Biological Forum – An International Journal **14(3): 409-416(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Biological Management of Dry Root Rot of Groundnut using *Trichoderma harzianum* and *Pseudomonas fluorescens* under Glasshouse conditions

M. Mahendra[1*], M. Reddi Kumar[2], C.P.D. Rajan[3] and P. Sumathi[4]
[1]M.Sc. Scholar (Ag.), Department of Plant Pathology,
S.V. Agricultural College, Tirupati ANGRAU, Guntur (Andhra Pradesh), India.
[2]Associate Professor, Plant Pathology,
S.V. Agricultural College, Tirupati, ANGRAU (Andhra Pradesh), India.
[3]Principle Scientist and University Head, Plant Pathology,
Agricultural Research Station, Nellore, ANGRAU (Andhra Pradesh), India.
[4]Assistant Professor, Statistics and Mathematics,
College of Agril. Engineering, Madakasira (Andhra Pradesh), India.

(Corresponding author: M. Mahendra)*
(Received 12 May 2022, Accepted 08 July, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Despite being major oil seed crop in Andhra Pradesh, groundnut has been suffering from recurrent incidence of dry rot disease. The pathogen being soil borne forms resistant propagules which can survive adverse environmental conditions and added regularly into the soil making the soil sick for economic cultivation. The chemical management practices though provide instant results were found to be ineffective in complete management of the disease besides posing environmental risks. To manage this major disease of groundnut, the current investigation involved combined application of bioformulations of *Pseudomonas fluorescens* and *Trichoderma harzianum* under glasshouse conditions. During the experiment we isolated six isolates of *Trichoderma* spp. and five isolates of *P. fluorescens* from the groundnut rhizosphere and tested their effectiveness *in vitro* against groundnut dry root rot pathogen, *Macrophomina phaseolina*. The outperforming isolates of antagonists were tested *in vivo* (pot culture) against the dry root rot disease. Among the six *Trichoderma* isolates tested against *M. phaseolina in vitro*, isolate GRT5 was found superior with highest mean inhibition (59.48%) when compared to the rest of the isolates but did not combine well with the bacterial antagonist. Among five isolates of *P. fluorescens* assessed *in vitro* against *M. phaseolina*, isolate PF4 recorded highest mean inhibition (36.11%). The glasshouse pot culture results indicated that, combined application of *P. fluorescens* @ 10 g + *T. harzianum* @ 8 g kg^{-1} of seed as seed treatment application coupled with soil application of consortia of the same @ 2 L + 80 kg of FYM + 5 kg of neem cake acre^{-1} outperformed other treatments with the highest plant biometrical parameters i.e., shoot length (22.39 cm), root length (34.47 cm), fresh weight (10.7 g) and dry weight (2.20 g). The treatment also yielded maximum initial and final plant populations (9.67, 8.67 respectively) with increased germination percentage (96.67%) and least PDI (3.70%).

Keywords: *Tricoderma harzianum*, *Pseudomonas fluorescens*, *Macrophomina phaseolina*, Per cent Disease Incidence (PDI).

INTRODUCTION

Groundnut is one of the major edible oil seed crops of Andhra Pradesh, occupying 0.66 M ha of area with 0.85 M t of average production and 1.28 t ha^{-1} of average productivity. Majority of groundnut crop in Andhra Pradesh is cultivated during kharif in Anantapuramu, Chittoor, Kurnool and Kadapa districts. Several biotic and abiotic factors are the causes of low productivity (1.28 t ha^{-1}) of groundnut in A.P (Directorate of Economics and Statistics, 2019-20).

Pattee and Young (1982) reported that, *M. phaseolina* can cause pod, root, peg, stem and leaf spots on older and younger seedlings. The congenial conditions for the development of the disease are soil temperature of 80-95°F prevailing for two to three weeks. The pathogen is primarily soil borne and transport of implements, irrigation water and grazing animal from the infected

field to healthy field transmits the disease. The air borne pycniospores also acts as potential dispersing agent for the pathogen (Rangaswami and Mahadevan 2008).

The current indiscriminate use of toxic chemicals alone to reduce the disease incidence resulted in environmental deterioration, additional financial burden to the farmer and fungicidal resistance development in the pathogen (Rudresh *et al.,* 2005).

Considering the above demerits of using toxic chemicals to manage the disease, inclusion of biological control in the disease management has the good potential to ameliorate the environmental risks, provides long lasting management and reduces the chances of resistance build up in the target pathogen.

Use of consortia formulation of potential biocontrol agents like PGPR bacteria and antagonistic fungi is the recent advancement in the field of plant protection. This approach besides controlling the diseases, increases the nutrient uptake, phosphorous solubilization and uptake, plant growth and finally the yield.

MATERIALS AND METHODS

Pathogen, *M. phaseolina* isolation. The standard tissue segment method as described by Rangaswamy and Mahadvan, (1999) was followed for isolation of the pathogen from the infected plant sample.

The isolation includes cutting a small bit of host root tissue having both infected and healthy portions using sterilized scalpel. The cut pieces were kept for two minutes in one per cent sodium hypochlorite and washed thrice with sterile distilled water. The resultant root pieces were inoculated on to Petri plates containing sterilized potato dextrose agar (PDA) medium and incubated at 28 ± 2°C. The Petri plates were regularly inspected for the growth of the fungal pathogen and pure culture of the same was maintained following single hyphal tip method. PDA slants were used to store the pure cultures of the test pathogen.

Identification and morphological characterization of *M. phaseolina.* The morphological description of Barnett and Hunter, (1972) were followed for identification of *M. phaseolina*. The compound microscope (ACCU SCOPE-EX 30) was used to study the hyphal branching pattern, size and shape of microsclerotia.

Biocontrol agents isolation. The *Trichoderma* spp. were isolated form the rhizosphere soil of groundnut following serial dilution technique on Trichoderma Specific Medium (TSM) (Johnson and Curl 1977).

The bacterial biocontrol agent, *P. fluorescens* isolates were isolated form the rhizosphere soil of groundnut and were purified following streak plate method. The isolated bacteria was found gram negative and emitted fluorescent light when illuminated with UV light. (Manjunatha *et al*., 2012).

***Trichoderma* spp. taxonomic identification**

The six isolates of *Trichoderma* spp. were classified up to species level using the Trichoderma morphological descriptions of Bisset, (1984, 1991 a, b, c). To do so, the characters of conidiophore branching, phialide shape, phialide grouping, chlamydospore formation, spore balls, conidial shape and sterile appendages were considered.

In vitro* dual culture studies of biocontrol agents against *M. phaseolina

***In vitro* fungal antagonist-test pathogen interaction.** The standard dual culture technique described by Morton and Straube (1955) was followed for assessment of effectiveness of *Trichoderma* isolates against *M. phaseolinain vitro*. The test was conducted by inoculation of 5 mm mycelial discs of both the test pathogen and the fungal biocontrol agents opposite to each other form one cm away from the periphery of Petri plate containing PDA.

When the test pathogen was fully occupied the control plate, the results of the dual culture were recorded.

Bacterial antagonist-test pathogen interactions *in vitro.* The *P. fluorescens* isolates were tested for their effectiveness against *M. phaseolinain vitro* following dual culture method.

In a Petri plate containing equal amounts of PDA and NA, the antagonistic bacterium was inoculated as two streaks of 5 cm opposite to each other one cm away form the periphery and the test pathogen was inoculated as five mm mycelial disc in the middle. The monoculture of the test pathogen was maintained as the control for comparison. The results were recorded as the zone of inhibition in the dual culture plates when the pathogen in the monoculture was fully grown.

The formula given by Vincent (1927) was used to obtain the per cent inhibition exhibited by the bacterial biocontrol agent against mycelial growth of test pathogen in the dual culture plates.

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

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.

***In vitro* compatibility studies between bacterial and fungal antagonists**

Dual culture studies were conducted to test the compatibility between bacterial and fungal antagonists as described by Morton and Stroube (1955).

The Petri plate containing equal amounts of PDA and NA was inoculated with fungal and bacterial biocontrol agents as 5 mm mycelial disc in the middle and 5 cm streaks at one cm away from the periphery of the plate on either side of the fungal mycelial disc respectively. The Petri plates containing the pathogen was treated as control. The plates were incubated at 25 ± 2°C and observations from the dual cultured plates were recorded when the pathogen in the control plates was fully grown.

The standard formula by Vincent (1927) was used to obtain the percent inhibition of fungal biocontrol agent by bacterial biocontrol agent.

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

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.

***M. phaseolina* mass multiplication.** Sterilized sorghum grains were used as substrate for mass multiplication of *M. phaseolina*. The clean sorghum grains were soaked in solution containing 4 per cent dextrose for overnight. Later the solution was drained and the grains were dried to appropriate moisture levels. The dried sorghum grains were filled up to 2/3 volume of 250 ml conical flasks and autoclaved at 15 p.s.i. for twenty minutes. The sterilized sorghum grains were inoculated with four day old 5 mm mycelial discs of test pathogen and kept for incubation.

Talc based formulations of potential antagonists. The sterilized potato dextrose broth (PDB) was inoculated with three-day old *Trichoderma* culture and kept agitated by placing inside shaking incubator for seven days to get increased biomass production.

Nutrient broth (NB) was inoculated with two loops full of bacterial antagonist and kept in shaking incubator for continuous agitation for three days for increased biomass production.

The standard procedure was followed to develop talc formulations of both bacterial and fungal biocontrol agents (Vidhyasekaran and Muthamilan, 1995).

Effective *Trichoderma* isolate formulation in paraffin oil + soybean oil (1:1). Effective *Trichoderma* sp. isolate was formulated using paraffin oil + soybean oil (1:1) liquid carrier material. For this, dry spores of *Trichoderma* were harvested from solid substrate (*Trichoderma* mass multiplied on sorghum grains) after 12 days of incubation using 100 mesh sieve. Two grams of dry spore was aseptically transferred into pre-sterilized 100 ml of liquid carrier containing paraffin oil + soybean oil (1:1). Later the formulation was poured into glass bottles and stored at 4°C in a refrigerator (Sathiyaseelan *et al*., 2009).

Formulation of effective *P. fluorescens* isolate in glycerol. Solution containing two per cent glycerol in NB was used as liquid carrier material for formulation of effective isolate of *P. fluorescens*. The formulation was prepared by mixing 1 ml of effective bacterial antagonist at log phase and kept for incubation for two days. Later the formulation was stored in refrigerator at 4°C (Manikandan *et al.,* 2010).

Glass house studies on management of *M. phaseolina* using effective isolates of bacterial and fungal antagonists. The experiment was designed to test the performance of chemical and formulation of biocontrol agents in managing dry root of groundnut in pot culture. The table below shows the design of the CRD.

Completely Randomized Design (CRD) statistical method was followed with groundnut test variety Narayani for finding effectiveness of above treatments in the management of groundnut dry root rot. The observations recorded were, PDI, final plant population, initial plant population, dry weight and fresh weight, shoot length and root length of groundnut plants.

Treatment Number	Treatment
T1	Seed treatment with *P. fluorescens* @ 5 g + *Trichoderma* spp. @ 4 g kg^{-1} of seed
T2	Seed treatment with *P. fluorescens* @ 10 g + *Trichoderma* spp. @ 8 g kg^{-1} of seed
T3	Seed treatment with *P. fluorescens* @ 5 ml + *Trichoderma* spp. @ 3 ml kg^{-1} of seed
T4	Soil treatment with combined bioformulation @ 2 kg + 80 kg of FYM + 5 kg of neem cake $acre^{-1}$
T5	Soil treatment with combined bioformulation @ 2 L+ 80 Kg of FYM + 5 kg of Neem cake $acre^{-1}$
T6	Seed treatment with *Trichoderma* spp. @ 8 g kg^{-1} of seed
T7	Seed treatment with *P. fluorescens* @ 10 g kg^{-1} of seed
T8	T1 + T4
T9	T2 + T4
T10	T2 + T5
T11	Seed treatment with Tebuconazole @ 1.5 g kg^{-1} of seed
T12	Control

RESULTS AND DISCUSSION

The pathogen, *M. phaseolina*

The disease samples of groundnut dry root rot were collected from Sangam, Somasila areas of SPSR Nellore Dt. and R.A.R.S Tirupati, Rangampeta areas of Chittoor Dt. of Andhra Pradesh. The isolates collected from Sangam, Somasila, R.A.R.S Turuapti, Ramgampeta are named as SgMp, SmMp, TpMp and RgMp respectively. The different isolates of *M. phaseolina* were isolated following tissue segment

method and single hyphal tip purification technique on PDA (Ghewande *et al*., 2002).

The isolates RgMp, SgMp and SmMp produced partially fluffy mycelium whereas isolate TpMp produced highly fluffy mycelium in culture. Morphological studies to characterize *M. phaseolina* isolates were conducted by observing 10 d old culture grown on PDA medium. Mycelia of for RgMp, TpMp and SgMp appeared white fluffy in the beginning and grey at maturity but the mycelia of SmMp appeared black at maturity. The microscopic examination of fungal mycelia revealed characteristic right angular branching pattern of *M. phaseolina*. The average microsclerotial size of SmMp was 94.30 µm which was bigger among four isolates followed by RgMp (91.80 µm) and TpMp (85.80 µm). Smaller microsclerotia were observed in isolate SgMp (70.82 µm). The microsclerotia of all isolates were blackish brown in colour.

The test pathogen exhibiting all the above characteristics was identified as *M. phaseolina* (Tassi.) Goid. The results were similar with the works of Subramanayam (1971) who morphologically characterized *M. phaseolina*.

Native rhizosphere isolates of antagonistic *Trichoderma* spp. Six isolates of *Trichoderma* spp. were isolated from different soil samples of Chittoor and SPSR Nellore districts. The Trichoderma selective medium was used for initial selective isolation and PDA was used for further purification and storage of the fungal antagonist. The isolates of Chittoor were named as GRT1, GRT2, GRT3 and Nellore as GRT4, GRT5 and GRT6.

Using ACCU-SCOPE-EX 30 microscope, the *Trichoderma* isolates were characterized up to species level by considering the characters of conidiophore branching, chlamydospore formation, structure and distribution of phialides, size and shape of conidia. Form the study conducted, two species of *Trichoderma* were identified i.e., *T. viride* (GRT2, GRT5) and *T. harzianum* (GRT1, GRT3, GRT4 and GRT6).

Native rhizosphere isolates of antagonistic *P. fluorescens*. Five isolates of antagonistic *P. fluorescens* were isolated from rhizosphere soil samples of groundnut collected from different places in Chittoor (PF1, PF2, PF3) and SPSR Nellore (PF4, PF5) districts. King's B medium was used for selective isolation of *P. fluorescens* and PDA was used for further purification and maintenance. The bacterial isolates were confirmed as *P. fluorescens* as the cells were gram negative and emitted fluorescens under UV light. The results were similar with the findings of Elangovan and Gnanamanickam (1990).

Interaction between *Trichoderma* spp. and *M. phaseolina*. Dual culture technique was performed to study interaction between *Trichoderma* spp. and *M. phaseolina in vitro*. Two factorial CRD was used to analyze the results as shown in Table 1.

we observed *Trichoderma* isolates GRT5 (67.92%), GRT6 (61.25%), GRT1 (59.17%) and GRT4 (60.42%) recorded maximum inhibition percentage when tested against RgMp, TpMp, SmMp and SgMp. *Trichoderma* isolates, GRT1, GRT2, GRT4 and GRT 5 were found effective in inhibiting the mycelial growth of *M. phaseolina* while the pathogen isolate SmMp appeared as virulent pathogen by showing minimal inhibition percentage (56.81 %).

Kumari *et al.* (2022) recorded highest mycelial growth inhibition of *M. phaseolina*, the causal agent of dry root of chickpea by *T. harzianum* (73.33) which outperformed over other biocontrol agents under the study.

Interaction between *P. fluorescens* and *M. phaseolina.* Dual culture technique was performed with five isolates of *P. fluorescens* and four isolates of *M. phaseolina* to find the effective isolates of bacterial antagonist *in vitro*. Two factorial CRD was used for analysis of results as presented in the Table 2.

We observed that, PF4 isolate of *P. fluorescence* recorded maximum inhibition percentage against RgMp (37.04%), SgMp (38.52%), SmMp (31.85%) and PF3 isolate against TpMp (39.26%).

The two isolates of antagonist *i.e.,* PF3 and PF4 were found effective in inhibiting the growth of *M. phaseolina* with maximum inhibition while the pathogen isolated SmMp was identified as virulent pathogen isolate with minimum inhibition (15.26 %). In a study conducted by Shanmugam *et al.* (2002), it was found that, Pf 1 isolate of *P. fluorescens* was significant in reducing the growth of *M. phaseolinain vitro*.

Compatibility studies between effective isolates of *P. fluorescens* and *Trichoderma* sp. We obtained successful combination of antagonistic fungal (GRT4) and bacterial (PF4) isolates with minimum negative interaction (21.48 %) from the dual culture studies *in vitro*.

Mishra *et al.* (2013) reported that the PBAP-27 isolate of *Pseudomonas* and PBAT-43 isolate of *Trichoderma* were found most compatible with least negative interactions *in vitro*. The same combination was used for developing mixed formulation.

Formulations of isolate GRT4 of *T. harzianum* and isolate PF4 of *P. fluorescens*. For solid formulations of bacterial and fungal antagonists talc was used as solid carrier material and paraffin oil + soybean oil (1:1) and glycerol as liquid carrier material.

For control of chickpea root rot Gaur *et al*. (2005) used talc formulation of *T. harzianum* and obtained better results.

Management of groundnut dry root rot using bioformulations of *P. fluorescens* (PF4) and *T. harzianum* (GRT4)

The mass multiplied test pathogen on sorghum grains was applied as 100 g kg^{-1} of soil in pots and treatments were imposed in different combinations of biocontrol agents and chemical to test for the best performing treatment.

Table 1: Interaction between *Trichoderma* spp. and *M. phaseolinain vitro*.

Treatment	RgMp		TpMp		SgMp		SmMp		Mean A	
	Pathogen growth in cm	PI (%)	Pathogen growth in cm	PI (%)	Pathogen growth in cm	PI (%)	Pathogen growth in cm	PI (%)	Pathogen growth in cm	PI (%)
GRT1	3.30	58.75 (50.02)	3.30	58.75 (50.02)	3.17	60.42 (50.99)	3.27	59.17 (50.26)	3.26	59.27^{abc} (50.32)
GRT2	2.97	62.92 (52.47)	3.57	55.42 (48.09)	3.17	60.42 (51.00)	3.30	58.75 (50.02)	3.25	59.38^{ab} (50.39)
GRT3	3.17	60.42 (51.00)	3.47	56.67 (48.81)	3.33	58.33 (49.78)	3.47	56.67 (48.81)	3.36	58.02^{de} (49.60)
GRT4	3.07	61.67 (51.73)	3.37	57.92 (49.54)	3.17	60.42 (50.99)	3.53	55.83 (48.33)	3.28	58.96^{abcd} (50.15)
GRT5	2.57	67.92 (55.48)	3.33	58.33 (49.78)	3.53	55.83 (48.33)	3.53	55.83 (48.33)	3.24	59.48^{a} (50.48)
GRT6	3.40	57.50 (49.30)	3.10	61.25 (51.48)	3.27	59.17 (50.27)	3.63	54.58 (47.61)	3.35	58.13^{de} (49.66)
Mean B	3.08	61.53^{a} (51.67)	3.36	58.06^{c} (49.62)	3.27	59.10^{b} (50.23)	3.46	56.81^{d} (48.89)		
M. phaseolina monoculture	8.00	0.00								
Factors	**C.D (P=0.01)**	**C.D (P=0.01)**	**SEm±**	**SEm±**						
Trichoderma spp. Isolates	0.09	1.12	0.03	0.39						
M. phaseolina Isolates	0.07	0.91	0.03	0.32						
Interactions	0.18	2.24	0.06	0.78						

* Values are means of three replications; Values in the parenthesis are angular transformed values; Values with common letter are not significantly different; PI = Per cent inhibition.

Table 2: Interaction between *P. fluorescens* and *M. phaseolinain vitro*.

Treatment	RgMp		TpMp		SgMp		SmMp		Mean A	
	Pathogen growth in cm	PI (%)	Pathogen growth in cm	PI (%)	Pathogen growth in cm	PI (%)	Pathogen growth in cm	PI (%)	Pathogen growth in cm	PI (%)
PF1	3.17	29.63 (32.96)	3.27	27.41 (31.55)	3.23	28.15 (32.03)	3.77	16.30 (23.76)	3.36	25.37^{c} (30.08)
PF2	3.10	31.11 (33.88)	3.03	32.59 (34.79)	3.27	27.41 (31.55)	4.50	0.00 (0.00)	3.48	22.78^{d} (25.06)
PF3	2.97	34.07 (35.70)	2.73	39.26 (38.78)	2.87	36.30 (37.02)	3.23	28.15 (32.03)	2.95	34.45^{b} (35.88)
PF4	2.83	37.04 (37.47)	2.83	37.04 (37.46)	2.77	38.52 (38.35)	3.07	31.85 (34.34)	2.88	36.11^{a} (36.90)
PF5	4.50	0.00 (0.00)	4.50	0.00 (0.00)	4.50	0.00 (0.00)	4.50	0.00 (0.00)	4.50	0.00^{e} (0.00)
Mean B	3.31	26.37^{a} (28.00)	3.27	27.26^{a} (28.52)	3.33	26.08^{a} (27.79)	3.81	15.26^{b} (18.03)		
M. phaseolina monoculture	4.50	0.00								
Factors	**C.D (P=0.01)**	**C.D (P=0.01)**	**SEm±**	**SEm±**						
P. fluorescens Isolates	0.07	1.47	0.02	0.51						
M. phaseolina Isolates	0.06	1.31	0.02	0.46						
Interactions	0.13	2.93	0.05	1.02						

* Values are means of three replications; Values in the parenthesis are angular transformed values; Values with common letter are not significantly different; PI = Per cent inhibition.

At 45 DAS the biometric observations like root length, shoot length, dry weight, fresh weight were recorded along with germination percentage, final plant population, initial plant population and per cent disease incidence (PDI) of dry root rot.

Impact of treatments on biometric parameters of groundnut plant

(a) Root length. It was observed that, treatment T10 showed increased root length (34.44 cm) followed by T9 and T8 (31.97, 29.99 cm respectively). Jayasree *et al.* (2000) observed similar effects on shoot and root lengths of sesame and black gram with combined application of bacterial and fungal biocontrol agents.

(b) Shoot length. Similar with the root length, the treatment T10 again recorded highest shoot length (22.39) followed by treatments T9, T8 and T2 (22.16, 21.92 and 19.57 cm respectively). As presented in the Table 4, T12, the control treatment recorded 6.04 cm of shoot length which was lowest among all treatments tested. Aiswarya *et al.* (2022) reported that seed

treatment of groundnut seeds with *T. viride* @ 10 g kg^{-1} and *P. fluorescens* @ 10 g kg^{-1} against collar rot pathogen *Aspergillus flavus* showed increased seedling lengths of 17.07 cm and 16.89 cm respectively over control (16.04 cm).

(c) Fresh weight and dry weight. Treatment T10 recorded highest fresh weight of 10.72 g and treatment T9, T8 and T11 found next best treatments with 9.42, 8.91 and 7.75 g respectively.

Table 3: Effect on plant population of groundnut.

Treatment	Germination percentage	Initial plant population	Final plant population	PDI
T1: Seed treatment with *P. fluorescens* @ 5 g + *Trichoderma* spp. @ 4 g kg^{-1} of seed	73.33 (58.98)	7.33 (15.70)	5.33 (13.29)	27.38 (31.02)
T2: Seed treatment with *P. fluorescens* @ 10 g + *Trichoderma* spp. @ 8 g kg^{-1} of seed	83.33 (66.12)	8.33 (16.77)	7.00 (15.34)	15.74 (23.17)
T3: Seed treatment with *P. fluorescens* @ 5 ml + *Trichoderma* spp. @ 3 ml kg^{-1} of seed	80.00 (63.90)	8.00 (16.40)	6.33 (14.56)	20.50 (26.77)
T4: Soil treatment with combined bioformulation @ 2 kg + 80 kg of FYM + 5 kg of neem cake $acre^{-1}$	76.67 (61.90)	7.67 (16.02)	6.00 (14.04)	23.15 (28.23)
T5: Soil treatment with combined bioformulation @ 2 L+ 80 Kg of FYM + 5 kg of Neem cake $acre^{-1}$	63.33 (52.75)	6.33 (14.56)	3.33 (10.40)	47.62 (43.61)
T6: Seed treatment with *Trichoderma* spp. @ 8 g kg^{-1} of seed	70.00 (56.98)	7.00 (15.31)	4.33 (11.99)	37.90 (37.96)
T7: Seed treatment with *P. fluorescens* @ 10 g kg^{-1} of seed	66.67 (54.76)	6.67 (14.95)	4.00 (11.47)	38.89 (38.02)
T8: T1 + T4	90.00 (74.98)	9.00 (17.43)	8.00 (16.40)	11.20 (19.53)
T9: T2 + T4	93.33 (77.69)	9.33 (17.78)	8.67 (17.09)	7.41 (12.98)
T10: T2 + T5	96.67 (83.85)	9.67 (18.10)	9.33 (17.76)	3.70 (6.49)
T11: Seed treatment with Tebuconazole @ 1.5 g kg-1of seed	86.67 (68.83)	8.67 (17.11)	7.33 (15.67)	15.74 (22.97)
T12: Control	53.33 (46.90)	5.33 (13.34)	1.33 (5.24)	76.67 (66.13)
SEm±	4.71	0.47	0.66	7.30
CD (0.05)	13.84	1.38	1.94	21.43

*Values are means of three replications; Values in the parenthesis are angular transformed values; PDI = Per cent disease incidence.

Table 4: Effect on biometric parameters of groundnut.

Treatment	*Shoot length (cm)	*Root length (cm)	*Fresh weight (g)	*Dry weight (g)
T1: Seed treatment with *P. fluorescens* @ 5 g + *Trichoderma* spp. @ 4 g kg^{-1} of seed	13.57 (21.54)	23.69 (29.10)	5.02 (12.91)	1.07 (5.92)
T2: Seed treatment with *P. fluorescens* @ 10 g + *Trichoderma* spp. @ 8 g kg^{-1} of seed	19.57 (26.20)	26.69 (30.86)	7.08 (15.38)	1.44 (6.88)
T3: Seed treatment with *P. fluorescens* @ 5 ml + *Trichoderma* spp. @ 3 ml kg^{-1} of seed	18.42 (25.41)	24.82 (29.78)	5.81 (13.78)	1.31 (6.48)
T4: Soil treatment with combined bioformulation @ 2 kg + 80 kg of FYM + 5 kg of neem cake acre-1	17.20 (24.49)	23.99 (29.30)	5.71 (13.81)	1.29 (6.50)
T5: Soil treatment with combined bioformulation @ 2 L+ 80 Kg of FYM + 5 kg of Neem cake acre-1	10.57 (18.89)	18.38 (25.37)	4.38 (12.03)	0.89 (5.36)
T6: Seed treatment with *Trichoderma* spp. @ 8 g kg^{-1} of seed	13.43 (21.34)	22.03 (27.93)	4.95 (12.82)	0.93 (5.49)
T7: Seed treatment with *P. fluorescens* @ 10 g kg^{-1} of seed	12.27 (20.48)	21.72 (27.69)	4.59 (12.32)	0.92 (5.46)
T8: T1 + T4	21.92 (27.90)	29.99 (33.13)	8.91 (17.32)	1.86 (7.82)
T9: T2 + T4	22.16 (28.03)	31.97 (34.34)	9.42 (17.86)	2.16 (8.45)
T10: T2 + T5	22.39 (28.23)	34.47 (35.83)	10.72 (19.07)	2.20 (8.52)
T11: Seed treatment with Tebuconazole @ 1.5 g kg-1of seed	10.38 (18.77)	15.70 (23.29)	7.75 (16.15)	1.54 (7.12)
T12: Control	6.40 (14.62)	12.07 (20.27)	3.44 (10.60)	0.76 (4.91)
SEm±	1.28	2.94	0.65	0.13
C D (0.05)	3.75	8.63	1.90	0.38

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

The dry weight was highest in the pots imposed with treatment T10 followed by T9 and T8 (2.16, 1.86 g respectively). Treatment T12, the control yielded lowest dry weight of 0.76 g. Martínez-Salgado *et al.* (2021) reported increased growth of groundnut plants (1417.60 g) and low disease incidence (76 %) with the application of *T. koningiopsis* (T-K11) @1 × 10^8 conidia mL^{-1} at 16 days after sowing on seedlings.

Germination percentage. The treatment T10 recorded highest germination percentage of 96.67 per cent and treatments T9, T8, T11 and T2 stood next with germination percentages of 93.33, 90.00, 86.67 and 83.33 per cent respectively as represented in Table 3. The germination percentage was very poor in the control treatment T12 (53.33 %). Mishra *et al.* (2013) found that higher germination percentage (72.11 %) was recorded in soybean and chickpea plants treated with combined bioformulation of PBAT-43, PBAP-27 isolates of *Trichoderma* and *Pseudomonas* respectively.

Effect on groundnut plant population. The treatment T10 recorded highest initial (9.67) and final (9.33) plant population followed by T9, T8, T11 and T2. The control treatment T12 recorded least plant population (1.33).

Effect on dry root rot disease. Lowest dry root rot disease incidence (3.70%) was observed in T10 treatment and T9, T8 treatments were also shown to be promising treatments with minimal disease incidence of 7.41 and 11.20 per cent respectively. The disease incidence was very high (76.67 %) in control treatment. Ramesh and Korikantthimath (2006) observed same effect on groundnut dry root incidence with combined use of *P. fluorescens* and *T. viride*.

CONCLUSION AND FUTURE SCOPE

Groundnut dry root has become major disease with greater yield losses every year. Chemical control found ineffective in managing the disease besides polluting the environment which is current burning issue in the human society. To address this problem the current investigation was made on utilization of rhizosphere antimicrobial agents such as *Trichoderma* spp. and *P. fluorescens*. The research started with isolation of biocontrol agents from the rhizosphere and plant pathogen from the diseased sample. The isolated biocontrol agents were further subjected to confirmational studies on suitable media. The effective combination of two or many biocontrol agents gives the advantage of synergism and complete control of pathogen as observed in the current study where GRT4 and PF4 were shown highly compatible under *in vitro* using dual culture technique. The treatment T10 which included both fungal and biocontrol agents performed better among other treatments, which is an indication of synergism exhibited by two varied biocontrol agents in pathogen management along with improved plant growth. Though biological control of plant disease appears promising with less environmental pollution and sustainable management of plant pathogens, often it fails to achieve the target due to inconsistency in its performance over wide range of ecological conditions. The future research is needed to address this problem of inconsistent performance of biocontrol agents which makes them then the real alternatives of chemical management of plant diseases.

Acknowledgement: The research assistance provided by Department of Plant Pathology, S.V. Agricultural College, Tirupati, Acharya N.G. Ranga Agricultural University is highly acknowledged

Conflict of Interest. None.

REFERENCES

Aiswarya, N., Bhattiprolu, S. L., Bayyapu Reddy K. and Rama Rao G. (2022). Efficacy of Fungicides and Bio-control Agents Against *Aspergillus flavus*. *Biological Forum – An International Journal*, *14*(2): 698-703.

Barnett, H. L. and Hunter, B. B. (1972). *Illustrated genera of imperfect fungi*. Burgess Publishing Company, 273-275.

Bisset, J. (1984). A revision of the genus *Trichoderma* I. Section Longibrachiatum. *Canadian Journal of Botany, 69*(1): 2373-2417.

Bisset, J. (1991a). A revision of the genus *Trichoderma* II Infragenric classification. *Canadian Journal of Botany, 69*(1): 2357-2417.

Bisset, J. (1991b). A revision of the genus *Trichoderma* III Section. Pachybasium. *Canadian Journal of Botany, 69*(1): 2373-2417.

Bisset, J. (1991c). A revision of the genus *Trichoderma* III Additional notes on section Longibrachium. *Canadian Journal of Botany, 69*(1): 2418-2420.

Directorate of Economics and Statistics, Ministry of Agriculture and Farmer's Welfare (2019). Area, production and productivity of groundnut, http://eands.dacnet.nic.in

Elangovan, C. and Gnanamanikam, S. S. (1990). Incidence of *Pseudomonas fluorescens* in rhizosphere of rice and their antagonism towards *Sclerotium oryzae*. *Indian Phytopathology, 45*(1): 358-61

Gaur, R. B., Sharma, R. N., Sharma, R. R. and Gautam, V. S. (2005). Efficacy of *Trichoderma* for Rhizoctonia root rot control in chickpea. *Journal of Mycology and Plant Pathology, 35*(1):144-150.

Ghewande, M.P., Desai, S. and Basu, M.S. (2002). Diagnosis and management of major diseases of groundnut. *National Research Center for Groundnut,* 1(1): 36-37.

Jayasree, K., Shanmugham., Raguchander, T., Ramanatham, A. and Samiyappan, R. (2000). Evaluation of *Pseudomonas fluorescens*-1 (Pf-1) against blackgram and sesame root rot disease. *Journal of Biological Control, 14*(2): 55-61.

Johnson, L. F. and Curl, E. A. (1977). *Methods for research on the ecology of soil borne plant pathogens*. Burgess Publishing Company. Minneapolis, 27-35.

Kumari, N., Meena, A.K., Godara, S. L., Gahlot V. S. and Dilip Kumar Reddy S. (2022). Evaluation of Bio-Agents, Botanicals and Fungicides under in vitro Conditions against *Macrophomina phaseolina* (Tassi) Goid Causing Charcoal Rot of Cowpea. *Biological Forum – An International Journal*, *14*(2): 84-90.

Manikandan, R., Saravanakumar, D., Rajendran, L., Raguchander, T. and Samiyappan, R. (2010).

Standardization of liquid formulation of *Pseudomonas fluorescens* Pfl for its efficacy against Fusarium wilt of tomato. *Biological Control, 54*(1): 83–89.

Manjunatha, H., Naik, M. K., Patil, M. B., Lokesha R. and Vasudevan, S. N. (2012). Isolation and characterization of native fluorescent pseudomonads and antagonistic activity against major plant pathogens. *Karnataka Journal of Agricultural Sciences, 25*(3): 346-349.

Martínez-Salgado, S. J., Andrade-Hoyos, P., Parraguirre Lezama, C., Rivera-Tapia, A., Luna-Cruz, A. and Romero-Arenas, O. (2021). Biological Control of Charcoal Rot in Peanut Crop through Strains of *Trichoderma* spp., in Puebla, Mexico. *Plants, 10*: 2630.

Mishra, D. S., Kumar. A., Prajapati, C. D., Singh, A. K. and Sharma, S. D. (2013). Identification of compatible bacterial and fungal isolates and their effectiveness against plant diseases. *Journal of Environmental Biology, 34*(1): 183-189.

Morton, D. J. and Straube, W. H. (1955). Antagonistic and stimulatory effect of soil microorganisms upon *Sclerotium rolfsii. Phytopathology, 45*: 417-420.

Pattee, H. E., Young, C. T., Pearson, J. L., Singleton, J. A. and Giesbrecht, F. G. (1982). Storage and moisture effects on peanut composition and roasted flavor. *Peanut Science, 9*(2): 98-101.

Rajasekhar, L., Sain, S.K. and Divya, J. (2016). Evaluation of microbial consortium for 'plant health management' of pigeon pea. *International Journal of Plant, Animal and Environmental Sciences, 6*(2): 107-113.

Ramesh, R. and Korikanthimath, V. S. (2006). Management of groundnut dry root rot by *Trichoderma viride* and *Pseudomonas fluorescens* under rain fed conditions. *Indian Journal of Plant Protection, 34*(2): 239-241.

Rangaswami, G. and Mahadevan, A. (1999). *Diseases of crop plants in India*, 4th ed. Prentice Hall of India Pvt. Ltd, New Delhi. 607.

Rangaswami, G. and Mahadevan, A. (2008). *Diseases of crop plants in India*, 4th ed. Prentice Hall of India Pvt. Ltd, New Delhi. 275- 278.

Rudresh, D. L., Shivaprakash, M. K. and Prasad, R. D. (2005). Potential of *Trichoderma* spp as biocontrol agents of pathogens involved in wilt complex (*Cicer arietinum* L). *Journal of Biological Control, 19*(2): 157-166.

Sathiyaseelan, K., Sivasakthivelan, P. and Lenin, G. (2009). Evaluation of antagonistic activity and shelf life study of *Trichoderma viride*. *Botany Research International, 2*(3): 195-197.

Shanmugam, V., Senthil, N., Raguchander, T., Ramanathan, A. and Samiyappan, R. (2002). Interaction of *Pseudomonas fluorescens* with *Rhizobium* for their effect on the management of peanut root rot. *Phytoparasitica, 30*(2): 169-176.

Subramanayam, C. V. (1971). *Hyphomycetes*. ICAR, New Delhi. 881-882.

Vidhyasekaran, P. P. and Muthamilan, M. (1995). Development of formulations of *Pseudomonasa fluorescens* for control of chick pea wilt. *Plant Disease, 79*(2): 782-786.

Vincent, J. M. (1927). Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, *159*: 850.

How to cite this article: M. Mahendra, M. Reddi Kumar, C.P.D. Rajan and P. Sumathi (2022). Biological Management of Dry Root Rot of Groundnut using *Trichoderma harzianum* and *Pseudomonas fluorescens* under Glasshouse conditions. *Biological Forum – An International Journal*, *14*(3): 409-416.