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Survey of groundnut diseases and collection of plant growth promoting rhizobacteria (PGPR) against soil borne pathogens

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Abstract
Plant growth promoting rhizobacteria (PGPR) is living to roots to give beneficial effect against soil borne pathogens and plant growth promotions, during survey of southern Tamil Nadu for major disease of groundnut and collection of PGPR under rainfed conditions during 2020-2021 survey southern district of Tamil Nadu. Groundnut root rot (24.35%) high at Chettinad, Stem rot (18.38%) at Sevur, late leaf spot (23.56%) and rust disease (32.42%) high in Sivagangai district comparing to other district. In PGPR Sivagangai, Tirunelveli, Tuticorin, and Tenkasi Soil antagonistic microbes *viz*., *Pseudomonas* sp. and *PGPR*. were isolated from the rhizosphere soil using King's B medium and Nutrient agar medium (NA) respectively and tested against *Macrophomina phaseolina* and *Sclerotium rolfsii* among the effective PGPR isolates of GNP4 highest percent mycelia growth inhibition of *M. Phaseolina* (48.31.%) and *S. rolfsii* (40.47), these isolate further confirmed and sequenced for PGPR is Pseudomonas sp (OM908755.1).

Keywords: Survey, soil borne pathogens, PGPR, *Pseudomonas* sp and groundnut

Introduction
Groundnut is an important oilseed crop in India which occupies the first position in terms of area and second position in terms of production next to soyabean. China ranks first in terms of groundnut production with 17.57 million tonnes followed by India 6.73 million tonnes, According to the 1st advance estimates, groundnut production estimate during kharif season was 82.54 lakh tonnes for 2021-2022 again 85.56 million tonnes in 2020-2021 (kharif). Among the states (Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra) stem rot & root rot are the major diseases causing severe yield loss in Groundnut. A study on the management of major Soil borne diseases of groundnut was carried through PGPR.

In Southern districts such as Madurai, Virudhunagar, Sivaganga, Ramanathapuram and Pudukottai districts groundnut was cultivated in 56,163 hectares. Diseases cause a considerable yield losses in groundnut. Fungal, virus and bacterial pathogens attack the crop at various stages of growth and affects quality. The major soil borne diseases of groundnut caused by fungi are stem rot/Sclerotium wilt which is caused by *Sclerotium rolfsii*, dry root rot/dry wilt caused by *Macrophomina phaseolina* dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. is considered as the most devastating disease in all the groundnut growing areas of Rajasthan. *Trichoderma viride, T. harzianum, T. atroviride, Pseudomonas fluorescens* and *Bacillus subtilis* gave distinct antagonistic reactions, showing stunting of *Macrophomina phaseolina* colony and clear zone of inhibition between colonies of antagonist and the pathogen was developed Pancham *et al*, (2017) [7]. A talc-based powder formulation of the highly effective strain, B. subtilis G-1, was developed and its efficacy in controlling groundnut stem rot was determined under greenhouse conditions. Shifa *et al.* (2015) [11].

Materials and Methods
Disease assessment
An intensive systematic survey was conducted to assess the disease incidence in different Groundnut growing areas of Tamil Nadu viz., Sivagangai. Tirunelveli, Tenkasi and Tuticorin districts of Tamil Nadu. to assess the incidence of Important foliar and soilborne disease of Groundnut based on the external symptoms. In each field, five plots each with 5x5 m area were selected. Among the five plots one plot was fixed at the centre of the field and the remaining four plots were fixed at random in different places in the field avoiding border rows.

The root rot incidence was assessed by counting the number of affected plants out of total number of plants in each plot ($25m^2$). In each area three fields were assessed and the mean disease incidence was calculated. Percent disease incidence was calculated by using the formula.

Isolation of pathogen from Groundnut plants

The pathogen was isolated from the affected portion of the diseased plants collected from different districts separately by tissue segment method (Rangaswami, 1958) [9] on sterile Potato Dextrose Agar (PDA) medium. The infected plants were pulled out with intact root showing the presence of white mycelial mat with small round brown sclerotia near the collar region are collected and gently tapped to remove the soil and dirt particle. The Infected portions of diseased plants collected from different area were cut into small pieces of 1 to 1.5cm separately using sterilized scalpel and these were surface sterilized with 0.1 percent mercuric chloride for one min. and then washed in sterile distilled water thrice and then placed in a Petriplate at equidistance onto previously poured and solidified in Petriplate containing potato dextrose agar (PDA) medium. These plates were incubated at room temperature (28 ± 2 °C) for five days and observed for the growth of the fungus. The hyphal tips of fungi growing from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogen was identified based on the morphological characters as described by Punja (1985) [8].

Utilization of carbon and nitrogen sources

The Richard's agar medium was substituted with different carbon sources viz., carboxy methyl cellulose, glucose, fructose, manitol, sucrose, and starch and different nitrogen sources such as ammonium nitrate, ammonium molybdate, ammonium oxalate, ammonium sulphate, urea, thiourea, sodium nitrate, and potassium nitrate. All the nitrogen sources were dissolved and sterilized. The medium containing without nitrogen and carbone source served as control. The sterilized warm medium was poured in the sterilized Petri plates and allowed to solidify and this inoculated with five-days-old 10 mm culture disc of the pathogen. Then these plates were incubated at room temperature (28 ± 2 °C) for ten days. The diameter of mycelial growth was recorded. Three replications were maintained in each treatment.

Effect on linear growth of *Sclerotium rolfsii* and *Macrophomina phaseolina*

Fourteen isolates of PGPR were streaked in a four cm line (1 cm away from the edge of the plate) on each PDA medium. A nine mm mycelial disc of *S. rolfsii* and *Macrophomina phaseolina* was placed to the most distal point of the Petri dish perpendicular to the bacterial streak. The plates were incubated at room temperature for Five days and mycelial growths of the pathogen and inhibition zone (cm) were measured.

Effect on germination of sclerotia

Six isolates of PGPR (GNP 4, GNP 5, GNP 6 GNP 8 GNP 12 GNP 14) were tested for their effect on germination of sclerotia in natural soil plates. Fifty gram of field soil, adjusted to 80% moisture content were evenly distributed Petri dishes and slightly compacted. Before placement on the soil, sclerotia were immersed for 24 h in culture filtrates of the isolates *PGPR* separately. Sclerotia (20 numbers per plate) were equally placed on the soil surface and pushed into the soil gently with glass rod so that only their tops remain exposed. Plates were incubated at room temperature for seven days and examined for its germination. (Henis *et al.,* 1984) [3].

Identification of bacterial isolates

Characterization of the different cultures of antagonistic bacteria was done according to the methods recommended in the laboratory guide for identification of plant pathogenic bacteria published by the American Phytopathological Society. For each test, 24 to 48 h old cultures were used.

Results

An intensive systemic survey on Groundnut disease was conducted in different Groundnut growing areas of Tamil Nadu during 2020 to 2021. The incidence of root rot in groundnut ranged from 12.00 to 25.35 percent (Fig1). Maximum incidence of 25.35 percent root rot was recorded at Chettinad in Sivagangai district followed by Maranthai in Tirunelveli district. The incidence of stem rot in groundnut ranged from 8.1 to 18.38 percent. Maximum incidence of 18.38 percent stem rot was recorded at Sevur in Sivagangai district followed by Manur in Tirunelveli district. Among the foliar disease the late leaf spot (23.56) and rust (32.42) disease incidence was observed in Chettiand of Sivagangai district.

Carbon sources

The growth of the pathogen in different carbon sources exhibited significant differences among the treatments. Starch as carbon source promoted significant mycelial growth and mycelial dry weight of 8.80cm and 348.49mg followed by glucose (7.56 cm and 213.95mg) while in carboxy methyl cellulose the growth of *S. rolfsii* was low (4.53cm and 57.83mg of dry weight) (Table 1: Fig 2.).

Nitrogen sources

The growth of different nitrogen sources exhibited significant differences among the treatments. Potassium nitrate as nitrogen source promoted significant mycelial growth (8.51 cm) and mycelial dry weight 348.49 mg of the pathogen, followed by sodium nitrate (8.22 cm and 283.50 mg) while in urea (3.19 cm) and in thiourea (3.69 cm) the growth of *S. rolfsii* was low (Fig.3).

Efficacy of PGPR against soil borne pathogens

Among the fourteen isolates of PGPR tested for their antagonistic activity against *M. phaseolina* and *S. rolfsii* by dual culture, the GNP4 were found to be effective significantly exerted highest percent mycelial growth inhibition of 48.31 and 40.47 percent mycelial growth inhibition over the control (Table 2).

Number of sclerotia

Among the Six PGPR microorganisms GNP 4 showed the maximum reduction of sclerotial production which was recorded 70.65 percent followed by GNP 6 and GNP 14 which recorded 58.05 and 56.30 percent reduction respectively (Table 3).

Sclerotial germination

Among the *Six PGPR* microorganisms GNP 4 recorded maximum sclerotial germination inhibition of 58.67 percent

followed by GNP 6 and GNP 14 which recorded 52.67 and 51.67 percent inhibition respectively and these were on par with each other (Table 3)

Genomic DNA was extracted from the PGPR GNP 4 isolate of and the 16S rDNA region was amplified using 27 F and 1115 R primers with the size of 1000 bp approximately. Further sent to sequence Eurobins sequence India ltd its confirmed as *Pseudomonas* sp.

Discussion

These inherent ill effects associated with the use of chemicals in plant disease management forced the plant pathologists all over the world to search for safer alternatives with a little or no negative impact on the environment. The biological control of plant pathogens which apply fits in the framework of the integrated disease management.

Recently, new approaches involving biocontrol agents are considered as an alternative to chemical fungicides due to their target specificity, economical to use, no chance for development of resistance by pathogens and increases the plant growth and yield potential of crops. Development of biocontrol formulations leads to environmentally friendly plant disease management.

In the present study, PGPR biocides were evaluated for their antifungal activity against root rot and stem rot disease of groundnut Detailed studies were conducted on the antagonism of biocides against the pathogen *Macrophomina phaseolina* and *Sclerotium rolfsii*.

Groundnut cultivation is hampered by root rot and stem rot in all groundnut growing areas. The recent survey on root rot and stem rot Groundnut root rot (24.35%) chettinad, Stem rot (18.38%) at Sevur, late leaf spot (23.56%) and rust disease (32.42%) high in Sivagangai district comparing to other district of Tamil Nadu. Divya Rani *et al*., (2016) [1] conducted survey in major groundnut growing areas of Andhra Pradesh during 2012 & 2013 to assess the distribution and the incidence of collar rot and stem rot diseases. The highest incidences of stem rot and collar rot were observed in Chittoor district of Andhra Pradesh. Palaiah *et al*., (2019) reported that The average highest collar rot incidence was noticed in Kalaburgi (16.00%), dry root rot incidence in Koppal (25.25%) followed by Tumkur (24.37%), stem rot incidence in Chitradurga (22.72%) followed by Tumkur (21.22%) districts of Karnataka. The information generated could be useful for making the ecosystem specific management strategy to reduce the impact of soil borne diseases of groundnut in different districts of Karnataka Groundnut stem rot, caused by *Sclerotium rolfsii*, is a serious soil-borne disease. During the 2018–2019 calendar year, In the investigated area, disease incidence ranged from 10.71 to 18.50%. (Hawaladar *et al*. 2021) [2].

The PGPR isolates of GNP4 recorded 48.31 (*M. phaseolina*) and 40.47 (*S. rolfsii*) percent reduction of mycelial growth over control. *Pseudomonas fluorescens* strain Pf1. Effectively inhibited the mycelia growth of *M. phaseolina* the pathogen causing dry root rot in groundnut, Application of Pf1 as seed treatment 10g/kg seed followed by soil application (2.5kg/ha) against root rot effectively supported higher plant growth and grain yield (Shanumugam *et al*., 2003) [10]. The antagonistic bacterial population in the groundnut rhizosphere against *S. rolfsii* majorly belongs to *Bacillus*, *Pseudomonas* and *Burkholderia* (Le *et al*. 2018) [4] Mahendra *et al*., 2022 [5] 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%).

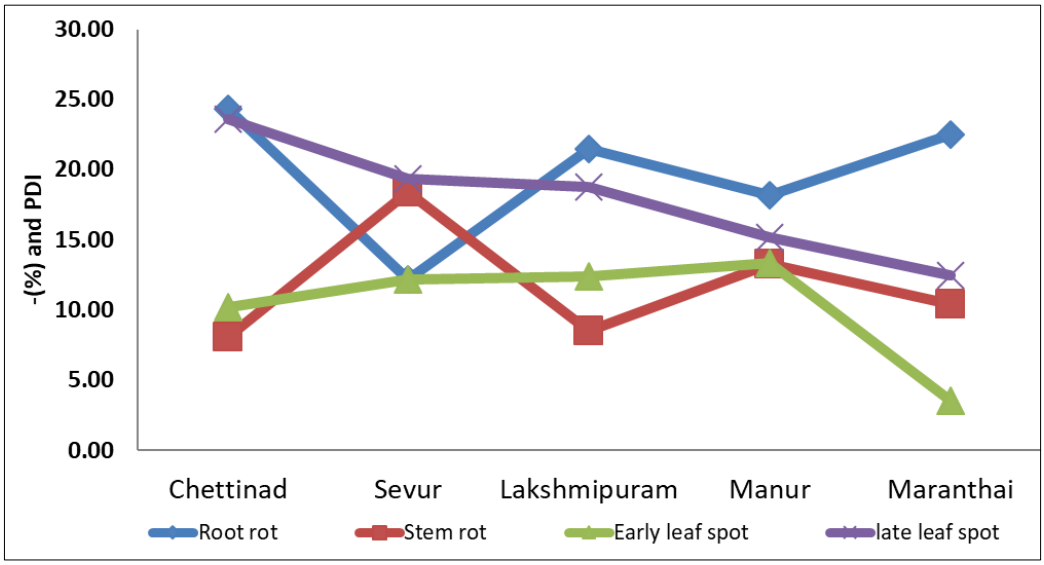

Fig 1: Survey of Groundnut disease in Tamil Nadu

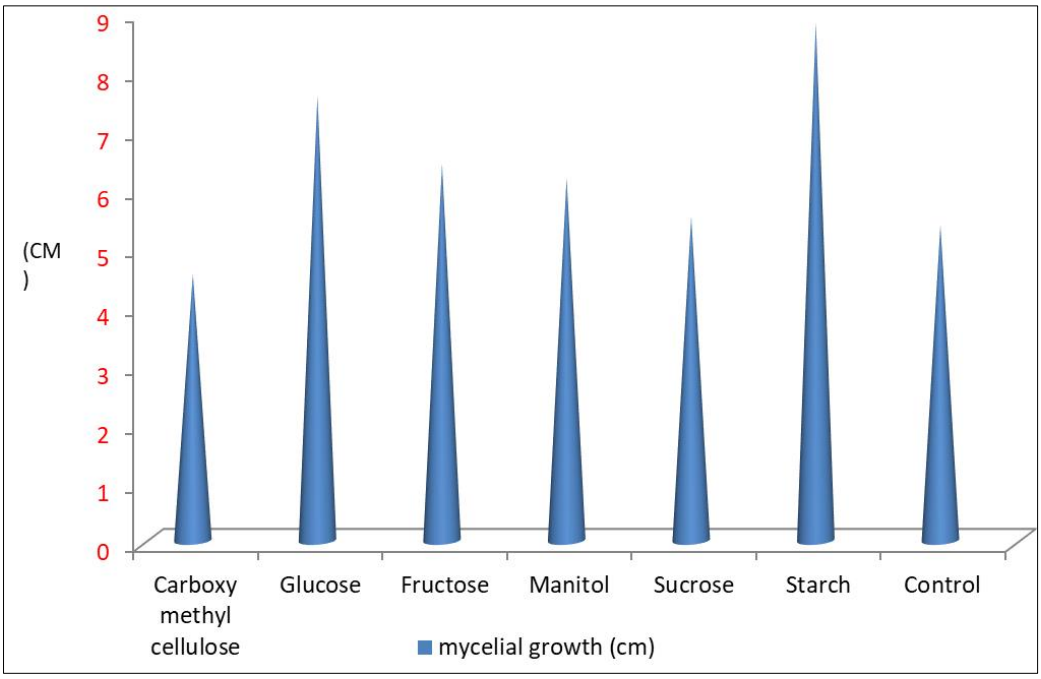

Fig 2: Effect of different carbon sources on growth of *S. rolfsii in vitro*

Table 1: Effect of different carbon and nitrogen sources on growth of *S. rolfsii in vitro*

S. No	Carbon sources	Mycelial dry weight (mg)*	Nitrogen sources	Mycelial dry weight (mg)*
1	Carboxy methyl cellulose	57.83	Ammonium nitrate	71.49
2	Glucose	213.95	Ammonium oxalate	66.54
3	Fructose	144.72	Ammonium sulphate	48.48
4	Manitol	151.59	Potassium nitrate	348.49
5	Sucrose	125.30	Sodium nitrate	283.50
6	Starch	348.49	Urea	58.65
7	Control	48.04	Control	50.32
	CD (P=0.05)	4.90	CD (P=0.05)	4.16

*Mean of three replications

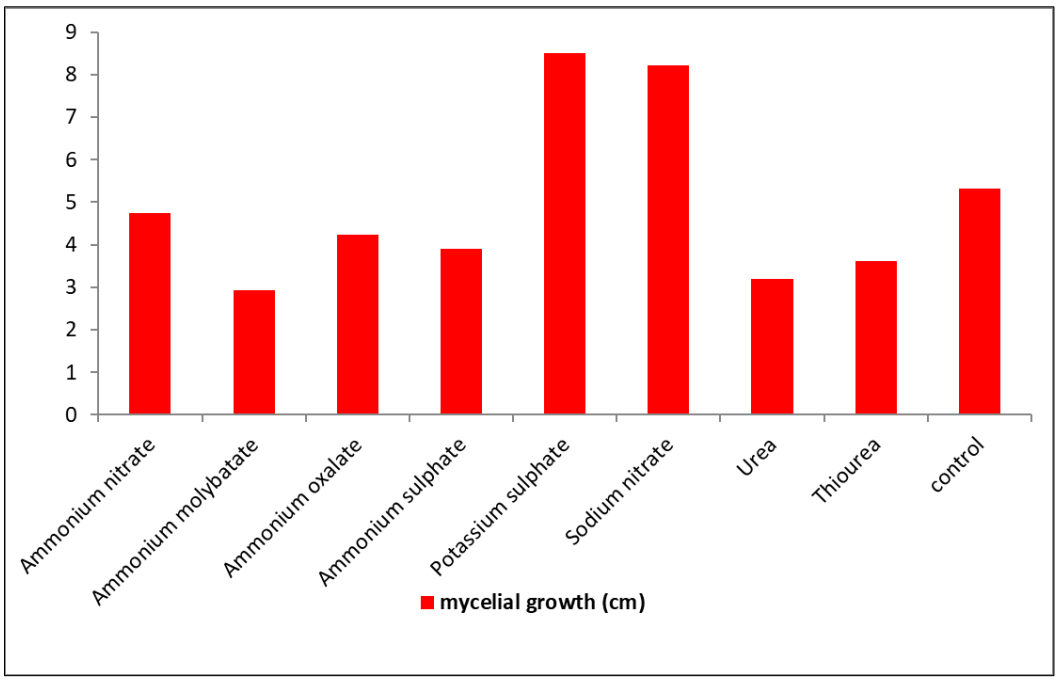

Fig 3: Effect of different nitrogen sources on growth of *S. rolfsii in vitro*

Table 2: Effect of PGPR against *Sclerotium rolfsii* and *Macrophomina phaseolina*

S. No	PGPR isolates	Mycelial growth of *M. phaseolina* (cm)	Percent reduction over control (%)	Mycelial growth of *S. rolfsii* (cm)	Percent reduction over control (%)
1.	GNP 1	8.6	3.37	8.9	0.00
2.	GNP2	8.7	2.24	8.9	0.00
3.	GNP 3	6.0	32.58	7.8	12.35
4.	GNP4	4.6	48.31	5.3	40.47
5.	GNP5	4.7	47.19	6.0	32.58
6.	GNP6	5.3	40.44	5.6	37.07
7.	GNP7	8.6	3.37	8.9	0.00
8.	GNP8	5.8	34.83	6.8	23.59
9.	GNP9	8.7	2.24	8.9	0.00
10.	GNP10	8.8	1.12	8.9	0.00
11.	GNP11	8.7	2.24	8.9	0.00
12.	GNP12	4.9	44.94	6.7	24.71
13.	GNP13	8.6	3.37	8.7	2.24
14.	GNP14	5.2	41.57	5.8	34.83
15	Control	8.9		8.9	
CD (P=0.05)		0.62		0.66	

Table 3: Antagonistic effect of PGPR biocides against *S. rolfsii in vitro*

S No	Treatments	Mycelial growth*		Number of sclerotia*		Sclerotial germination* (%)	
		Mycelial growth (cm)	Growth inhibition (%)	No. of sclerotia/ plate	Percent reduction	Sclerotial germination (%)	Percent reduction
1	GNP 4	5.13	41.90	61.33	70.65	41.33	58.67
2	GNP5	6.33	28.31	181.33	13.23	67.33	32.67
3	GNP 6	5.33	39.00	87.67	58.05	47.33	52.67
4	GNP8	6.93	21.48	192.33	7.97	65.33	34.67
5	GNP12	6.77	23.32	189.67	9.25	61.66	38.34
6	GNP14	5.37	39.18	91.33	56.30	48.33	51.67
7	Control	8.83	-	209.00	-	100	
	CD (P=0.05)	0.68		2.23		2.66	

* Mean of three replications

Conclusion

The root rot and stem rot diseases greater yield losses of every year in Groundnut, the management using chemical is major environmental pollution causing to human beings to ill health. to address the reduced this problems to current investigation on utilization of rhizosphere antimicrobial agents like GNP4 (*Pseudomonas* sp) reduces the pathogen growth in dual culture and increasing plant growth and Though biological control of plant disease appears promising with less environmental pollution.

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