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Management of Stem Rot of Groundnut caused by *Sclerotium rolfsii* Sacc. with Actinomycetes

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A B S T R A C T

Sclerotium rolfsii Sacc. is an destructive soil borne fungal pathogen with wide host range that includes groundnut, an important oil seed crop in India. Biological control offers an interesting alternative to fungicides for sustainable management of soil borne diseases. A total of ten isolates of actinomycetes were collected from different districts of Telangana and tested for their inhibitory activity against *Sclerotium rolfsii* Sacc., the stem rot of pathogen. These isolates were identified as *Streptomyces* spp. on the basis of standard bacteriological test and 16S RNA gene sequence analysis. Among the various isolates tested *in vitro*, three isolates (RGP-49%, NDG- 47.7%, YLD-45% inhibition) were found effective in inhibiting the mycelial growth of *Sclerotium rolfsii* in dual culture assay. These three potential isolates had shown 100% compatibility with the systemic fungicides (carbendazim, tebuconazole, propiconazole and azoxystrobin) whereas less compatibility with the non-systemic fungicides (captan and mancozeb) when tested by disc diffusion method. Seeds of groundnut when treated with *Streptomyces* spp. increased root length, shoot length and seedling vigour. Greenhouse studies revealed that talc-based formulations of *Streptomyces* spp. increased the germination percentage by RGP- 89%, NDG- 85%, YLD-84%. Among the three potential isolates maximum disease control was obtained when seed treatment and soil application of isolate RGP (*Streptomyces parvulus*) and YLD (*Streptomyces werraensis)* was used in controlling stem rot under greenhouse conditions.

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop in the world belonging to the Leguminosae family. It is one of the essential food and cash crops of our country and is considered as the 'king' of oilseeds. It is also known as wonder nut, peanuts or monkey nuts and provides over 30 essential nutrients. It is a valuable source of protein, niacin, fiber, vitamin E and anti-oxidants. The seeds are valued for high 40-48 per cent edible oil

content, 22-26 per cent digestible protein content, 26 per cent carbohydrate, 3 per cent fat and high calcium, thiamine and niacin contents, which make a substantial contribution of protein for human and animal nutrition. They can be consumed directly or processed into oil meal or cake which serves as an important source of protein for livestock or further processed into confectionery products or snack food. The oil cake is rich in nitrogen, potassium, phosphorous and can be used as fertilizers. Immense health benefits are due to the presence of antioxidants and resveratrol. The groundnuts are also used in the manufacturing of soaps, shaving creams, cosmetics and lubricants.

Groundnut is cultivated worldwide in an area of 28 M ha with a total production of 47 Mt averaging a productivity of 1.6 t ha^{-1} (FAO, 2017). The leading producers of the groundnut crop include China (54%), India (22%), and USA (9.03%). In India, the crop is mostly grown in the states of Gujarat, Andhra Pradesh, Telangana, Tamil Nadu, Karnataka, Rajasthan and Maharashtra constituting about 80 percent of the total area and production of groundnut.

In India a large number of diseases attack groundnut (Mayee and Datar, 1988). Among many soil borne diseases, stem rot or white mold caused by *Sclerotium rolfsii* Sacc. (Saccardo, 1911) is an important disease causing significant yield losses in several groundnut growing countries (Mehan *et al.,* 1994). The pathogen attacks host plant during at all stages when conditions are favourable (Punja, 1985) and yield losses over 25% have been reported (Mayee and Datar, 1988).

The pathogen survives as resistant structures called sclerotia in the soil which serves as primary source of inoculum for disease in the absence of susceptible host and remain viable for a long period (Backman and Brenneman, 1997; Aycock, 1966). High temperature of 30°C, dense planting, frequent irrigation, moist conditions favours infection and fungal mycelial spread within and between plants (Aycock, 1966; Paolo, 1933; Punja, 1985).

The stem rot infected plant produces typical symptoms which include dark brown lesions on the stem just below the soil surface followed by drooping and wilting of entire plant (Garren, 1959; Backman and Brenneman, 1997). Such wilted plants show cottony mycelial growth of the fungus, girdling of basal part of the stem, spreading in both directions covering the stem and roots followed by formation of light to dark brown sclerotia (Mehan *et al.,* 1995). Whitish mycelial growth with sclerotial bodies resembling mustard seeds can be observed at collar region. Infected seeds appear to be bluish-black, reducing its economic value (Garren and Higgins, 1947, Subrahmanyam *et al.,* 2012).

Sclerotium rolfsii causes immediate knock down effect on plant by producing organic acids like oxalic acid and enzymes like cellulases (Bateman, 1970; Bateman, 1972; Bateman and Beer, 1965) Hence, yield losses caused by stem rot are directly proportional to the disease incidence. Further, once established in the soil, the soil turns sick, and hence management of the disease is very difficult.

Different practices are recommended for management of groundnut stem rot such as deep summer ploughing, destruction of plant debris, crop rotation with jowar and bajra, seed treatment with carbendazim or captan or mancozeb or tebuconazole, soil drenching with hexaconazole, application of ammonium sulphate or calcium ammonium nitrate instead of urea and application of gypsum at flowering stage. Management of diseases caused by these soil borne plant pathogenic

fungi by chemicals are not practicable owing to high cost besides causing environmental pollution and resistance development in target fungus (Biswas and Sen, 2000; Pant and Mukopadhyay, 2001; Patibanda *et al.,* 2002; Uma Maheshwari *et al.,* 2002; Rudresh *et al.,* 2005). Further, no single treatment is full proof and disease continues to cause losses in farmers' fields.

At this juncture, biological control seems to offer a practicable approach as it has several advantages (when applied either alone or in combination with other management practices) like eco-friendliness, effective against soil borne disease, growth promoting activity etc., which cannot be possible by chemicals.

Actinobacteria are excellent choices as plant disease control agents due to their ability to produce fungicidally important compounds that antagonize many phytopathogens (Xiao *et al.,* 2002; Meschke *et al.,* 2012).

These are a diverse group of free living saprobic mycelial bacteria present abundantly in the soil, maintaining the structure and integrity of soil. They also take part in the recycling of soil nutrients. However, reports on the role of actinomycetes as biocontrol agents is meagre. Keeping this in view the objectives formulated were:

To isolate and identify actinomycetes from the rhizosphere soil of groundnut crop.

To screen the actinomycete isolates against *Sclerotium rolfsii* under *in vitro* conditions.

To study the compatibility of actinomycetes isolates with commonly used fungicides.

To evaluate the potential isolates of actinomycetes against *Sclerotium rolfsii* causing stem rot of groundnut in glass house.

Materials and Methods

Isolation of pathogen and actinomycetes

The fungus *Sclerotium rolfsii*, was isolated from a stem rot infected plants collected from the groundnut fields of Seed Technology Research Centre (SRTC), Rajendranagar, Hyderabad and maintained on potato dextrose agar (PDA) plates incubated at 25°C in a BOD incubator for five days. The pathogenicity of the fungal culture was proved by using Koch's postulates. After 14 days sclerotia were harvested from plates and stored at 4°C for further studies.

Actinomycetes were isolated from rhizosphere soils of groundnut collected from different districts of Telangana state (Table 1). Soil samples were suspended in sterile water (10gm in 100 ml) and agitated for 45 min at 160 rpm.

The supernatants were serially diluted upto 10^{-6} and spreaded (0.1 ml) evenly on the actinomycetes isolation agar medium incubated at 28 ± 2°C for 15 days. Subculturing was done on starch casein agar medium to get pure colonies. These isolates were identified as *Streptomyces* spp. on the basis of standard bacteriological test and 16S RNA gene sequence analysis.

Phenotypic Characterization of Actinomycetes

After the incubation period the plates were examined for typical actinomycetes colonies such as round, small, opaque, compact, frequently pigmented (white, brown, gray pink or other colors) and appear dull looking (Praveen *et al.,* 2017). The phenotypic features such as aerial mass colour, colony shape, colony surface, colony colour, spore formation of actinomycetes isolates was examined on starch ceasin medium.

Biochemical Characterization of Actinomycetes

Biochemical characterization of actinomycetes isolates were studied to understand the basic physiology of the soil actinomycetes. Biochemical tests analysis such as oxidase test, catalase test, voges prausker's test, methyl red test, indole test, gelatin liquefaction, hydrolysis of startch and hydrogen sulphide test were done ((Holt, 1989).

Molecular Characterization of *Actinomycetes*

The molecular characterization of pure cultures of actinomycetes was done at ICRISAT. Sequenced DNA data were complied and analyzed with a Basic local alignment search tool (BLAST) (www.ncbi.nlm.nih.gov). The genomic DNA sequence was used for species identification and 16S rDNA phylogenetic tree was built by Neighbor-Joining method constructed with MUSCLE and the evolutionary analyses were conducted in MEGA X.

In vitro screening of actinomycetes against *S. rolfsii*

Ten isolates of actinomycetes were tested for antifungal activity against *S. rolfsii* under *in vitro* conditions using a dual culture assay (Anjaiah *et al.,* 2003 and Adhilakshmi *et al.,* 2013). Fresh cultures of actinomycetes were streaked on one end of the starch casein agar media plates and incubated for 96 h at 28 ± 2 ºC. After incubation period, a mycelial plug (5 mm diameter) from a 3-days-old *S. rolfsii* culture was placed on the opposite side, perpendicular to the actinomycetes in the media plates. Control plates were maintained with *S. rolfsii* mycelial discs alone. The Petri dishes were incubated for 7 days at 28 ± 2 ºC, until the fungus completely covered the control plates. Three replications were maintained for each isolate. Per cent inhibition (I %) of the pathogen by the antagonistic isolates was calculated with the formula (Dennis and Webster 1971). Per cent inhibition (I %) = (C – T) / C x 100, where, I = Per cent reduction in growth of the pathogen, C = Radial growth of pathogen in control, T = Radial growth of pathogen in treatment

Preparation of talc based formulation of potential *Streptomyces* spp.

Talc-based formulations of effective *Streptomyces* spp. (RGP, NDG and YLD) were prepared on carrier material, talcum powder. One hundred grams of carrier material was taken and the pH was adjusted to 7 by adding $CaCO_3$ at the rate of 15g/kg. Carboxy methyl cellulose (CMC) was then added at the rate of 10g/kg and mixed well. The mixtures was then autoclaved for 30 min at 121^{o}C (15lb/inch^{2}). After autoclaving, 400 ml of bacterial suspension (1×10^{8} cfu/ml) was added to the sterilized carrier material (1kg) and thorough mixed followed by drying aseptically and then grounded to powder. They were then packed in sterile polythene bags and stored at 4°C (Vidhyasekaran and Muthamilan,1995). The concentration of colony-forming units was obtained using the formula: Number of cfu/g = Number of colonies / (Amount of sample plated x Dilution) X 100

Efficacy of *Streptomyces* spp. on plant growth

The influence of *Streptomyces* spp. (RGP, NDG and YLD) on germinability and vigor of peanut seedlings was evaluated using the Ragdoll method (Chamblee and Green 1995) with slight modifications. K-6 variety seeds were surface sterilized using 2.5 % sodium hypochlorite solution for 2 min and washed several times with distilled water. Seed treatments (ST) were prepared by treating surface sterilized seeds with talc formulation

of *Streptomyces* spp. Treated peanut seeds were placed on germination towels and incubated in growth chambers at 28 °C for 15 days. Four replications were kept for each treatment, with 10 seeds per replication.

Seeds soaked in sterile distilled water served as control. Seven days after incubation, number of germinated seeds was recorded (Sai *et al.,* 2018). At the end of 15 days, root and shoot lengths of seedlings was recorded and the seedling vigour index was calculated as per the following formula: Vigour index = germination per cent X (root length + shoot length)

Compatibility of actinomycetes with fungicides in vitro

Streptomyces spp. isolates were tested *in vivo* for their compatibility with the fungicides by Disc diffusion method. A loop full of actively growing culture of *Streptomyces* spp. isolates was added into starch casein broth and incubated at 28 ± 2^{o}C for 3 days at 180 r min^{-1}. After incubation period, 0.1 ml of *Streptomyces* spp. isolate suspension was uniformly spreaded over the starch casein agar media plates. Fungicide solutions of required concentrations *i.e.* 50, 100, 250 and 500 ppm, a.i. for systemic fungicides (propiconazole, tebuconazole, azoxystrobin, carbendazin) and 1000, 1500, 2000 and 2500 ppm a.i. for non-systemic fungicides (captan and mancozeb) were prepared in separated test tube. 5mm diameter sterile filter paper discs were dipped in fungicide solutions at required concentrations and placed on the starch casein agar. Discs dipped in normal water and placed on the starch casein agar served as control. For each treatment three replications were maintained and incubated at 28 ± 2 ºC. After 14 days Per cent inhibition of *Streptomyces* spp. with certain concentration was recorded with the formula (Louis *et al.,* 2016): Per cent inhibition (I %) = (C – T) / C x 100, where, I = Per cent reduction in growth of the *Streptomyces* spp., C = Radial growth of *Streptomyces* spp. in control, T = Radial growth of *Streptomyces* spp. in treatment

Biological control assay with *Streptomyces* spp. formulation

Pot culture studies were conducted to test the antagonists for their efficacy *in vivo*. Two different application methods i.e., seed treatment and soil application were used to assess the biocontrol potential in decreasing stem rot of groundnut caused by *S. rolfsii* (Adhilakshmi *et al.,* 2013 and Jacob *et al.,* 2018).

S. rolfsii, mass multiplied on sorghum *grains was* mixed with sandy loam soil @ 4g kg^{-1} soil in the pots and were incubated for twenty four hours before sowing.

Groundnut seeds of K-6 variety were treated with talc based formulation of potential *Streptomyces* spp. @ 10 g kg^{-1} of seed in the infested soil.

For soil applications, the talc power formulations of potential *Streptomyces* spp. were added to FYM and neem cake mixture (in 2:90:8 ratio) and incubated for 15 days. Such antagonist fortified FYM was added to soil @ 2g/pot before sowing groundnut seeds on the top 2" layer. For treatments involving seed treatment with fungicide and biocontrol agent. First seeds were coated with fungicide followed by *Streptomyces* spp. talc powder and were sown twenty four hours after *S. rolfsii* inoculation.

Pathogen inoculated and uninoculated pots were maintained as checks and three replications were maintained for each treatment. Seeds were sown @ 5 per pot. Irrigation was done as and when necessary to maintain soil moisture. Data on number of seeds germinated and number of plants survived was recorded 30 days after sowing as

per the formula: Per cent germination = Number of seeds germinated in each treatment / Total number of plants assessed X 100 and Per cent disease incidence = Number of infected plants / Total number of plants assessed X 100

Results and Discussion

Biological control of stem rot of groundnut has been pursued by using antagonistic microorganism (Rakh *et al.*, 2011, Jacob *et al.*, 2016).

In the present investigation ten actinomycetes isolates were isolated, screened for their biocontrol potential against groundnut stem rot pathogen *Sclerotium rolfsii in vitro* and the selected potential isolates were further assessed for the compatibility with commonly used fungicides and in pot culture against groundnut stem rot.

Phenotypic Characterization of Actinomycetes

Ten pure and clear distinguishable colonies were isolated from 10 soil samples, these strains were slow to moderate growing and showed colony colours like white and grey.

Binocular compound microscope (100X) studies were used to examine the spore surface and spore shape. (Table.1).

Biochemical Characterization of Actinomycetes

The biochemical characterization of the potential isolate, NDG and YLD included oxidase test, catalase test, gelatin liquefaction, hydrolysis of startch and hydrogen sulphide test (Table.2).

Molecular Characterization of Actinomycetes

16S rRNA gene sequencing established revealed that the nine isolates belong to the genus *Streptomyces* except isolate LKP which was identified as *Nocardiopsis* of Phylum Actinobacteria (Table.3). The 16S rRNA gene sequences were submitted to the Gen Bank with the accession numbers.

Table.1 Collection of isolates of actinomycetes from the rhizosphere of groundnut plants from different districts of Telangana

Village	District	Latitude	Longitude	Isolate code
Kadarigudem	Warangal	17.812097	79.591928	KDG
Yellanda	Warangal	17.790303	79.593930	YLD
Dharmapur	Warangal	17.589325	79.395619	DMP
Ramojikummarigudem	Warangal	17.772540	79.575977	RKG
Palem	Mahabubnagar	16.555308	78.209277	PLM
Nandigama	Mahabubnagar	18.049111	79.826284	NDG
Dasaripally	Mahabubnagar	17.990992	79.860546	DSP
Rangapur	Ranga Reddy	17.544298	79.522845	RGP
Lenkallapally	Nalagonda	18.027734	79.856665	LKP
Kamapally	Nizamabad	17.986614	79.858700	KMP

Table.2 Phenotypic characterization of different isolates of actinomycetes

S.no	Strain	Aerial mass colour	Colour of substrate mycelium	Melanoid pigment	Diffusible pigment	Colony Shape	Colony Surface	Colony colour	Light microscopy	Spore surface morphology	Spore shape	Speed of growth
1	KDG	White(W)	Moderate yellow	None	None	Round	Smooth	White	Flexible(F)	Smooth	Rod shaped	Moderate
2	RGP	White(W)	Greyish brown	Brown	Pale Brown	Irregular	Rough	White	Spira(S)	Smooth	Rod shaped	Slow
3	YLD	White(W)	Red	None	None	Round	Powdery	White	Spira(S)	Smooth	Rod shaped	Moderate
4	DMP	White(W)	Greyish yellow	Brown	None	Round with lines	Rough	White	Spira(S)	Smooth	Rod shaped	Good
5	PLM	White(W)	Bright yellow	None	None	Round radiated	Smooth	White	Spira(S)	Smooth	Rod shaped	Good
6	LKP	White(W)	Light Yellow	None	None	Round with feathers	Smooth	White	Spira(S)	Smooth	Rod shaped	Good
7	NDG	White(W)	Brownish yellow	Brown	None	Button with feathers	Powdery	White	Spira(S)	Smooth	Rod shaped	Good
8	RKG	White	Pale yellow	None	None	Double ring	Powdery	White	Spira(S)	Smooth	Ovoid shaped	Good
9	DSP	Gray	Yellowish gray	None	None	Two round circles	Rough	White and grey	Retinaculum Apertum(RA)	Smooth	Rod shaped	Good
10	KMP	Light gray	Light gray	None	None	Round	Smooth	White and grey	Spira(S)	Smooth	Rod shaped	Good

Table.3 Biochemical characteristics of different isolates of actinomycetes

Isolate	Gram's staining	Oxidase test	Catalase test	Voges Prausker's test	Methyl red test	Indole test	Gelatin liquefaction	Hydrolysis of starch	Hydrogen sulphide production	Coagulation of milk
KDG	+	+	+	-	+	-	-	+	-	-
RGP	+	+	+	-	-	-	+	+	+	+
YLD	+	+	+	-	+	-	+	+	+	+
DMP	+	+	+	-	+	-	+	+	+	+
PLM	+	+	+	-	+	-	-	+	+	-
LKP	+	+	+	-	+	-	+	+	-	+
NDG	+	+	+	-	-	-	+	+	+	+
RKG	+	+	+	-	+	-	+	+	+	+
DSP	+	+	+	-	+	-	+	+	+	+
KMP	+	+	+	-	-	-	+	+	+	+

(+) Presence / Positive
(-) Absence / Negative

Table.4 Molecular characterization of actinomycetes isolates

Isolate	Identification of the actinomycete through 16S rRNA gene sequencing	Type strain	Accession number	Sequence similarity
DSP	*Streptomyces griseostramineus*	NBRC 12781	MW070527	97.07
LKP	*Nocardiopsis synnemataformans*	DSM 44143	MW070526	97.07
NDG	*Streptomyces werraensis*	NRBC 13404	MW070529	98.19
KDG	*Streptomyces aureofaciens*	DSM 40127	MW070532	98.24
PLM	*Streptomyces albaduncus*	JCM 4715	MW070531	97.33
RGP	*Streptomyces parvulus*	NBRC 13193	MW073390	97.01
YLD	*Streptomyces aurantiacus*	NBRC 13017	MW073391	98.12
KMP	*Streptomyces cacaoi*	DSM 40057	MW070530	97.23
DPM	*Streptomyces griesus*	DSM 40226	MW073392	97.38
RKG	*Streptomyces lusitanus*	NRBC 13464	MW070528	98.27

Table.5 Effect of different isolates of *Streptomyces* on radial growth of *S. rolfsii* in dual culture assay

Isolate	Radial growth of *Sclerotium rolfsii*							
	Day 2		Day 4		Day 6		Day 8	
	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)
KDG	1.36	10.82	3.23	3.03	5.33	4.20	7.20	0
RGP	1.13	26.09	2.03	39.00	3.03	45.51	3.66	49.06
YLD	1.13	26.09	2.43	27.00	3.73	32.94	3.96	44.90
DMP	1.23	19.56	2.83	15.00	5.43	2.40	7.20	0.00
PLM	1.36	10.82	3.03	9.00	5.53	0.61	7.20	0.00
LKP	1.13	26.09	2.63	21.00	5.36	3.59	7.20	0.00
NDG	1.23	19.56	2.23	33.00	3.03	45.51	3.76	47.75
RKG	1.23	19.56	3.23	3.03	5.41	2.40	7.20	0.00
DSP	1.20	21.72	3.06	7.98	5.36	3.59	7.20	0.00
KMP	1.23	19.56	3.13	6.06	5.53	0.61	7.20	0.00
Control	1.53	-	3.33	-	5.56	-	7.20	-
C.D.	0.10	-	0.09	-	0.10	-	0.05	-
SE(m)	0.03	-	0.03	-	0.03	-	0.01	-

Note: Data are the mean of three replications

Table.6 Compatibility of different concentrations of fungicides with isolate RGP, YLD and NDG of *Streptomyces* spp.

Fungicide A= Systemic B= Non-systemic	Isolate	Per cent inhibition of *Streptomyces* with fungicide Concentration				Mean bioagent YLD inhibition
		A=50ppm	A=100 ppm	A=250 ppm	A=500 ppm	
		B=1000 ppm	B=1500 ppm	B=2000 ppm	B=2500 ppm	
Captan (B)	RGP	0 (0.0)	0 (0.0)	0.833 (5.234)	0.933 (5.540)	0.442 (2.693)
	YLD	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	NDG	0 (0.0)	0 (0.0)	0.767 (4.908)	0.9 (5.36)	0.408 (2.586)
Mancozeb (B)	RGP	0 (0.0)	0 (0.0)	0.867 (5.338)	1.0 (5.732)	0.467 (2.767)
	YLD	0 (0.0)	0 (0.0)	0.667 (4.679)	0.833 (5.234)	0.375 (2.478)
	NDG	0 (0.0)	0 (0.0)	0.767 (5.019)	0.933 (5.540)	0.425 (2.640)
Carbendazim (A)	RGP	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	YLD	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	NDG	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Propiconazole (A)	RGP	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	YLD	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	NDG	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tebuconazole (A)	RGP	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	YLD	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	NDG	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Azoxystrobin (A)	RGP	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	YLD	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	NDG	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Concentration mean (%)	RGP	0 (0.0)	0 (0.0)	0.283 (1.762)	0.322 (1.879)	-
	YLD	0 (0.0)	0 (0.0)	0.111 (0.779)	0.138 (0.872)	
	NDG	0 (0.0)	0 (0.0)	0.250 (1.654)	0.306 (1.829)	
Factors		Fungicide (F)	Concentration(C)	F X C		
C.D.	RGP	0.024 (0.071)	0.019 (0.058)	0.048 (0.141)		
	YLD	0.014 (0.046)	0.011 (0.038)	0.027 (0.092)		
	NDG	0.024 (0.074)	0.019 (0.006)	0.048 (0.148)		
SE(d)	RGP	0.012 (0.035)	0.01 (0.029)	0.024 (0.020)		
	YLD	0.007 (0.028)	0.006 (0.019)	0.014 (0.046)		
	NDG	0.012 (0.037)	0.010 (0.030)	0.024 (0.074)		
SE(m)	RGP	0.008 (0.141)	0.007 (0.070)	0.017 (0.050)		
	YLD	0.005 (0.016)	0.004 (0.013)	0.010 (0.030)		
	NDG	0.008 (0.016)	0.007 (0.013)	0.017 (0.052)		

Notes: Data are mean of three replications.
Figures in parentheses are arc sine transformed values.

Table.7 Efficacy of *Streptomyces* spp. on plant growth

Streptomyces sp. strains	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
RGP	99 (84.30)	14.6	25.833	4,003.73
YLD	97 (80.06)	13.667	23.167	3,573.00
NDG	97.66 (81.24)	13.9	23.833	3,685.13
Control	96 (78.50)	12.333	19	3,008.83
C.D.	1.745	1.142	1.851	283.811
SE(m)	0.527	0.345	0.559	85.698
SE(d)	0.745	0.488	0.791	121.195
C.V.	0.937	4.383	4.217	4.16

Notes: Data are mean of three replications.
Figures in parentheses are arc sine transformed values.

Plate.1 Dual culture technique employed with isolates of actinomycetes against *S. rolfsii*

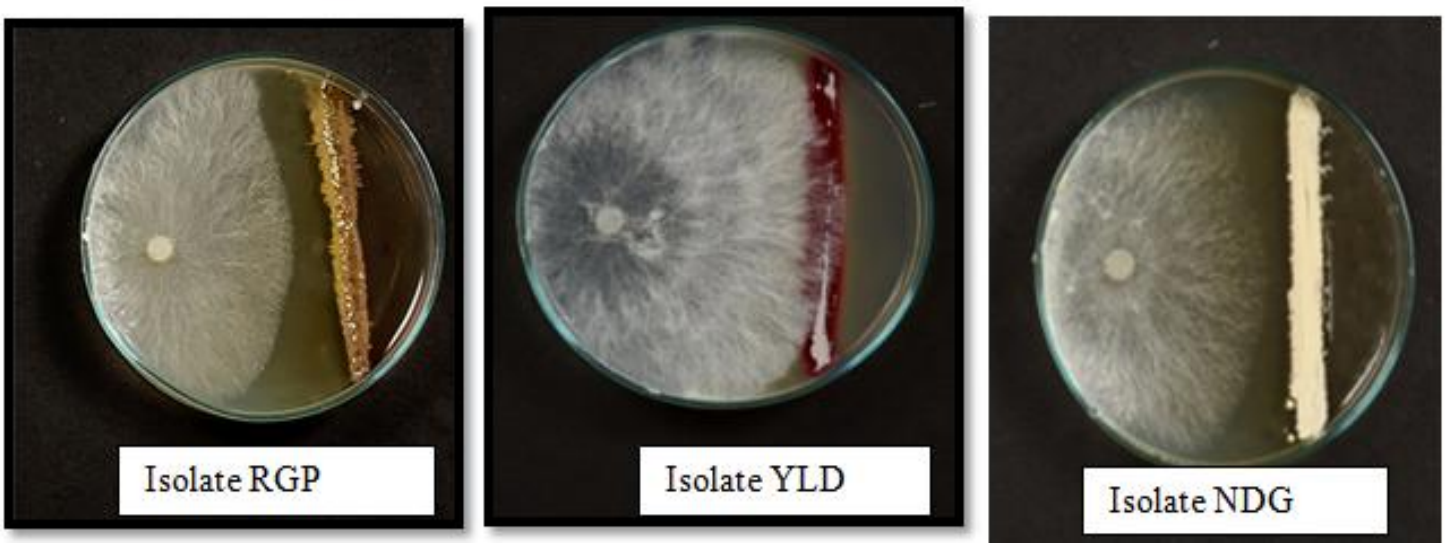

Table.8 Evaluation of biocontrol potential of isolates of *Streptomyces* spp. against *S. rolfsii in vivo* on per cent germination and disease control

Treatment	Treatments	Germination %	Disease incidence %	Disease control %
T1	Seed treatment (ST) with isolate RGP of *Streptomyces* spp.	80 (63.42)	14 (21.93)	86 (68.03)
T2	Soil application (SA) of isolate RGP of *Streptomyces* spp.	78 (62.007)	14.167 (22.09)	85.833 (67.86)
T3	Seed treatment + Soil application (ST+SA) of isolate RGP of *Streptomyces* spp.	89 (70.61)	0 (0.0)	100 (90.00)
T4	Seed treatment of Carbendazim @ 0.1% + ST with isolate RGP of *Streptomyces* spp.	80 (63.42)	14 (21.95)	86 (68.00)
T5	Seed treatment (ST) with isolate YLD of *Streptomyces* spp.	77.66 (61.78)	20 (26.55)	80 (63.41)
T6	Soil application (SA) with isolate YLD of *Streptomyces* spp.	75.5 (60.308)	20.333 (26.78)	79.667 (63.18)
T7	Seed treatment + Soil application (ST+SA) of isolate YLD of *Streptomyces* spp.	84 (66.42)	4 (11.51)	96 (78.44)
T8	Seed treatment of Carbendazim @ 0.1%) + ST with isolate YLD of *Streptomyces* spp.	76.333 (60.87)	20.667 (24.01)	79.333 (62.94)
T9	Seed treatment (ST) with isolate NDG of *Streptomyces* spp.	77.667 (61.77)	16 (23.56)	84 (66.40)
T10	Soil application (SA) with isolate NDG of *Streptomyces* spp.	76 (60.64)	16.3 (23.79)	83.7 (66.16)
T11	Seed treatment + Soil application (ST+SA) of isolate NDG of *Streptomyces* spp.	84.667 (66.93)	0 (0.0)	100 (90.00)
T12	Seed treatment of Carbendazim @ 0.1% + ST with isolate NDG of *Streptomyces* spp.	76.667 (61.09)	16.333 (23.82)	83.667 (66.13)
T13	Seed treatment of Carbendazim @ 0.1%	66.667 (54.71)	45.42 (42.35)	54.58 (47.13)
T14	Pathogen inoculated control (Pathogen check)	52.667 (44.71)	80.333 (63.66)	19.667 (26.30)
T15	Un-inoculated control (Absolute check)	70 (56.77)	0 (0.0)	100 (90.00)
	C.D.	2.393	1.77	1.770
	SE(m)	0.824	0.61	0.610

Notes: Data are mean of three replications;
Figures in parentheses are arc sine transformed values.

In vitro screening of actinomycetes against *S. rolfsii*

Dual culture studies revealed that both the interacting fungi and the actinomycetes isolates could come together after eight days of inoculation. Among the 10 isolates, 3 isolates had shown inhibition aganist growth of *S. rolfsii*. Isolate RGP produced a zone of inhibition of 49.06 per cent whereas, isolate

NDG and isolate YLD produced zone of inhibition of 47.75 per cent and 44.9 per cent indicating that secondary metabolites of *Streptomyces* spp. might be involved in inhibiting the growth of *S. rolfsii* (Table.4, Plate.1).

Efficacy of *Streptomyces* spp. on plant growth

Streptomyces spp. produce growth-promoting factors such as auxins and gibberellins which promote plant growth (Jacob *et al.,* 2018). The study indicated that the potential isolates RGP, NDG and YLD of *Streptomyces* spp. significantly increased per cent seed germination, root length, shoot length and seedling vigour over control. Among the three potential isolates tested, the isolate RGP was the most effective in promoting growth of plant followed by NDG and YLD. Significant increase in vigour index was recorded in seeds treated with talc formulation of the isolate RGP (4003.73) followed by the isolate NDG (3685.13) and YLD (3573) than that of the untreated control (3008.83) (Table.5).

Compatibility of actinomycetes with fungicides in vitro

Among the 10 isolates, only 3 isolates had shown inhibitory effect against *S.rolfsii* i.e isolates RGP, NDG and YLD. Hence, these 3 isolates were used to check the combatibility of different commonly used fungicides. These isolates had shown 100 per cent compatibility with the systemic fungicides *viz.*, carbendazim, tebuconazole, propiconazole and azoxystrobin at all concentrations whereas less compatibility was shown by non-systemic fungicides (captan and mancozeb). Thus the present study indicates the possibilities of combined use of systemic fungicides with bio control agent *Streptomyces* sp. for the effective management of stem rot of groundnut (Table.6).

Green house experiment

The freshly prepared antagonists bio agents must be used immediately hence, talc formulations were used for easy storage and proper handling for commercial use. The talc based formulations of three *Streptomyces* spp. isolates were used for their efficacy in controlling *S. rolfsii* under greenhouse conditions. Inoculation of *S. rolfsii* in groundnut had caused disease incidence upto 80 per cent under pot culture experiments. Pot culture studies revealed that seed treatment with *Streptomyces* isolate RGP, isolate NDG and isolate YLD prevented seed rot, seedling rot and collar rot caused by *S. rolfsii* in groundnut. All the treatments proved better in reducing stem rot of groundnut in comparison to pathogen inoculated check.

Seed treatment, soil application or combination of fungicide and biocontrol agent were found effective in increasing seed germination per cent.

But, higher seed germination results were obtained when both seed treatment and soil application of potential biocontrol agents were used (ST+SA) when compared with absolute check.

Disease control obtained when the isolate RGP was used as talc formulation is 86% followed by NDG (84%) and YLD (80%). But, the maximum disease control was obtained when combination of both seed treatment and soil application (ST +SA) of isolate RGP and NDG were used and had shown 100% disease control in green house studies.

No additional benefit could be derived by combination treatments of fungicide and biocontrol agent indicating that biocontrol agent can successfully be substituted for fungicidal treatment. Integration of chemical

and biocontrol agents did not give any additional benefit in decreasing the groundnut stem rot with germination on par with absolute check (Table.7).

Results indicated that seed treatment or soil application of powder formulations of all the three strains of *Streptomyces* sp. effectively reduced the incidence of stem rot under greenhouse conditions. Among them, RGP (*Streptomyces parvulus*) and YLD (*Streptomyces werraensis)* were found highly effective in controlling stem rot under greenhouse conditions.

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