International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 28-11-2021, Accepted: 14-12-2021, Published: 29-12-2021
Volume 6, Issue 6, 2021, Page No. 1556-1559

Antagonistic potential of bio-control agent *Trichoderma* spp. against stem rot of groundnut incited by *Sclerotium rolfsii* Sacc

A Praveen[1], C Kannan[2*], V Jaiganesh[2], R Anandan[3]

[1] Department of Plant Pathology, Faculty of Agriculture, Annamalai University, New Delhi, Delhi, India
[2] Assistant Professor, Department of Plant Pathology, Faculty of Agriculture, Annamalai University, New Delhi, Delhi, India
[3] Assistant Professor, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

Abstract

Groundnut is infected by several fungal, bacterial and viral diseases but Stem rot disease considered as one of the most devastating diseases in the groundnut growing areas. Stem rot of groundnut caused by *Sclerotium rolfsii* which causes yield losses upto 25% and the losses may be accounted for 40-50% in terms of mortality of crop, particularly in kharif season when the climatic conditions are more favourable for pathogens. Management of soil borne pathogens were found difficult, uneconomical and harmful for the environment. Biological control is a great renaissance of interest and research in microbiological balance to control soil-borne plant pathogens and leads to the development of a better farming system. In biological control, genus *Trichoderma* serves as one of the finest bioagents, which is found to be effective against a wide range of soil borne pathogens. *Trichoderma* is a soil inhabiting filamentous fungus, which belongs to the division Ascomycota. The biocontrol potential of *Trichoderma* spp. is due to their complex interaction with plant pathogens either by parasitizing them, secreting antibiotics or by competing for space and nutrients. In the present study, to evaluate the *in vitro* efficiency of fungal antagonist *Trichoderma* spp. against mycelial growth of *S. rolfsii*. Tr-3 isolate reported maximum percent inhibition of 73.81%, whereas least inhibition was noticed in the isolate Tr-10 with 41.05% inhibition when compared over control.

Keywords: groundnut, stem rot, *Sclerotium rolfsii*, bio-control, *Trichoderma*

Introduction

Groundnut or peanut (*Arachis hypogeae* L.) is an important edible leguminous oilseed crop cultivated throughout the tropical and sub-tropical regions of the world. It is the fourth important source of edible oil and third most source of vegetable protein (Baskey S *et al*., 2020) [3]. Different biotic and abiotic factors are affected the groundnut production. The crop is susceptible to losses incited by soil borne pathogens due to the close association of the pods with the soil. The roots and pods are attacked by numerous soil borne fungi causing quantitative and qualitative losses of yield (Ramanjineyulu P *et al*., 2021) [14]. Stem rot of groundnut caused by *Sclerotium rolfsii* Sacc. is one of the major constraint to groundnut production in many countries in warm and humid areas (Bagwan, 2011) [2]. The average yield losses due to this disease are known from 25%, which goes up to 80% in severe cases. *S. rolfsii* also causes indirect losses such as reduction in both dry weight and oil content of groundnut kernels besides downgrading the quality of pod and fodder. Stem rot is a persistent soil borne disease throughout India and its prevalence is increasing gradually even at maturity stage of the groundnut crop. Though S. *rolfsii* resides both on seed and soil, soil-borne nature of the disease is more widespread than seed borne (Kumar *et al*., 2013) [11]. The occurrence of the disease is more visible at 30 to 45 days after germination and at the time of harvest under rain-fed situations due to low and erratic distribution of rainfall. Punja (1985) [12] reported that increase in temperatures and damp conditions enhance sclerotial germination. The pathogen survives in the soil as resistant structures called sclerotia that are found associated with plant debris or near the soil surface remaining viable for a long period in the absence of a susceptible host, serving as primary inoculum for disease.

The development of safer and environmentally feasible plant disease control alternative has become a top priority. In this context, biological control becomes an urgently needs for modern agriculture. Fungi of the genus *Trichoderma* are potential biocontrol agents of several soil born phytopathogens (Hassan *et al*., 2014) [8]. Moreover, all *Trichoderma* isolates exhibited inhibition to the mycelial growth of various soil borne pathogens. This could be due to the production of diffusible components, such as lytic enzymes or water-soluble metabolites (Anees *et al*., 2010) [1]. *Trichoderma* strains are free-living fungi that are common in soil and root ecosystems. They are highly interactive in root, soil and foliar environments. They produce or release a variety of compounds that induce localized or systemic resistance responses in plants. *Trichoderma* spp. is able to produce unpredictable antibiotics in agar and their culture filtrates can also be used for control of fungal growth (Rekha *et al*., 2012) [15]. The aim of the current investigation was to examine the antagonistic potential of different *Trichoderma* species for bio control of *S. rolfsii*.

Materials and Methods

Isolation and identification of pathogenic culture

The pathogen was isolated from the groundnut plants showing typical symptoms of stem rot disease by tissue segment method (Rangaswami, 1958). The infected portion of the stem was cut into small bits, surface sterilized in 0.1 percent sodium hypochlorite solution for 1 min., washed in repeated changes of sterile distilled water and plated onto PDA medium in sterilized petridishes. The plates were incubated for room temperature 28±2°C for five days and were observed the fungal growth. The fungal isolates were purified by single hyphal tip method. The purified isolates were identified as *Sclerotium rolfsii* based on morphological and colony characteristics.

Isolation of fungal antagonistic culture

Ten *Trichoderma* spp. was isolated from different groundnut growing areas by soil dilution plate technique (Dhingra and Sinclair, 1995) [5] using *Trichoderma* specific medium (TSM) (Elad and Chet, 1983) [7]. The isolate were purified by single hyphal tip method and the culture were stored in test tube slants at 4°C for further studies. Identification of antagonists strains were confined by morphological and cultural characteristics of the colonies, measurement of hyphal diameter, conidiophores and conidia dimensions (Rifaee, 1969).

Efficacy of antagonistic *Trichoderma* spp. against *Sclerotium rolfsii*

In vitro antagonistic potential of *Trichoderma* spp. were evaluated against the virulent *Sclerotium rolfsii* isolate through dual culture technique (Dennis and Webster, 1971) [6]. Seven day old cultures of both pathogenic and antagonistic fungi were inoculated on PDA plates at periphery. In control plate only pathogenic fungi were inoculated. Three replications were maintained in each treatment. Plates were incubated at 28^{o}c. Observations of colony growth were recorded. Diameter of colony was measured in cm and percent inhibition was calculated by using following formula suggested by Pandey *et al*., (2000).

Percent inhibition (I) = C-T/C × 100

Where,
I = Per cent inhibition in growth of test pathogen
C = Radial growth in control
T = Radial growth in treatment

Preparation of the culture filtrates of *Trichoderma spp.*

The effective five *Trichoderma* isolates were grown for 15 days at room temperature (28 ± 2°C) in Erlenmeyer flasks containing 50 ml of sterilized potato dextrose broth. Then the cultures were filtered through bacteriological filter under vacuum and the filtrates thus obtained were used for the studies.

Effect of antagonistic culture filtrates on the mycelial growth of *Sclerotium rolfsii* (Poisoned food technique) (Groover and Moore, 1962)

The culture filtrates of the antagonists were separately incorporated into sterile PDA melted medium at 5, 10, 15 and 20 per cent concentration by means of a sterile pipette. The amended media were transferred to sterile Petri dishes separately @ 15 ml and allowed to solidify. The PDA medium without the culture filtrate served as control. Each plate was inoculated at the Centre with a five days old PDA culture disc of *Sclerotium rolfsii*. Three replications were maintained for each treatment. The diameter of the mycelial growth (mm) of *S. rolfsii* was measured after 5 days of incubation. The per cent inhibition of the test fungi was calculated by the formula of Vincent (1927) [18].

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

Where,
I = Per cent inhibition of fungal growth
C = Radial Growth in Control
T = Radial Growth in treatment

Result and discussion

A field survey was conducted in major groundnut growing areas of Tamil Nadu to collect the 20 different stem rot infected samples. To evaluate the virulence among 20 isolates, pathogenicity test was conducted in pots and SrALR recorded the highest disease incidence. Based on the morphological and molecular characters, SrALR isolate was identified as *Sclerotium rolfsii*. The culture sequence was deposited in NCBI (National Centre for Biotchnology Information) genbank with Accession no: MZ920139.

Cultural characteristics of different *Trichoderma* spp. isolates

Ten different *Trichoderma* isolates were isolated from different locations and determined the growth patterns of the different isolates which observed by the colony growth, colony color, conidiophore, conidial color, conidia shape, conidial breadth and length. The petri plates were fully occupied with the mycelial growth of the *Trichoderma* spp. within five days and colonies were observed on whitish green, dull green, light green, dark green, pale white and pale yellow in color with compact or floccose in mycelial form (table 1a).

Conidial characters were observed and recorded on the table 1b. Conidiophores are highly branched with regular or irregular manner and some of the species were noticed on moderately branched conidiophores. Shape of the conidia observed in globose, ellipsoidal and obovoid in nature with dark or dull green in color. Conidial length and breadth were measured in the range of 2.09-3.75(µ) and 1.52-4.18(µ) respectively. Conidiophores of *Trichoderma virens* were smoothly bent gather and not spread to top. Conidia broadly rounded to obovoid, both ends broadly rounded or with the base Narrower (Shah *et al*., 2012) [17]. 6 *Trichoderma* isolates (PB 10, 13, 23, 26, 27 and 28) were exhibited hyaline conidiophores arising in clusters from aerial mycelium, branching toward the tip, each branch terminating in a penicillus of 3-6 closely appressed and divergently branched phialides towards the apex, with a sterile stipe (Sharma and Singh, 2012). Isolates GRT-1, GRT-6 and GRT-9, colony showed dark green to dark bluish green sporulation, colony reverse was amber or uncolored. Conidiophore usually long, infrequently branched, verticillate conidiophores. Phialides are frequently paired, lageniform convergent (GRT-6 and GRT-9) or divergent (GRT-1). Conidial shape was globose to ellipsoidal (Chandra sekar *et al*., 2017).

Table 1: Morphological and cultural characteristics of different Trichoderma spp.

Isolates	Locality	Mycelial characters		
		Growth rate (cm)	**Colony color**	**Mycelial form**
Tr-1	Pinnalur	9	Whitish green to dull green	Compact and cottony
Tr-2	Veppanthatti	9	Light Green to bright green	Floccose
Tr-3	Parigam	9	Dark green	Floccose
Tr-4	Nedungur	9	Dark green to whitish green	Compact and cottony
Tr-5	Kadathur	9	Whitish green to dull green	Floccose
Tr-6	Nachiyarpettai	9	Yellow to green	Arachnoid
Tr-7	T-Palur	9	Pale yellow green	Scattered
Tr-8	Sengam	9	Light Green to bright green	Floccose to Arachnoid
Tr-9	Melpadai	9	Pale white to dull green	Compact colony
Tr-10	Thurinjapuram	9	Whitish green to dull green	Floccose to Arachnoid

Table 2: Morphological and cultural characteristics of different *Trichoderma* spp.

Isolates	Locality	Conidial characters				
		Conidiophore	**Conidial shape**	**Conidial color**	**Conidial length (µ)**	**Conidial breadth (µ)**
Tr-1	Pinnalur	Moderately branched, Regular	Globose	Dull green	2.09 –3.05	1.65 – 3.55
Tr-2	Veppanthatti	Highly branched, Regular	Ellipsoidal	Green	2.34 –3.21	2.34 – 3.52
Tr-3	Parigam	Highly branched	Globose	Dark green	2.27 –3.42	2.55 – 3.68
Tr-4	Nedungur	Few lateral branches at apex, Irregular	Sub globose to obovoid	Dark green	2.51 –3.03	2.14 – 3.32
Tr-5	Kadathur	Highly branched, Irregular	Ellipsoidal	Dull green	2.38 –3.43	1.52 – 3.03
Tr-6	Nachiyarpettai	Branched regular	Globose to ellipsoidal	Green	2.98 –3.57	2.55 – 4.18
Tr-7	T-Palur	Moderately branched, Regular	Narrow ellipsoidal	Dark green	2.72 –3.24	2.11 – 3.62
Tr-8	Sengam	Branched regular	Globose	Dark green	2.33 –3.54	1.85 – 2.89
Tr-9	Melpadai	Few lateral branches at apex, Irregular	Obovoid	Dull green	2.48 –3.75	2.34 – 3.44
Tr-10	Thurinjapuram	Rarely branched, Regular	Globose	Light green	2.25 –3.20	2.90 – 3.03

In vitro efficiency of *Trichoderma* spp. against *Sclerotium rolfsii* (SrALR)

Ten *Trichoderma* isolates were collected from different Groundnut growing areas of Tamil Nadu, which tested against the pathogenic culture of *Sclerotium rolfsii* by dual culture technique (table 2). Among the ten isolates, the isolate Tr-3 recorded the maximum percent inhibition of 73.81% followed by Tr-7 which recorded 70.02 % inhibition, whereas least inhibition was noticed in the isolate Tr-10 with 41.05% inhibition over control.

Three different *Trichoderma* spp. were tested against *Sclerotium rolfsii*, *Trichoderma hamatum* is most efficacious with 72.2% mycelial growth inhibition, 89.02% sclerotial inhibition followed by *Trichoderma harzianum* with 74.7% mycelial growth inhibition, 59.84% sclerotial inhibition and *Trichoderma viride* with 71.9% mycelia growth inhibition, 59.99% sclerotial inhibition (Vineela DRS *et al*., 2017) [19]. Kannan C *et al*., 2020 [10] reported that *T. asperellum* (Ta2) recorded the maximum inhibition zone (72.98) followed by *T. asperellum* (Ta5) which recorded 70.72 per cent inhibition on the mycelial growth and the isolate *T. asperellum* (Ta9) recorded the minimum inhibition zone (36.08).

Table 3: *In vitro* efficacy of *Trichoderma* spp. against *Sclerotium rolfsii* (SrALR)

Sl. No	Isolates	Mycelia growth (cm)		Percent inhibition over control
		***Trichoderma* spp.**	***Sclerotium rolfsii***	
1	Tr-1	6.09	2.91	67.31c (55.12)
2	Tr-2	5.48	3.52	60.28e (50.94)
3	Tr-3	6.66	2.34	73.81a (59.21)
4	Tr-4	4.89	4.12	53.58g (47.06)
5	Tr-5	5.12	3.88	44.10i (41.61)
6	Tr-6	4.40	4.60	49.32h (44.60)
7	Tr-7	6.33	2.67	70.02b (56.79)
8	Tr-8	4.00	5.00	56.47f (48.68)
9	Tr-9	5.62	3.38	62.56d (52.30)
10	Tr-10	3.72	5.28	41.05j (39.82)
11	Control	-	9.00	-

Mean of three replications Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

Efficacy of Non- Volatile compounds produced by *Trichoderma* spp. against *Sclerotium rolfsii* (SrALR)

The best five virulent *Trichoderma* spp. were selected in the dual culture technique and study the antagonistic potential against pathogenic culture of *Sclerotium rolfsii* by poison food technique. The results represented in the table 3 showed that all the five isolates were significantly inhibited the mycelial growth of *S. rolfsii* when compared to control.

Among the five isolates were tested, the isolate Tr-3 recorded least mycelial growth of 0.98cm at 30% concentration of the culture filtrate. Based on the morphological and molecular characters, Tr-3 isolate was identified as *Trichoderma asperellum.* The culture Sequence was deposited in NCBI genbank with accession no: OL872253. Hirpara *et al*., 2017 [9] stated that the mycoparasitism is one of the main mechanisms used by *Trichoderma* isolates to control *Athelia rolfsii* because they can produce chitinase and β-1, 3-glucanase, which are involved in mycoparasitism. Efficacy of volatile compounds produced by seven *Trichoderma* spp. were tested, the maximum percentage inhibition was recorded with isolate ThrG1 (30.00 per cent) and minimum was recorded with isolate ThrG7 (20.00 per cent) (Rashmi KH *et al*., 2017) [13].

Table 4: Efficacy of Non-Volatile compounds produced by *Trichoderma* spp. against *Sclerotium rolfsii* (SrALR)

Sl. No	Isolates	Mycelial growth (cm) (5DAI)							
		5%	Percent inhibition over control	10%	Percent inhibition over control	15%	Percent inhibition over control	20%	Percent inhibition over control
1	Tr-1	4.60	48.89^{c} (43.92)	3.63	59.67 ^{b} (50.48)	3.46	61.56 ^{c} (51.51)	2.28	74.67 ^{c} (59.45)
2	Tr-2	6.00	33.34^{e} (35.50)	5.28	41.33 ^{d} (40.08)	4.94	45.11 ^{e} (41.82)	4.31	52.11 ^{e} (46.36)
3	Tr-3	3.02	66.45^{a} (54.50)	2.40	73.34 ^{a} (59.00)	1.81	79.89 ^{a} (63.00)	0.98	89.11 ^{a} (71.12)
4	Tr-7	4.43	50.78^{b} (45.33)	3.56	60.44 ^{b} (51.27)	2.73	67.45 ^{b} (55.20)	1.96	78.22 ^{b} (62.19)
5	Tr-9	5.05	43.89^{d} (41.30)	4.49	50.11 ^{c} (45.24)	4.02	55.33 ^{d} (48.02)	3.59	60.11 ^{d} (51.12)
6	Control	9.00							

Mean of three replicationsValues in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

References

1. Anees M, Tronsmo A, Hermann V, Hjeljord LG, Heraud C, Steinberg C. Characterization of field strains of Trichoderma antagonistic against *Rhizoctonia solani*. Fungal Biology,2010:114:691-701.
2. Bagwan NB. Morphological variation in *Sclerotium rolfsii* Sacc. isolates causing stem rot in groundnut (*Arachis hypogaea* L.). Int J Plant Prot,2011:4(1):68-73.
3. Baskey S, Khalko S, Hembram S, Sharma BR, Ali S. Survey for the Incidence of Stem Rot of Groundnut in North Bengal Districts of West Bengal, India. Int.J.Curr.Microbiol.App.Sci,2020:9(1):328-333.
4. Chandra Sekhar Y, Khayum Ahammed S, Prasad TNVKV, Sarada Jayalakshmi Devi R. Identification of *Trichoderma* species based on morphological characters isolated from rhizosphere of groundnut (*Arachis hypogaea* L.) International Journal of Science, Environment and Technology,2017:6(3):2056-2063.
5. Dhingra OP, Sinclair JB. Basics Plant Pathology methods, 2ndedn. CRC press, Bocca Raton, America, 1995.
6. Dennis C, Webster J. Antagonists properties of species groups of *Trichoderma* Production of non-volatile antibiotics. T. Brit. Mycol. Soc,1971:57:25-39.
7. Elad Y, Chet I. Improved selective media for isolation of *Trichoderma* spp and *Fusarium* spp. Phytoparasit,1983:11:55-58.
8. Hassan MM, Gaber A, El-Hallous EI. Molecular and Morphological Characterization of *Trichoderma harzianum* from different Egyptian Soils. Wulfenia Journal,2014:21:80-96.
9. Hirpara DG, Gajera HP, Hirpara HZ, Golakiya BA. Antipathy of *Trichoderma* against *Sclerotium rolfsii* Sacc. Evaluation of cell wall-degrading enzymatic activities and molecular diversity analysis of antagonists. J. Mol. Microbiol. Biotechnol,2017:27(1):22-28.
10. Kannan C, Praveen A, Jaiganesh V, Senthil Murugan S. Evaluation of fungal and bacterial antagonist against *sclerotium rolfsii* in groundnut. Plant Archives,2020:20(2):5087-5090.
11. Kumar N, Dagla MC, Ajay BC, Jadon KS, Thirumalaisamy PP. *Sclerotium* stem rot: A threat to groundnut production. Popular Kheti,2013:1: 26-30.
12. Punja ZK. The biology, ecology, and control of *Sclerotium rolfsii*. Annual Review of Phytopathology,1985:23:97-127.
13. Rashmi KH, Bireswar Sinha, Pramesh KH, Sobita devi PH. Native *Trichoderma* spp for the management of stem rot of groundnut caused by *Sclerotium rolfsii* Sacc in Manipur, India. International journal of current microbiology and applied sciences,2017:6:1343-1351.
14. Ramanjineyulu P, Viswanath K, Kiran Kumar N, Nagamani P. Characterization of pod rot disease associated pathogens of groundnut (*Arachis hypogaea* L.). The Pharma Innovation Journal,2021:10(5):623-629.
15. Rekha D, Patil MB, Shridhar S, Swamy P, Rajini K, Gamanagatti B. In vitro screening of native *Trichoderma* isolates against *Sclerotium rolfsii* causing collar rot of ground nut. Pak. J. Bot,2012:34:117-120.
16. Sharma KK, Singh US. Cultural and morphological characterization of rhizospheric isolates of fungal antagonist *Trichoderma*. Journal of Applied and Natural Science,2014:6(2):451-456.
17. Shah S, Nasreen S, Sheikh PA. Cultural and morphological characterization of *Trichoderma* spp. associated with green mold disease of *Pleurotus* spp. in Kashmir. Research Journal of Microbiology,2012:7:139-144.
18. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature,1927:59:850.
19. Vineela DRS, Beura SK, Dhal A, Swain SK, Sethi D. Efficacy of chemicals, bio-agents and their compatibility in management of stem rot disease of groundnut. International Journal of Chemical Studies,2017:5(5):443-446.