.e.* shoot and root length in groundnut was also reported by [18-20] that were corroborated to our findings. The treatment effect on biomass of groundnut plants was also evaluated in present study. The bio-agent treatments used alone and in combination positively influenced the fresh weight and dry weight of groundnut plants. Talc based formulation of *T. harzianum* (Th-BKN) used as soil application with FYM promoted maximum fresh and dry weight followed by *T. viride* (Tv-BKN) and *B. subtilis* (Bs-BKN). While in case of bio-agent as combination with seed treatment and soil application, *T. harzianum* (Th-BKN) significantly increased fresh and dry weight of groundnut as compare to other three bio agents *i.e.* *T. viride* (Tv-BKN), *B. subtilis* (Bs-BKN) and *P. fluorescens* (Pf-BKN). Similar results were evaluated by seven isolates of two different *Trichoderma spp.* that were isolated from the rhizosphere of groundnut from different locations of Manipur. *In vivo* study on efficacy of two potent isolates showed reduction in stem rot of groundnut among the different treatments (seed, soil and seed plus soil). The treatment with seed + soil of *Trichoderma* isolates TvG1 and TvG2 recorded the highest plant height, plant canopy, fresh weight, dry weight and maximum pod yield [21].

Efficacy of different bio-agents on per cent disease incidence and control of stem rot and pod yield of groundnut was recorded. The results depicted from **Table 2** revealed that all the module consisting with bioagents either alone or with combinations were found superior in reducing the stem rot disease incidence and impart maximum pod yield as compared to control. In fungal bioagents, the disease incidence, disease control and pod yield in kg ha^{-1} were significantly higher (7.51 %, 86.30% and 2173 kg ha^{-1}) in module consisting with treatment of T₉- SA with *T. harzianum* (Th-BKN) @ 10 kg ha^{-1} + FYM @ 10 tonn. ha^{-1} followed by (11.08%, 79.78% and 1933 kg ha^{-1}) in module comprises with treatment of T₁₀- SA with *T. viride* (Tv-BKN) @ 10 kg ha^{-1} + FYM @ 10 tonn. ha^{-1} respectively. Similarly, the effect of bacterial bio-agents on disease incidence, disease control and pod yield in kg ha^{-1} were found maximum (13.72%, 74.96% and 1833 kg ha^{-1}) in module consisting with treatment T₁₂- SA with *B. subtilis* (Bs-BKN) @ 10 kg ha^{-1} + FYM @ 10 tonn. ha^{-1} ; followed by (18.05%, 67.06% and 1586 kg ha^{-1}) in module comprises with treatment of T₁₁- SA with *P. fluorescens* (Pf-BKN) @ 10 kg ha^{-1} + FYM @ 10 tonn. ha^{-1} respectively. Conclusively, the bio-agents used as soil application in combined with FYM reduced the disease incidence more effectively as compared to seed treatment and combination with seed treatment and soil application of bio-agents. All the bioagents treatments significantly enhanced the crop production, minimize seedling mortality percentage and increase per cent survival of groundnut plants.

Table 2 Management of stem rot of groundnut through important bio- agents

Treatment	Disease incidence (%)	Disease control (%)	Yield (kg ha^{-1})
T ₁ -ST with <i>T. harzianum</i> (Th-BKN) @ 10 g kg^{-1}	24.34 (29.56)*	55.58	1400
T ₂ - ST with <i>T. viride</i> (Tv-BKN) @ 10 g kg^{-1}	25.83 (30.55)	52.86	1320
T ₃ - ST with <i>P. fluorescens</i> (Pf-BKN) @ 10 g kg^{-1}	28.10 (32.01)	48.72	1166
T ₄ - ST with <i>B. subtilis</i> (Bs-BKN) @ 10 g kg^{-1}	27.13 (31.39)	50.49	1273
T ₅ - ST with <i>T. harzianum</i> (Th-BKN) @ 10 g kg^{-1} + SA with <i>T. harzianum</i> (Th-BKN) @ 10 kg ha^{-1}	14.55 (22.42)	73.44	1766
T ₆ - ST with <i>T. viride</i> (Tv-BKN) @ 10 g kg^{-1} + SA with <i>T. viride</i> (Tv-BKN) @ 10 kg ha^{-1}	16.17 (23.71)	70.49	1700
T ₇ - ST with <i>P. fluorescens</i> (Pf-BKN) @ 10 g kg^{-1} + SA with <i>P. fluorescens</i> (Pf-BKN) @ 10 kg ha^{-1}	22.16 (28.08)	59.56	1433
T ₈ - ST with <i>B. subtilis</i> (Bs-BKN) @ 10 g kg^{-1} + SA with <i>B. subtilis</i> (Bs-BKN) @ 10 kg ha^{-1}	19.9 (26.49)	63.68	1573
T ₉ - SA with <i>T. harzianum</i> (Th-BKN) @ 10 kg ha^{-1} + FYM @ 10 tonn. ha^{-1}	7.51 (15.91)	86.30	2173
T ₁₀ - SA with <i>T. viride</i> (Tv-BKN) @ 10 kg ha^{-1} + FYM @ 10 tonn. ha^{-1}	11.08 (19.44)	79.78	1933
T ₁₁ - SA with <i>P. fluorescens</i> (Pf-BKN) @ 10 kg ha^{-1} + FYM @ 10 tonn. ha^{-1}	18.05 (25.14)	67.06	1586
T ₁₂ - SA with <i>B. subtilis</i> (Bs-BKN) @ 10 kg ha^{-1} + FYM @ 10 tonn. ha^{-1}	13.72 (21.74)	74.96	1833
T ₁₃ - Control (Treatment with water)	54.80 (59.45)	-	1080
SEm (\pm)	0.89		78.82
CD (P=0.05)	2.75		242.2
CV (%)	7.77		10.04

*Figures in parentheses are angular transformed values; ST- Seed treatment; SA- Soil application

Singh and Singh (2012) and Upadhyay and Mukopadhyay (1983) [19, 22] also found significant effect of treatments with respect to reduce disease incidence, disease control and enhanced the plant yield production that were supported to our investigation. Potentiality of many bio-agents viz., *Trichoderma* spp., *Gliocladium virens*, *Bacillus subtilis*, *Streptomyces* spp., etc against soil borne pathogens including groundnut in field conditions has been reported in many host pathogen interaction by earlier workers [23, 24]. Biswas and Sen (2000) [25] reported that stem rot of groundnut caused by *S. rolfisii* was significantly reduced by *T. harzianum* when delivered as seed dressing or soil application in the pot trials. Per cent disease reduction through seed dressing was 33 to 50 per cent and through direct soil application it was 72 to 83 per cent over control. Similarly, Ganesan *et al.* (2007) [26] reported that the integrated management of stem rot disease (*Sclerotium rolfisii*) of groundnut (*Arachis hypogaea* L.) using *Rhizobium* and *Trichoderma harzianum* (ITCC - 4572). In the present study the combined application of *Rhizobium* and *Trichoderma harzianum* (ITCC – 4572) was performed. The results indicated that the application of these native micro-organisms successfully decreases the stem rot incidence and also increases the growth of the groundnut plants. The plant growth promoting activity and disease control ability of these microbial agents are discussed. Karthikeyan *et al.* (2006) [27] reported that one among the three isolates of *T. viride*, one isolate each of *T. harzianum* and *Pseudomonas fluorescens* were inhibitory to the growth of *S. rolfisii* the causal agent of stem rot of groundnut that are also supported to our study. The effect of different bio-agent treatments on grain yield of groundnut was also recorded during the present investigation. The study clearly showed that groundnut grain yield was considerably enhanced due to bio-agent treatments used as seed treatment, in combination with seed treatments and soil application and combined as soil application with FYM. The grain yield was highest (2173 kgha⁻¹) in *T. harzianum* (Th-BKN) used as soil application (10 kgha⁻¹) with FYM (10 tonn. ha⁻¹) followed by *T. viride* (Tv-BKN) combined with FYM, Besides, the combination treatment of *T. harzianum* (Th-BKN) used as seed treatment and soil application led to significant increase in grain yield. The reduction in soil borne plant diseases and subsequent enhancement in the yield of different crops after treatment with formulations of *T. harzianum* has been reported by several workers [21, 28, 29].

Based on our present studies, it is concluded that the treatment module consisting with soil application with *T. harzianum* (Th-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹ gave highest root length, shoot length, fresh weight, dry weight, disease control, pod yield in kgha⁻¹ and lowest disease incidence (9.90 cm, 21.05 cm, 222.60 gm, 65.20 gm, 86.30%, 2173 kgha⁻¹ and 7.51 %) followed by in module consisting with treatment of soil application with *T. viride* (Tv-BKN) @ 10 kgha⁻¹ + FYM @ 10 tonn.ha⁻¹ (9.10 cm, 20.03 cm, 202 gm, 55.03 gm, 79.78%, 1933 kgha⁻¹ and 11.08%,) respectively.

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