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Research Article

Glass House Studies on Management of Groundnut Stem Rot using *Trichoderma harzianum* and *Pseudomonas fluorescens*

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ABSTRACT

In the present investigation on "Efficacy of combined bioformulation of Pseudomonas *fluorescens and Trichoderma spp. in the management of stem rot of groundnut", six native rhizosphere isolates of Trichoderma spp. and five native rhizosphere isolates of P. fluorescens were isolated, screened for their biocontrol potential against Sclerotium rolfsii, incitants of stem rot of groundnut under in vitro condition. The potential isolates were further assessed in vivo in pot culture. Among six Trichoderma isolates tested, isolate GRT4 recorded highest mean inhibition (74.69%) followed by GRT2 (73.85%) which were on par with each other. Out of five isolates of P. fluorescens tested, isolate PF3 was superior with highest mean inhibition (40.93 %) which was significantly differed with the rest of the isolates. The efficacy of potential fungal antagonist Trichoderma isolate GRT4, bacterial antagonist PF4 was tested in pot culture against stem rot of groundnut. Among the twelve treatments imposed, treatment* T_{10} *i.e. seed treatment with P. fluorescens @ 10 g + Trichoderma spp. @ 8 g* kg^{-1} *of seed along with soil treatment with combined bioformulation @ 2 L + 80 kg of FYM + 5 kg of neem cake* $acre^{-1}$ *was found to be superior as it recorded the highest germination percentage (93.33%), highest initial population (9.33), final population (8.33) and least per cent disease incidence of 11.11 per cent. This treatment also recorded maximum shoot length (25.28 cm), root length (30.79 cm) and maximum fresh and dry weights i.e. 11.35 g and 2.11 g, respectively when compared to other treatments.*

Keywords: *Tricoderma harzianum, Pseudomonas fluorescens, Sclerotium rolsfii, Percent Disease Incidence (PDI).*

INTRODUCTION

Groundnut is one of the major oil seed crops grown in India in an area of 4.89 M ha with a production and productivity of 9.25 M t and 1.89 t ha^{-1}, respectively. In Andhra Pradesh, it is grown over an area of 0.74 M ha with a production of 1.05 M t and productivity of 1.42 t ha^{-1}. Anantapuramu, Chittoor, Kurnool and Kadapa are the major groundnut growing districts of Andhra Pradesh during *kharif* (Directorate of Economics and Statistics, 2017-18).

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Several factors such as water stress, pests, and diseases are responsible for the low productivity of groundnut in A.P (1.42 t ha-1) compared with national average (1.89 t ha-1). Stem rot of groundnut is one of the major soil borne diseases of groundnut with considerable yield losses.

The pathogen *S. rolfsii* Sacc., is a soil borne plant pathogen that commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, stem rot, wilt and foot rot on more than 500 plant species including almost all the agricultural and horticultural crops (Aycock, 1966 & Domsch et al., 1980). Stem rot causes pod yield losses of 10-25 per cent but under severe diseased conditions yield losses may range to up 80 per cent (Rodriguez Kabana et al., 1975).

S. rolfsii being soil borne necrotroph with very high competitive saprophytic ability causes immediate knock down effect on plant by producing organic acids like oxalic acid and enzymes like cellulases. Hence, yield losses caused by stem rot are directly proportional to the disease incidence. Once established in the soil, the soil turns sick and management of the disease becomes exceedingly difficult.

Biocontrol agents like *P. fluorescens* and *Trichoderma* spp. have been assessed for their efficacy against *S. rolfsii* (Khalili et al., 2016). Application of biocontrol agents gives long term protection from the soil borne pathogens besides eliminating the problem of environmental pollution which is the major setback of chemical management.

Application of two different biocontrol agents or two strains of the same biocontrol agent with different mechanisms of action gives the advantage of complementing each other in nullifying the deleterious effect of plant pathogens (Rajasekhar et al., 2016).

MATERIALS AND METHODS

Isolation and Morphological Characterization of *S. rolfsii*:

The pathogen *S. rolfsii* was isolated from sclerotial bodies of pathogen by surface sterilizing with 1 per cent sodium hypochlorite for 2 minutes, followed by three washings in sterile distilled water to remove traces of sodium hypochlorite on sclerotia. Later, the sterilized sclerotial bodies were placed on Petri plates containing 20 ml of sterilized potato dextrose agar (PDA) media, incubated at 28 ± 2^{o}C in an incubator and observed periodically for growth of the fungus. The fungal cultures were purified by single hyphal tip method and maintained on PDA. The test pathogen was identified based on their mycelial and sclerotial characters described by Barnett and Hunter, (1972).

Morphological characterization *S. rolfsii* was done by observing colony characters and sclerotial size, shape and colour.

Isolation of Biocontrol Agents

For isolating *Trichoderma* spp., soil sample was collected from rhizosphere of healthy groundnut plants. Later, the biocontrol agents were obtained on *Trichoderma* selective medium by performing serial dilution technique (Johnson & Curl, 1977). The fungi were further purified following single hyphal tip method and maintained on PDA slants.

The native antagonistic *P. fluorescens* were isolated from collected soil samples following serial dilution technique on King's B medium (Johnson & Curl, 1977). One day old colonies of bacteria were picked up, purified by streak plate method and maintained on nutrient agar (NA) slants.

Identification of Biocontrol agents

Taxonomic identification of six isolates of *Trichoderma* spp. upto species level was done based on morphological characteristics. For microscopic study, lactophenol blue staining was done for proper visualization of characteristic features. The microscopic images of *Trichoderma* spp. were taken and identified by using available literature (Bisset, 1984, 1991 a, b, c).

Identification of P. fluorescens isolates was done by following Gram's staining and visualization under U.V. light for florescent pigmentation (Manjunatha et al., 2012).

Antagonistic Potential of Fungal and Bacterial Antagonists *In Vitro*

Dual culture technique was used to identify the potential antagonists from rhizosphere of groundnut (Morton & Straube, 1955).

For finding the potential fungal antagonist against test pathogen, 5 mm mycelial disc of fungal antagonist was placed at 1 cm away from the periphery of 9 cm sterile Petri plate containing 20 ml of sterile PDA. Then, 5 mm mycelial disc of the test pathogen was placed opposite to the mycelial disc of fungal antagonist at 1 cm away from the periphery of Petri plate. The plates were kept in incubator at 25 ± 2^{o}C for incubation. Readings were recorded when the pathogen in the monoculture control grown fully.

For finding the potential bacterial antagonist, the bacterium was streaked as 5 cm line at 1 cm away from the periphery of Petri plate containing 20 ml of equal amounts of PDA and NA. Then, 5 mm mycelial disc of test pathogen was placed in the centre of the Petri plate. The plates were kept in incubator at 25 ± 2^{o}C for incubation. Readings were taken when the pathogen in the monoculture control grown fully.

Per cent inhibition of mycelial growth of test pathogen over control for both fungal and bacterial biocontrol agents was calculated using the formula given by Vincent (1927).

$$I=\frac{C-T}{C}\times 100$$

where,

I = Per cent reduction in growth of test pathogen.

C = Radial growth (mm) in monocultured check.

T = Radial growth (mm) in dual cultured plates.

Compatibility Between Fungal and Bacterial Antagonists *In Vitro*

The compatibility between potential fungal and bacterial antagonists was determined by dual culture technique (Morton & Stroube, 1955) maintaining separate controls for bacteria and fungus under *in vitro* conditions.

For finding the compatibility between potential fungal and bacterial antagonists, 5 mm mycelial disc of fungal antagonist was placed in the centre of the Petri plate containing equal amounts of PDA and NA. Then, the bacterial antagonist was streaked as 5 cm line at 1 cm away from the periphery of 9 cm Petri plate on both sides of the fungal antagonist. The Petri plate containing only fungal antagonist was treated as control. The plates were kept in incubator at 25 ± 2°C, observations were recorded as zone of inhibition till the fungal antagonist completely occupied the plate in monoculture check.

Per cent inhibition of mycelial growth of fungal antagonist over control was calculated using the formula given by Vincent (1927).

$$I=\frac{C-T}{C}\times 100$$

where,

I = Per cent reduction in growth of test pathogen.

C = Radial growth (mm) in monocultured check.

T = Radial growth (mm) in dual cultured plates.

Mass Multiplication of Potential Biocontrol Agents

Potential *Trichoderma* isolate was mass multiplied on potato dextrose broth (PDB) by inoculating 4-5 discs of three day old *Trichoderma* culture and incubated in a shaking incubator at 25 ± 2°C for 7 days.

Potential *P. fluorescens* isolate was mass multiplied on nutrient broth (NB). For this, two loops full of bacterial culture was added to the medium and incubated in a shaking incubator at 25 ± 2°C for three days.

Mass Multiplication of *S. rolfsii*

The test pathogen *S. rolfsii* was mass multiplied on sterilized sorghum seeds for pot culture studies. For this, 100g of sorghum seeds were washed thoroughly in tap water and soaked in water overnight with addition of 20 ml of 4 per cent dextrose. After removing the water, the seeds were half boiled using pressure cooker and dried on sterilized bench top until the moisture content of the seeds reach to twenty per cent. Later the seeds were transferred to 250 ml conical flasks, autoclaved for 20 minutes at 15 p.s.i. The flasks containing sorghum seeds were inoculated with 2-3 discs of 4 d old culture of test pathogen (*S. rolfsii*).

Formulation of Biocontrol agents

The potential isolates of both fungal and bacterial antagonistic isolates were formulated using talc as carrier material following procedure developed by Vidhyasekaran and Muthamilan (1995).

Liquid Formulations of potential Trichoderma sp. and P. fluorescens isolates was done using paraffin oil + soybean oil (1:1) and glycerol amended NB as liquid carrier materials, respectively (Sathiyaseelan et al., 2009 & Manikandan et al., 2010).

Pot Culture Studies on Management of Stem Rot of Groundnut using Biocontrol Agents

Pot Culture Studies

The experiment was conducted under glasshouse conditions. In this experiment, the mixed formulations of compatible antagonists along with effective fungicide was evaluated against stem rot of groundnut as per the treatments given below:

Treatment No.	Treatment
T_1	Seed treatment with *P. fluorescens* @ 5 g + *Trichoderma* spp. @ 4 g kg^{-1} of seed
T_2	Seed treatment with *P. fluorescens* @ 10 g + *Trichoderma* spp. @ 8 g kg^{-1} of seed
T_3	Seed treatment with *P. fluorescens* @ 5 ml + *Trichoderma* spp. @ 3 ml kg^{-1} of seed
T_4	Soil treatment with combined bioformulation @ 2 kg + 80 kg of FYM + 5 kg of neem cake acre^{-1}
T_5	Soil treatment with combined bioformulation @ 2 L+ 80 Kg of FYM + 5 kg of Neem cake acre^{-1}
T_6	Seed treatment with *Trichoderma* spp. @ 8 g kg^{-1} of seed alone
T_7	Seed treatment with *P. fluorescens* @ 10 g kg^{-1} of seed alone
T_8	$T_1 + T_4$
T_9	$T_2 + T_4$
T_{10}	$T_2 + T_5$
T_{11}	Seed treatment with Tebuconazole @ 1.5 g kg^{-1} of seed
T_{12}	Control

Variety: Narayani; Design: CRD; Replications: Three

Seed Treatment

Groundnut seeds were treated with talc based formulation of potential fungal and bacterial biocontrol agents individually and in different combinations. Later, the seeds were used for sowing. For treatment with fungicide, the groundnut seeds were treated with Tebuconazole @ 1.5 g kg^{-1} of seeds and sown in the pathogen infested soil in the pots @10 seeds per pot. Seeds sown without application of formulations of biocontrol agents and fungicide served as control.

Soil Application

The liquid formulations of potential fungal and bacterial biocontrol agents were applied @ 2 L + 80 kg of FYM + 5 kg of neem cake $acre^{-1}$ before sowing.

Observations

Observations on germination percentage, initial population, final population, per cent disease incidence (PDI), shoot length, root length, fresh weight and dry weight were made at 60 days after sowing (DAS) when the plants displayed stem rot symptoms.

RESULTS AND DISCUSSION

Isolation and Characterization of *S. rolfsii*

The pathogen *S. rolfsii* was isolated from sclerotial bodies collected near the collar region of the infected plants from Rangampeta (RgSr), R.A.R.S. fields of Tirupati (TpSr) in Chittoor and Damaramadugu (DmSr), Sangam (SgSr) regions in S.P.S.R. Nellore districts. In an earlier study, Gupta and Sharma (2004) isolated *S. rolfsii* from leaves, stem and pods of groundnut.

The mycelium of the fungus was silky white in colour later turned to dull white with radial spread giving fan like appearance. At maturity, small mycelial knots were formed in the culture which later gave rise to whitish sclerotial bodies again turned to deep brown to tan coloured mustard seed like structures. The matured sclerotia were shiny, hard and spherical in shape. Similar reports on mycological characters of *S. rolfsii* were observed by Hemalatha et al. (2006).

All the four isolates showed variability with respect to number of sclerotia per Petri plate, size and colour of sclerotia after ten days of incubation. The isolate SgSr formed maximum number of sclerotia (186) per Petri plate having highest diam of 0.90 mm with dark brown colour followed by RgSr (134) having 0.81 mm diam with dark brown sclerotia and DmSr (103) having 0.65 mm diam with light brown sclerotia. The isolate TpSr formed least number of sclerotia (86) per Petri plate having 0.84 mm diam with light brown colour. The mycelia of RgSr, SgSr and DmSr was partially fluffy while it was highly fluffy in TpSr.

The pathogen identification as *S. rolfsii* was confirmed based on observations of Domsch et al. (1980), who described that colonies of *S. rolfsii* was fast growing reaching about 9 cm diam in three days at 23°C. Colony is white with many hyphal strands. Sclerotia are superficial, abundantly produced near the colony margins, globose, smooth walled, brown in colour and size ranging from 1-2 mm diam (average 1.2 mm).

Isolation and Identification of *Trichoderma* Spp

A total of six rhizosphere soil samples were collected from healthy groundnut plants from different mandals of Chittoor and S.P.S.R. Nellore districts. From these soil samples, six *Trichoderma* isolates were isolated on *Trichoderma* selective medium following serial dilution technique and maintained on PDA slants for further experimental studies. The isolates obtained from Chittoor were indicated as GRT1, GRT2, GRT3 while, isolates obtained from S.P.S.R. Nellore districts were indicated as GRT4, GRT5 and GRT6.

Species-level identification of *Trichoderma* isolates was done based on the colour of the colony, formation of chlamydospores, branching of conidiophores, shape and disposition of phialides, shape of conidia as the main characters to identify the species (Gams & Bisset, 1998). Based on above characters under observations, isolates

GRT1, GRT3, GRT4, GRT6 were identified as *T. harzianum* and GRT2, GRT5 as *T. viride*.

In a similar study, Sundaramoorthy and Balabaskar (2013) isolated native fungal antagonists from tomato rhizosphere soils by serial dilution technique using *Trichoderma* selective medium and identified them as *T. hamatum*, *T. harzianum*, *T. koningi*, *T. longiconis* and *T. viride*.

Isolation and Identification of *P. fluorescens*

The native antagonistic *P. fluorescens* isolates were isolated from the soil samples collected from rhizosphere of healthy groundnut plants in different regions of Chittoor and S.P.S.R. Nellore districts on King's B medium following serial dilution technique. A total of five isolates were isolated from these soil samples, purified by streak plate method and maintained on NA slants for further experimental studies. Out of five isolates obtained, three isolates i.e. PF1, PF2, PF3 are from Chittoor district and two isolates (PF4 & PF5) are from S.P.S.R Nellore district.

The identification of *P. fluorescens* isolates was done based on Gram's staining followed by visualising under microscope. All the isolates were found Gram negative in reaction and emitted fluorescens when visualised under U. V. light. Based on these features the bacteria were identified as *P. fluorescens.*

The results were similar with the reports of Duffy et al. (1996), who found that in the Pacific Northwest, *P. fluorescens* have been associated with disease- suppression soils.

Screening of Antagonistic *Trichoderma* spp. Against *S. rolfsii*

Six isolates of *Trichoderma* spp. were assessed for their antagonist potential against four isolates of *S. rolfsii*, incitant of groundnut stem rot by following dual culture method *in vitro*. The results were analyzed using two factorial CRD and data was presented in table 1.

Among the six isolates of *Trichoderma* spp. tested against four isolates of *S. rolfsii*, maximum mean inhibition per cent (74.69%) was observed with the GRT5 isolate followed by GRT2 (73.85%), GRT1 (71.56%), GRT4 (70.42%) and GRT6 (67.81%). The isolate GRT5 was significantly differed with all the remaining isolates except GRT2 (73.85%). Lowest inhibition per cent (66.98%) was observed with the isolate GRT3 which was significantly differed with all the remaining isolates except isolate GRT6 (67.81%).

From the above study, better performing four isolates of *Trichoderma* which showed maximum mean inhibition per cent when dual cultured with *S. rolfsii viz.*, GRT5, GRT2, GRT1 and GRT4 were considered as effective *Trichoderma* isolates and taken for further studies. The isolate RgSr, which exhibited least minimum per cent inhibition (64.03%) when dual cultured with *Trichoderma* spp. was considered as a virulent pathogen isolate and taken for further studies.

These results were in the agreement with the findings of Deepthi et al. (2014), who reported Trichoderma isolate acted as a potential biocontrol agent against soil borne pathogenic fungi when treated in dual culture method.

Screening of Antagonistic *P. fluorescens* Against *S. rolfsii*

Five isolates of *P. fluorescens* were assessed for their antagonistic potential against four isolates of *S. rolfsii* causing groundnut stem rot by following dual culture method *in vitro*. The treatments were replicated thrice, results were analysed using two factorial CRD and data was presented in table 2.

Among the five isolates of *P. fluorescens* tested against four isolates of *S. rolfsii*, maximum mean inhibition per cent (40.93%) was observed with PF3 isolate, which is significantly differed with PF4 (34.82%), PF1 (24.26%) and PF2 (13.89%). Lowest mean inhibition per cent (4.81%) was observed with PF5 isolate.

Based on the above study two isolates of *P. fluorescens* (PF3 and PF4) that showed maximum mean inhibition per cent when dual cultured with *S. rolfsii* were considered as effective isolates and used for further studies. When mean inhibition per cent of pathogen isolate was observed, minimum mean inhibition per cent (18.82 %) was showed by RgSr isolate against all five bacterial biocontrol agents, which was significantly differed with all the remaining isolates of pathogen was considered as virulent pathogen isolate and used for further studies.

The results agreed with the findings of Ganesan and Sekar (2012), who evaluated biocontrol activity of eleven *Pseudomonas* isolates against *S. rolfsii*, causing stem rot disease in groundnut, by dual culture method. Among all, seven isolates showed above 68 per cent of inhibition. Mechanisms of biocontrol activity of the *Pseudomonas* isolates were determined by studying production of non-volatile compound (antibiosis), volatile compound (HCN production), siderophore (microbial iron transport agents) and chitinase activity (lysis).

Evaluation of Compatibility Between Potential *Trichoderma* spp. and *P. fluorescens*

The potential isolates of *Trichoderma viz.*, GRT1, GRT2, GRT4 and GRT5 were dual cultured with potential isolates of *P. fluorescens viz.*, PF3 and PF4 for testing the compatibility among them. The treatments were replicated thrice and the data was presented in the table 3.

Initially minimum inhibition per cent (21.48%) was observed in GRT4 (*T. harzianum*) and PF4 dual culture combination, which was significantly differed with all the remaining treatments. After prolonged incubation for ten days the inhibition zone disappeared and mycelium of GRT4 was overgrown on bacteria and occupied full plate. Therefore from the above study the GRT4 isolate (*T. harzianum*) and PF4 isolate of (*P. fluorescens*) were considered as most compatible combination and taken further for formulations preparation.

Similar reports were obtained by Mishra et al. (2013) who reported that *Trichoderma* isolate (PBAT-43) and *Pseudomonas* isolate (PBAP-27) emerged as most compatible and efficient combination in dual culture and therefore used in development of mixed formulations.

Mass Multiplication of *S. rolfsii*

The virulent pathogen isolate of groundnut stem rot (RgSr) from dual culture studies was mass multiplied on sorghum grains and added to the sterilized soil in pots @ 100 g kg^{-1} at the time of sowing.

In an earlier report, sterilized sorghum grains were used as substrate by Patibanda et al. (2002) for mass multiplication of *S. rolfsii*.

Solid formulation of *T. harzianum* Isolate GRT4 and *P. fluorescens* Isolate PF4

The potential isolates of both fungal and bacterial antagonists isolates were formulated using talc as carrier material following procedure developed by Vidhyasekaran and Muthamilan (1995). Gaur et al. (2005) used the talc based formulation of *T. harzianum* multiplied on yeast molasses broth containing 2 $\times 10^6$ cfu g^{-1} of talc for root rot control in chickpea.

Liquid formulation of *T. harzianum* Isolate GRT4 and *P. fluorescens* Isolate PF4

Liquid Formulations of potential *Trichoderma* sp. and *P. fluorescens* isolates were prepared using paraffin oil + soybean oil (1:1) and glycerol amended NB as liquid carrier materials, respectively (Sathiyaseelan et al., 2009 & Manikandan et al., 2010).

Efficacy of Combined Bioformulation against Stem Rot of Groundnut under Glasshouse Conditions

In the present investigation, pot culture study was conducted under glasshouse conditions by artificial inoculation of *S. rolfsii* inoculum mass multiplied on sorghum grains to the soil in pots @ 100 g kg^{-1} and the efficacy of combined bioformulation was analysed by imposing different treatments to the test pathogen. The observations were recorded at 60 DAS when the plants in control treatment started showing stem rot disease incidence.

Observations

The data on per cent germination, initial and final population of groundnut, per cent incidence of stem rot and plant growth parameters *viz*., shoot length, root length, fresh weight and dry weight of groundnut in each of the treatment were recorded and presented in tables 4 and 5.

Per cent Germination

Results from the pot culture studies on stem rot of groundnut revealed that maximum per cent germination (93.33 %) was recorded in treatment T_{10} (seed treatment with *P. fluorescens* @ 10 g + *Trichoderma* spp. @ 8 g kg^{-1} of seed along with soil treatment with combined bioformulation @ 2 L + 80 kg of FYM + 5 kg of neem cake $acre^{-1}$) which was on par with treatments T_9 (86.67 %) and T_8 (83.33 %). It is evident from the table 4 that lowest germination percentage (36.67 %) was recorded in control treatment (T_{12}).

Initial and Final Plant Population

From the data (Table 4) it is evident that, initial plant population of groundnut was highest in treatment T_{10} (9.33) which was on par with the treatments T_9 (8.67) and T_8 (8.33). Lowest plant population (3.67) was recorded in the control treatment (T_{12}). Similarly, regarding final plant population also highest plant population (8.33) was recorded in the treatment T_{10} which is on par with the treatments T_9 (7.33) and T_8 (6.67). Lowest plant population (1.00) was recorded in the control treatment (T_{12}). The final populations were in accordance with the per cent disease incidence in each treatment.

Per cent Disease Incidence

From the data (Table 4) it is evident that, minimum per cent disease incidence was recorded in the treatment T_{10} (11.11 %) followed by treatments T_9 (15.50 %), T_8 (19.91 %) and T_{11} (25.60 %). Maximum per cent disease incidence (75.00 %) was recorded in control treatment (T_{12}).

Results were similar with the findings of Manjula *et al*. (2004), who reported that combined application of *P. fluorescens* GB 10 with *T. viride* pq1 in protecting groundnut seedlings from stem rot infection had shown higher percentage of *S. rolfsii* mortality (78.0%) when compared to application of either *P. fluorescens* GB 10 (58.0%) or *T. viride* pq1 (70.0%) alone.

Effect of Different Treatments on Plant Growth Parameters of Groundnut

In the present investigation, an attempt was made to observe whether the treatments imposed have any stimulatory (or) inhibitory effect on mean shoot length, root length and fresh weight and dry weight of groundnut plants.

Shoot Length

Maximum shoot length (25.28 cm) was recorded in treatment T_{10} (seed treatment with *P. fluorescens* @ 10 g + *Trichoderma* spp. @ 8 g kg^{-1} of seed along with soil treatment with combined bioformulation @ 2 L + 80 kg of FYM + 5 kg of neem cake $acre^{-1}$) which was on par with treatments T_9 (24.55 cm), T_8 (23.98 cm), T_2 (22.82 cm) and T_{11} (22.59 cm). It is evident from the table 5 that minimum shoot length (14.18 cm) was recorded in control treatment (T_{12}).

b) Root Length

Maximum root length (30.79 cm) was recorded in treatment T_{10} which was on par with treatments T_9 (28.38 cm), T_8 (27.67 cm), T_2 (26.99 cm), T_{11} (26.89 cm), T_3 (25.73 cm), T_4 (25.39 cm) and T_1 (24.10 cm). It is evident from table 5 that minimum root length (12.20 cm) was recorded in control treatment (T_{12}).

Fresh Weight and Dry Weight

Maximum fresh weight (11.35 g) was recorded in treatment T_{10} which was on par with treatments T_9 (9.02 g), T_8 (8.70 g), T_{11} (8.52 g) and T_2 (8.13 g). It is evident from table 5 that minimum fresh weight (4.09 g) was recorded in the control treatment (T_{12}).

Maximum dry weight (2.11 g) was recorded in the treatment T_{10} which is on par with the treatments T_9 (2.10 g) and T_8 (1.97 g). Lowest dry weight (0.65 g) was recorded in control treatment (T_{12}).

Results were similar with the reports of Mathivanan et al. (2014), who reported that the highest germination percentage (98%), seedling growth (9.5 cm/seedling), vigour index 931 (seedling length x germination percentage) and dry weight (1.82 g/seedling) were recorded in groundnut seedlings grown with *Rhizobium* + *Pseudomonas* + *Bacillus*.

Table 1: *In vitro* evaluation of efficacy of antagonistic *Trichoderma* spp. isolates against *S. rolfsii* in dual culture technique

Treatments	RgSr		TpSr		SgSr		DmSr		Mean A	
	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control
GRT1	2.73	65.83 (54.21)	2.03	74.58 (59.70)	1.80	77.50 (61.66)	2.53	68.33 (55.74)	2.26	71.56[b] (57.83)
GRT2	2.63	67.08 (54.97)	1.47	81.67 (64.63)	2.00	75.00 (59.98)	2.27	71.67 (57.82)	2.09	73.85[a] (59.35)
GRT3	3.17	60.42 (51.00)	2.20	72.50 (58.35)	2.13	73.33 (58.93)	3.07	61.67 (51.73)	2.64	66.98[c] (55.00)
GRT4	3.17	60.42 (51.00)	1.13	85.83 (67.89)	1.70	78.75 (62.53)	3.48	56.67 (48.81)	2.37	70.42[b] (57.56)
GRT5	2.47	69.17 (56.25)	1.50	81.25 (64.34)	2.07	74.17 (59.43)	2.07	74.17 (59.43)	2.02	74.69[a] (59.86)
GRT6	3.10	61.25 (51.48)	2.03	74.58 (59.70)	2.03	74.58 (59.70)	3.13	60.83 (51.24)	2.56	67.81[c] (55.53)
Mean B	2.88	64.03[c] (53.15)	1.73	78.40[a] (62.44)	1.96	75.56[b] (60.37)	2.75	65.56[c] (54.13)		
Factors	**C.D (P=0.01)**	**C.D (P=0.01)**	**SEm±**	**SEm±**						
S. rolfsii monoculture	8.00	0.00								
Trichoderma spp. Isolates	0.11	1.33	0.04	0.47						
S. rolfsii Isolates	0.09	1.09	0.03	0.38						
Interactions	0.21	2.66	0.08	0.93						

* Values are means of three replications; Values in the parenthesis are angular transformed values; Values with common letter are not significantly different

Table 2: *In vitro* evaluation of efficacy of antagonistic *P. fluoresces* isolates against *S. rolfsii* in dual culture technique

Treatments	RgSr		TpSr		SgSr		DmSr		Mean A	
	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control	Radial growth of the pathogen (cm)	Per cent inhibition over control
PF1	3.40	24.44 (29.60)	3.57	20.74 (27.05)	3.27	27.41 (31.55)	3.40	24.44 (29.60)	3.41	24.26[c] (29.45)
PF2	4.50	0.00 (0.00)	3.57	20.74 (27.07)	3.63	19.26 (26.01)	3.80	15.56 (23.19)	3.88	13.89[d] (19.07)
PF3	2.67	40.74 (39.65)	2.53	43.70 (41.36)	2.60	42.22 (40.51)	2.83	37.04 (37.47)	2.66	40.93[a] (39.75)
PF4	3.20	28.89 (32.49)	2.80	37.78 (37.91)	3.00	33.33 (35.24)	2.73	39.26 (38.78)	2.93	34.82[b] (36.10)
PF5	4.50	0.00 (0.00)	4.40	2.22 (6.91)	4.43	1.48 (4.05)	3.80	15.56 (23.11)	4.28	4.81[e] (8.52)
Mean B	3.65	18.82[c] (20.35)	3.37	25.04[ab] (28.06)	3.39	24.74[ab] (27.47)	3.31	26.37[a] (30.43)		
S. rolfsii monoculture	4.50	0.00								
Factors	**C.D (P=0.01)**	**C.D (P=0.01)**	**SEm±**	**SEm±**						
P. fluorescens Isolates	0.08	1.70	0.03	0.59						
S. rolfsii Isolates	0.07	1.52	0.02	0.53						
Interactions	0.15	3.39	0.05	1.18						

* Values are means of three replications; Values in the parenthesis are angular transformed values; Values with common letter are not significantly different

Table 3: *In vitro* evaluation of compatibility between highly potential bacterial antagonists PF3, PF4 and fungal antagonists GRT1, GRT2, GRT4 and GRT5

Bacterial antagonist	GRT1		GRT2		GRT4		GRT5	
	*Radial growth of fungal antagonist (cm)	*Per cent inhibition over control	*Radial growth of fungal antagonist (cm)	*Per cent inhibition over control	*Radial growth of fungal antagonist (cm)	*Per cent inhibition over control	*Radial growth of fungal antagonist (cm)	*Per cent inhibition over control
PF3	1.93	57.04[a] (49.03)	2.17	51.85[b] (46.04)	2.53	43.70[d] (41.36)	2.73	39.26[e] (38.78)
PF4	2.30	48.89[c] (44.34)	2.37	47.41[c] (43.49)	3.53	21.48[g] (27.59)	3.23	28.15[f] (32.02)
Trichoderma monoculture	4.50	0.00 (0.00)						
SEm ±	0.08	0.56						
CD	0.25	1.69						

***** Values are means of three replications; Values in the parenthesis are angular transformed values; Values with common letter are not significantly different

Table 4: Effect of different formulations of biocontrol agents on *S. rolfsii* in pot culture

Treatment	Per cent germination	Initial population	Final population	Per cent disease incidence
T_1: Seed treatment with *P. fluorescens* @ 5 g + *Trichoderma* spp. @ 4 g kg^{-1} of seed	56.67 (48.83)	5.67 (13.75)	3.00 (9.88)	47.78 (43.66)
T_2 : Seed treatment with *P. fluorescens* @ 10 g + *Trichoderma* spp. @ 8 g kg^{-1} of seed	73.33 (59.19)	7.33 (15.67)	5.00 (12.87)	31.94 (34.33)
T_3 : Seed treatment with *P. fluorescens* @ 5 ml + *Trichoderma* spp. @ 3 ml kg^{-1} of seed	70.00 (56.98)	7.00 (15.31)	4.33 (11.74)	39.49 (38.22)
T_4 : Soil treatment with combined bioformulation @ 2 kg + 80 kg of FYM + 5 kg of neem cake acre^{-1}	63.33 (52.75)	6.33 (14.56)	3.67 (11.01)	41.27 (39.86)
T_5 : Soil treatment with combined bioformulation @ 2 L+ 80 Kg of FYM + 5 kg of Neem cake acre^{-1}	46.67 (43.06)	4.67 (12.46)	2.00 (8.13)	56.67 (48.83)
T_6 : Seed treatment with *Trichoderma* spp. @ 8 g kg^{-1} of seed alone	53.33 (46.90)	5.33 (13.34)	2.67 (9.36)	50.00 (44.98)
T_7 : Seed treatment with *P. fluorescens* @ 10 g kg^{-1} of seed alone	73.33 (59.19)	7.33 (15.67)	3.33 (10.49)	54.17 (47.39)
T_8 : T_1 + T_4	83.33 (66.12)	8.33 (16.77)	6.67 (14.95)	19.91 (26.27)
T_9 : T_2 + T_4	86.67 (72.77)	8.67 (17.07)	7.33 (15.65)	15.50 (22.91)
T_{10} : T_2 + T_5	93.33 (77.69)	9.33 (17.78)	8.33 (16.73)	11.11 (15.86)
T_{11} : Seed treatment with Tebuconazole @ 1.5 g kg^{-1}of seed	76.67 (61.20)	7.67 (16.06)	5.67 (13.75)	25.60 (29.98)
T_{12} : Control	36.67 (37.21)	3.67 (11.01)	1.00 (4.62)	75.00 (64.99)
SEm±	4.91	0.49	0.62	7.91
CD (0.05)	14.41	1.44	1.81	23.21

***** Values are means of three replications; Values in the parenthesis are angular transformed values

Table 5: Effect of different formulations of biocontrol agents on growth of groundnut against *S. rolfsii* in pot culture

Treatment	*Shoot length (cm)	*Root length (cm)	*Fresh weight (g)	*Dry weight (g)
T_1: Seed treatment with *P. fluorescens* @ 5 g + *Trichoderma* spp. @ 4 g kg^{-1} of seed	21.66 (27.72)	24.10 (29.32)	7.44 (15.69)	1.47 (6.87)
T_2 : Seed treatment with *P. fluorescens* @ 10 g + *Trichoderma* spp. @ 8 g kg^{-1} of seed	22.82 (28.48)	26.99 (31.27)	8.13 (16.37)	1.60 (7.23)
T_3 : Seed treatment with *P. fluorescens* @ 5 ml + *Trichoderma* spp. @ 3 ml kg^{-1} of seed	22.30 (28.16)	25.73 (30.33)	7.70 (16.05)	1.56 (7.17)
T_4 : Soil treatment with combined bioformulation @ 2 kg + 80 kg of FYM + 5 kg of neem cake acre^{-1}	22.00 (27.96)	25.39 (30.23)	7.54 (15.91)	1.55 (7.11)
T_5 : Soil treatment with combined bioformulation @ 2 L+ 80 Kg of FYM + 5 kg of Neem cake acre^{-1}	18.04 (25.08)	19.00 (25.74)	5.15 (13.00)	0.96 (5.49)
T_6 : Seed treatment with *Trichoderma* spp. @ 8 g kg^{-1} of seed alone	21.17 (27.37)	22.45 (28.26)	7.13 (15.36)	1.26 (6.39)
T_7 : Seed treatment with *P. fluorescens* @ 10 g kg^{-1} of seed alone	18.70 (25.59)	19.80 (26.28)	5.86 (13.93)	1.13 (5.90)
T_8 : T_1 + T_4	23.98 (29.30)	27.67 (31.71)	8.70 (17.13)	1.97 (8.05)
T_9 : T_2 + T_4	24.55 (29.66)	28.38 (32.13)	9.02 (17.38)	2.10 (8.31)
T_{10} : T_2 + T_5	25.28 (30.15)	30.79 (33.67)	11.35(19.68)	2.11 (8.33)
T_{11} : Seed treatment with Tebuconazole @ 1.5 g kg^{-1}of seed	22.59 (28.34)	26.89 (31.21)	8.52 (16.97)	1.86 (7.83)
T_{12} : Control	14.18(22.10)	12.20 (20.43)	4.09 (11.64)	0.65 (4.61)
SEm±	1.29	2.20	1.03	0.21
C D(0.05)	3.79	6.46	3.03	0.62

***** Values are means of three replications; Values in the parenthesis are angular transformed values.

CONCLUSION

Six *Trichoderma* spp. and five *P. fluorescens* isolates were obtained from rhizosphere of groundnut plants from different regions in Chittoor and S.P.S.R Nellore districts. To test the biocontrol potential of antagonists against *S. rolfsii,* dual culture study was conducted *in vitro*. Among the fungal and bacterial biocontrol agents tested *in vitro,* the fungal isolates GRT5, GRT2, GRT1, GRT4 and bacteria isolates PF3, PF4 recorded maximum mean per cent inhibition of *S. rolfsii* mycelial growth *in vitro*. The outperforming fungal and bacterial biocontrol agents were further tested for their compatibility *in vitro,* out of which, the fungal isolate GRT4 and bacterial isolate PF4 emerged as most compatible combination of biocontrol agents.

Similarly, the efficacy of potential fungal antagonist *Trichoderma* isolate GRT4, bacterial antagonist PF4 were tested in pot culture against stem rot of groundnut. Among the twelve treatments imposed, treatment T_{10} i.e. seed treatment with *P. fluorescens* @ 10 g + *Trichoderma* spp. @ 8 g kg^{-1} of seed along with soil treatment with combined bioformulation @ 2 L + 80 kg of FYM + 5 kg of neem cake $acre^{-1}$ was found to be superior as it recorded the highest germination percentage (93.33%), highest initial population (9.33), final population (8.33) and least PDI of 11.11 per cent. This treatment also recorded maximum shoot length (25.28 cm), root length (30.79 cm) and maximum fresh and dry weights i.e. 11.35 g and 2.11 g, respectively when compared to other treatments.

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