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Microbiological Management of Stem Rot Disease of Groundnut Caused by *Sclerotium rolfsii* Sacc., by Exploiting *Pseudomonas aeruginosa* AL98

Rakh R. R.*[1], S. M. Dalvi[2], L. S. Raut[3] and A. V. Manwar[4]

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ABSTRACT

Sclerotium rolfsii Sacc. is one of the major phytopathogen of Groundnut causing Stem rot disease, which was tough to control by conventional means by applying fungicides. Therefore, in the present investigation, an effort is being made to search for effective control measures in terms of biocontrol agent for the Stem rot disease. In the present research, 58 *Pseudomonas* strains isolated from rhizospheric niches were screened for their biocontrol activity against *S. rolfsii* under *in vitro* conditions. One of the identified strain, *Pseudomonas aeruginosa AL98* was selected for further studies because of its ability to content the mycelial growth of the pathogen significantly. During the *In vitro* dual culture study, the *Pseudomonas aeruginosa AL98* repressed the growth of *Sclerotium rolfsii* up to 94.44% in comparison to control. When the mechanism behind the biocontrol revealed that *Pseudomonas aeruginosa AL98* strain secreted Non-volatile diffusible metabolites, and volatile metabolites, which affects the growth of *Sclerotium rolfsii* in dual culture. While confirming the *in vitro* results, pot assay being conducted which shows decrease in the percent disease incidence of stem rot due to *Pseudomonas* treatment from 40.16 to 54.54%.

Key words: Groundnut, Rhizospheric niches, *Sclerotium rolfsii*, *Pseudomonas aeruginosa AL98*, Non-volatile diffusible metabolites, Volatile metabolites, Microbial control

Rhizospheric niches of plants is a good consortium where both kinds of microorganisms coexist i.e., phytopathogen and biocontrol agents. So, the rhizospheric niches of the plants serves as an excellent source for agents to control soil-borne plant pathogens. Various Bacterial species like *Bacillus*, *Pseudomonas*, *Serratia*, and *Arthrobacter* known to control the fungal diseases. Similarly, Bacteria present in the rhizospheric niches identified as plant growth-promoters as well as biocontrol strains which most often belong to the following genera: (i) *Bacillus spp.* [1-2], (ii) *Streptomyces spp* [3-5] and (iii) *Pseudomonas* [6-9] and *Trichoderma spp.* [10].

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The *Pseudomonas spp.* very well recognized as superior biocontrol agent because of their adaptive metabolism and their ability to produce a wide range of antifungal compounds [11]. Examples of antifungal and secondary metabolites produced by *Pseudomonas spp.* include phenazines [12], 2,4-diacetylphoroglucinol [13], pyoluteorin [14], pyrrolnitrin [15], cyclic lipopeptides [16], siderophores [17], volatile compounds [18], hydrolytic enzymes [19], and so on. Fluorescent pseudomonads, for example, *Pseudomonas aeruginosa* [20], *Pseudomonas putida* [21], and *Pseudomonas fluorescens* [22], are well-known to protect plants from fungal infections.

Sclerotium rolfsii Sacc is a soil-borne plant pathogen of worldwide importance with a very extensive host range including more than 500 plants species. Most *S. rolfsii* diseases have been reported on dicotyledonous hosts, but several monocotyledonous species are also being infected. *Sclerotium rolfsii* is especially severe on legumes, Solanaceous crops, cucurbits, and other vegetables grown in rotation with beans [23-24]. The traditional agricultural practice used to control the phytopathogen is by applying a wide variety of Fungicides e.g., Bavistin, Captan, etc. but a severe disadvantage of the traditional method, is that it is not effective to check the *Sclerotium* during the cropping duration (90-100 days) and is not eco-friendly. Hence, an alternative attempt has been made to provide an eco-friendly strategy for the control of *Sclerotium* during this work. The present research work mainly emphases

to i) isolate an efficient biocontrol agent in the form of *Pseudomonas spp*, from rhizospheric niches of healthy plants such as Neem, groundnut, soybean etc. ii) evaluate its potential in controlling the soil-borne pathogen, *Sclerotium rolfsii*, causing stem rot of groundnut, iii) finding out the mechanism of biocontrol and iv) Confirmation of biocontrol of stem rot disease of groundnut caused by *Sclerotium rolfsii* by applying the efficient *Pseudomonas spp*.

MATERIALS AND METHODS

Stem rot phytopathogen of groundnut

The stem rot phytopathogen of groundnut, i.e., *Sclerotium rolfsii* Sacc., was isolated previously during RGSTC (Rajiv Gandhi Science and Technology Commission, Government of Maharashtra) Project carried out in the Department of Microbiology, at Shri Guru Buddhiswami Mahavidyalaya, Purna (Jn.) Dist. Parbhani (Maharashtra), India. The fungal pathogen was maintained on potato Dextrose Agar medium at 30°C.

Isolation of Rhizospheric Pseudomonas spp.

The soil sample from rhizospheric niches of different healthy plants such as groundnut, soybean, neem, and tur, etc. was collected in poly-ethylene bags and brought to the research laboratory. A 1 gm of soil sample was inoculated into 100 ml Kings B broth and kept for incubation at room temperature for 24 h.

For isolation of *Pseudomonas spp.* 1ml of this Kings B broth was transferred to selective enrichment media, Cetrimide broth, and kept for incubation at room temperature for 24 h. From enriched Cetrimide broth, a loopful of culture was streaked on Cetrimide agar [25] and the plates were incubated at room temperature till colonies were observed (24 – 48 h). The isolated colonies developed were then purified on nutrient agar slants and used for screening against the phytopathogen for biocontrol ability. All the isolates were tentatively named during this research to avoid confusion.

Dual culture screening for potential biocontrol agents

The *Pseudomonas* isolates were screened for potential antagonistic activity against pathogenic fungi *S. rolfsii* on King's B agar by means of the dual culture technique [26]. In this modified method, the King's B agar plate was inoculated with 5 mm fungal disc (7-day-old culture) 10 mm away from the center of the agar plate. Correspondingly, 24 h old *Pseudomonas* culture was streaked in opposite direction, 10 mm away from the center of the agar plate to maintain the equidistance of phytopathogen and antagonist from the center of the agar plate. This dual culture experiment was performed in triplicates. A control plate was maintained without streaking bacterial culture. Both the test and control plates were incubated at room temperature for 7 days.

The antifungal activity was calculated by measuring the inhibition of radial mycelial growth of fungal pathogen in test as compared to control in terms of percentage inhibition of radial growth (PIRG) [27].

$$\text{Percentage inhibition of radial growth} = \frac{R_1 - R_2}{R_1} \times 100$$

Where;

R_1 – radius of the fungal phytopathogen colony in the control plate

R_2 – radius of the fungal phytopathogen colony in the direction of the antagonist colony.

Similarly, the inhibition zone of *S. rolfsii* under Shri observed under the binocular microscope

Identification of biocontrol agent

An effective *Pseudomonas* strain obtained after screening was identified according to Bergey's Manual of Systematic Bacteriology (1984) by using cultural, biochemical characteristics as well as 16s rRNA sequencing.

Revealing of biocontrol mechanism

To reveal the biocontrol mechanism, the efficient *Pseudomonas* isolate was analyzed for Non-volatile Metabolite and Volatile Metabolite.

Screening for non-volatile metabolite

For detection of non-volatile diffusible antibiotics, the experimental setup was done as per Montealegro *et al.* [28] with slight modification instead of PDA plates King's B plates were used. The plates covered with a cellulose nitrate membrane, were inoculated in the center with 100 µl of antagonistic bacterial suspension. After incubation for 72 hr at room temperature, the membrane with grown bacterial culture was removed, and the plate was inoculated in the center with a 5 mm disk of a pure culture of fungal pathogen and plates were further re-incubated at room temperature for 7 days and the growth of the pathogen was measured. Controls were run with uninoculated King's B plates containing plates on the cellulose nitrate membrane (replacing the bacterial suspension by sterile distilled water), and further incubated with pathogen *S. rolfsii*. The experiment was run in quadruplets. Results were expressed as means of % inhibition of fungal pathogens in the presence and absence of antagonistic bacterial isolate. Percent inhibition was calculated using the following formula [28]:

$$\text{Percentage inhibition} = 1 - \frac{\text{Fungal growth in test}}{\text{Fungal growth in control}} \times 100$$

Detection of volatile metabolites

To detect the secretion of Volatile metabolites by antagonistic *Pseudomonas spp.*, 100 µl of an antagonistic bacterial suspension was placed at the center of one-half Petri dish containing Kings B medium, and a 5 mm disk of pure culture of *S. rolfsii* was placed at the center of another Petri dish containing Kings B medium. Both half of plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension and were sealed to isolate the inside atmosphere and to prevent loss of volatiles formed. Plates were incubated at room temperature for 6 days and the growth of the pathogen was measured and compared to controls developed in the absence of the antagonistic. The experiment was run in quadruplets. Results expressed as means of inhibition (%) of fungal growth in the presence and absence of bacterial isolate. Percent inhibition was calculated using the following formula [28].

$$\text{Percentage inhibition} = 1 - \frac{\text{Fungal growth in test}}{\text{Fungal growth in control}} \times 100$$

Pot assay for biocontrol of stem rot disease (In vivo assay)

Mass multiplication of pathogen

The phytopathogen, *Sclerotium rolfsii* multiplied on sorghum grains. Sorghum grains were pre-soaked in 2 percent sucrose solution overnight, drained and boiled in fresh water for 30 minutes and drained again. This was transferred into 1000 ml flasks @ 400 g and autoclaved for 15 lb psi for 20 minutes. The flasks were allowed to cool at room temperature and inoculated with 5 mm discs of 3-to-4-day old culture of grown *Sclerotium rolfsii* on PDA. Seven discs per flask were added and the flasks were incubated for three weeks at 28 ± 2°C [29].

Preparation pots for in vivo pot assay

Soil was disinfected with formaldehyde for 3 days, after three days inoculum of *Sclerotium rolfsii*, pathogens multiplied on sorghum grains was mixed at the rate of 20g/kg of soil in upper 10 cm layer of pot soil. Pots were sprinkled with water and incubated for two days after covering with polythene bags [29]. In all three replications for each treatment were tried. The details of each treatment are as follows (Table 1).

Table 1 Treatment of microbiological control in oot assay

Treatments	Description
$T_{Pseudomonas}$	Pot with phytopathogen *Sclerotium rolfsii* and the seeds with treatment of biocontrol agents
Control	Pot with phytopathogen and the seeds without biocontrol agent treatment (Control)

Groundnut were cultivated for over a period of 60 days and the data on emergence of stem rot infection was recorded at 30 and 60 days. Seeds of Groundnut variety TAG24 used in this pot experiments and seed treatment with talc-based formulation of potential bacterial antagonist, *Pseudomonas* isolate was used and treated @ 10 g kg^{-1} of the seed using gum (5 ml kg^{-1}) as sticker. The treated seeds were spread over a clean paper and dried in a cool and shady place. The seeds were sown immediately after drying. Each pot was sown with 5 seeds. The pots were watered with tap water as an when required. The growth parameters like percent seed germination, shoot length, root length, and chlorophyll content were recorded after 30 and 60 days.

$$\text{Percentage germination} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds sown}} \times 100$$

$$\text{Percentage disease incidence} = \frac{\text{No. of infected plants}}{\text{Total No. of plants}} \times 100$$

RESULTS AND DISCUSSION

Isolation of Rhizospheric Pseudomonas sp.

During present research work, 58 *Pseudomonas sp.* were isolated from rhizospheric niches of healthy plants of soybean, neem, groundnut, tur, etc. all the rhizospheric *Pseudomonas* were tentatively named as shown in (Table 1) and maintained on Nutrient Agar slants for further screening.

Screening for potential biocontrol agents against phytopathogen

During the screening, it was observed that *Pseudomonas* 17 was found as an efficient antagonist against *Sclerotium rolfsii* in the dual culture technique (Plate 1, Table 2) while the other *Pseudomonas isolates* found ineffective to destroy the *Sclerotium rolfsii* (Table 2).

Table 2 *In vitro* screening for potential biocontrol agents against *Sclerotium rolfsii*

Isolate	Crop / Location	% Inhibition of *Sclerotium rolfsii*
Pseudomonas 1	Soybean/ Adgaon	7.77
Pseudomonas 2	Soybean/ Adgaon	11.11
Pseudomonas 3	Soybean/ Adgaon	70.00
Pseudomonas 4	Soybean/ Adgaon	63.33
Pseudomonas 5	Soybean/ Adgaon	55.55
Pseudomonas 6	Soybean/ Adgaon	52.22
Pseudomonas 7	Soybean/ Adgaon	44.44
Pseudomonas 8	Soybean/ Adgaon	11.11
Pseudomonas 9	Soybean/ Adgaon	22.22
Pseudomonas 10	Soybean/ Adgaon	7.77
Pseudomonas 11	Groundnut/ Mategaon	12.22
Pseudomonas 12	Groundnut/ Mategaon	18.88
Pseudomonas 13	Groundnut/ Mategaon	16.66
Pseudomonas 14	Groundnut/ Mategaon	68.88
Pseudomonas 15	Groundnut/ Mategaon	86.88
Pseudomonas 16	Groundnut/ Mategaon	91.11
Pseudomonas 17	Groundnut/ Mategaon	94.44
Pseudomonas 18	Groundnut/ Mategaon	14.44
Pseudomonas 19	Groundnut/ Mategaon	15.55
Pseudomonas 20	Neem/ Khujada	13.33
Pseudomonas 21	Neem/ Khujada	22.22
Pseudomonas 22	Neem/ Khujada	90.00
Pseudomonas 23	Neem/ Khujada	80.00
Pseudomonas 24	Neem/ Khujada	27.77
Pseudomonas 25	Neem/ Khujada	57.77
Pseudomonas 26	Neem/ Khujada	12.22
Pseudomonas 27	Neem/ Khujada	87.77
Pseudomonas 28	Neem/ Khujada	16.66
Pseudomonas 29	Neem/ Khujada	64.44
Pseudomonas 30	Tur/ Kandkheda	30.00
Pseudomonas 31	Tur/ Kandkheda	2.22
Pseudomonas 32	Tur/ Kandkheda	56.66
Pseudomonas 33	Tur/ Kandkheda	87.77
Pseudomonas 34	Tur/ Kandkheda	72.22
Pseudomonas 35	Tur/ Kandkheda	58.88
Pseudomonas 36	Tur/ Kandkheda	40.0
Pseudomonas 37	Groundnut/ Kandkheda	27.77
Pseudomonas 38	Groundnut/ Kandkheda	16.66
Pseudomonas 39	Groundnut/ Kandkheda	6.66
Pseudomonas 40	Groundnut/ Kandkheda	18.88
Pseudomonas 41	Groundnut/ Kandkheda	5.66
Pseudomonas 42	Groundnut/ Kandkheda	23.33
Pseudomonas 43	Neem/ Mategaon	54.44
Pseudomonas 44	Neem/ Mategaon	10.0
Pseudomonas 45	Neem/ Mategaon	28.88
Pseudomonas 46	Neem/ Mategaon	25.55
Pseudomonas 47	Neem/ Mategaon	7.77
Pseudomonas 48	Neem/ Mategaon	75.55
Pseudomonas 49	Neem/ Mategaon	74.44
Pseudomonas 50	Soybean/ Khujada	70.0
Pseudomonas 51	Soybean/ Khujada	56.66
Pseudomonas 52	Soybean/ Khujada	52.22
Pseudomonas 53	Soybean/ Khujada	22.22
Pseudomonas 54	Soybean/ Khujada	21.11
Pseudomonas 55	Soybean/ Khujada	4.44
Pseudomonas 56	Soybean/ Khujada	34.44
Pseudomonas 57	Groundnut/ Adgaon	80.00
Pseudomonas 58	Groundnut/ Adgaon	12.22

Mean of three replications

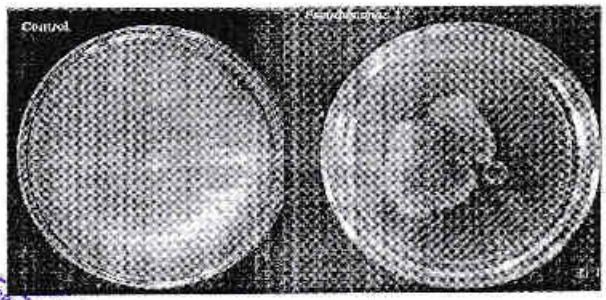

Plate 1 Screening for potential biocontrol agents by dual culture technique against *S. rolfsii*

It was revealed from the screening study that rhizospheric culture of *Pseudomonas* 17 was able to reduce *in vitro* growth of *Sclerotium rolfsii* up to 94.44%. Similarly, Singh, *et al.* [30] screened 186 bacterial strains of different morphological types for their biocontrol activity against *S. rolfsii* under *in vitro* conditions. Two *Pseudomonas* strains, namely *Pseudomonas fluorescens* NBRI-N6 and *P. fluorescens* NBRI-N, shown to inhibit the mycelial growth of the pathogen significantly having the zone inhibition upto 15 mm by NBRI-N6 and 11 mm by NBRI-N respectively, in comparison with the other strains screened. Also, Karthikeyan *et al.* [31] proved that in dual culture, one among the three isolates of *Trichoderma viride*, an isolate in each of *T. harzianum* and *Pseudomonas fluorescens* were inhibitory to the growth of *Sclerotium rolfsii* (Sacc.), the causal agent of stem rot of groundnut. The isolate Tv1 of *T. viride* caused 69.40% inhibition of the mycelial growth of the pathogen followed by *P. fluorescens* resulting in 64.40% inhibition. Ganesan and Gnanamanickam [32] also reported that Native strains of *Pseudomonas fluorescens* suppress the root and stem rot pathogen of peanut, *Sclerotium rolfsii*. *P. fluorescens* restricted the mycelial growth of *S. rolfsii* in in vitro plate tests. In in vitro plate assays, mycelial growth of *S. rolfsii* (three strains) was strongly restricted by the four strains of *P. fluorescens*. Clear inhibition zones ranging from 2.5 to 5.5 cm dia. were observed. Also in 2012, Ganesan and Sekar reported that six isolates of *Pseudomonas* showed about 68% of inhibition *S. rolfsii* in dual culture studies. Our results when compared with the results earlier reported by Kishore *et al.* [33], Karthikeyan *et al.* [34], Ganesan and Sekar [35] for control of *Sclerotium rolfsii* with *Pseudomonas aeruginosa* in dual culture. It was found that our results with *Pseudomonas* come about far better than the above-mentioned results because there was only 32-74% inhibition recorded where as in our results 94.44% inhibition of *Sclerotium rolfsii* was recorded. All this research indicates that rhizospheric niches contains tremendous number of different kinds of microorganisms which serves better for controlling the phytopathogen.

Further the inhibitory activity of *Pseudomonas* 17 was confirmed by microscopic observation of the clear zone, which discovered lysis of mycelium of *Sclerotium rolfsii* as shown in (Plate 2). Similar findings were also recorded by Sen *et al.* [36] where the microscopic study of mycelia from interacting zone showed hyphal shriveling, deformities, swelling, fragmentation and finally resulting into lysis. These results in accordance with our findings.

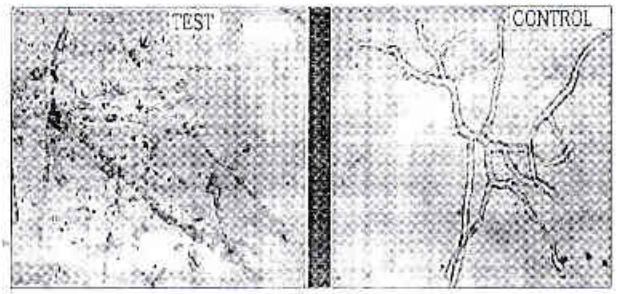

Plate 2 Microscopy of antagonistic effect of *Pseudomonas* 17 against *Sclerotium rolfsii*

Identification of biocontrol agent

The efficient *Pseudomonas* 17 isolate was identified by 16S rDNA gene sequencing. The sequence was aligned with sequence in the public domain, GenBank by BLAST Programm which showed 98% similarity with *Pseudomonas aeruginosa AL98* having accession number AJ249451.

Revealing of biocontrol mechanism

The biocontrol agents exercise an inhibitory effect towards the phytopathogen, *Sclerotium rolfsii*. Two major mechanisms have been anticipated to explain the suppressive and antagonistic effects of *Pseudomonads* in particular i.e., the phytopathogen is inhibited by competition for iron, as availability of Fe^{+++} in soil is low (10^{-17} M). Secondly, *Pseudomonas* inhibit the pathogens by producing secondary metabolites with antibiotic activity e.g., Phenazine, Pyrrolnitrin, Phenazines, 2,4-diacetylphloroglucinol and cyanides [37]. Nevertheless, growth suppression of pathogen remains a multifunctional attribute. Hence for determining the mechanism of action shown by the strains under study, variety of experiments were carried out during this research work. To reveal the biocontrol mechanism, the selected strain *Pseudomonas aeruginosa* AL98 was scrutinized for production of Non-volatile Metabolite and Volatile Metabolite. From the results, it was evident that *Pseudomonas aeruginosa* AL98 was able to produce variety of secondary metabolites namely non volatile diffusible antibiotic, and volatile metabolite.

Table 3 Inhibition of growth of *S. rolfsii* by non-volatile metabolite

Rhizospheric isolate	Fungal growth in test (mm)	Fungal growth in control (mm)	% Inhibition of pathogen
Pseudomonas aeruginosa AL98	6.5(±1.29)	90(±0)	92.77(±1.43)

± Represents standard deviation

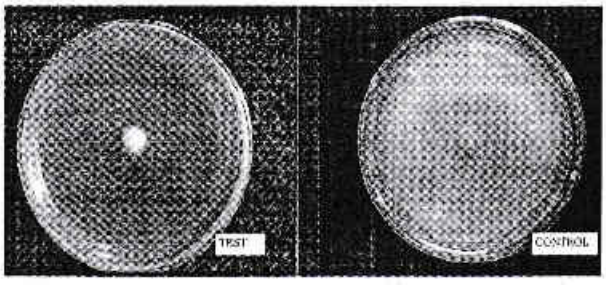

Plate 3 Action of non-volatile metabolites on growth of *Sclerotium rolfsii*

Screening for non-volatile metabolites

For screening the non-volatile metabolites, results were recorded after 7 days of incubation. Result displays that the *Pseudomonas aeruginosa* AL98 was able to secrete non-volatile diffusible metabolites and able to inhibits the *Sclerotium rolfsii*, 92.77% on King's B medium (Plate 3, Table 3).

Several researchers reported involvement of non-volatile metabolites in inhibition of plant pathogens. Ganesan and Sekar reported contribution of non-volatile metabolites produced by *P. fluorescence* in inhibition of *S. rolfsii*. Also, Rakh *et al.* [36] displayed inhibition of *Sclerotium rolfsii* by *Pseudomonas cf. monteilii* 9, producing non-volatile diffusible metabolites. Our results were in accordance with the results recorded earlier i.e., *Pseudomonas aeruginosa* AL98 produced non-volatile diffusible metabolites which inhibited *S. rolfsii*. All these results supported that *Pseudomonas aeruginosa* AL98 strains produced non-volatile metabolites which might include Phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (2,4-DAPG), Pyoluteorin (Plt), Pyrrolnitrin (Prn) which needs to be confirmed further.

Detection of volatile metabolites

After 6 days incubation period, results for volatile metabolites production by *Pseudomonas aeruginosa* AL98 was recorded. *Pseudomonas aeruginosa* AL98 secreted volatile metabolites in closed environment of petri plate which inhibited the growth of *S. rolfsii*, 93.88% as shown in (Plate 4, Table 4).

Table 4 Inhibition of growth of *S. rolfsii* by volatile metabolite

Rhizospheric isolate	Fungal growth in test (mm)	Fungal growth in control (mm)	% Inhibition of pathogen
Pseudomonas aeruginosa AL98	5.5(±0.57)	90(±0)	93.88(=0.64)

± Represents standard deviation

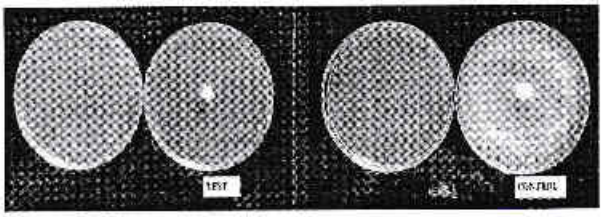

Plate 4 Influence of volatile metabolites on the growth of *S. rolfsii*

Volatile metabolites production by rhizosphere *Pseudomonas* was reported earlier by several workers [37] (Rengashwaran and Prasad 2000). Similarly, Rakh *et al.* [36] reported the volatile metabolite production by *Pseudomonas cf monteilii* 9 for growth inhibition of *S. rolfsii* upto 100%. All the six *Pseudomonas* isolates used by Ganesan and Sekar [35] showed 100% inhibition of the pathogen, *S. rolfsii* by volatile metabolite production in paired Petri plate technique. These literature survey reveals that our results were in accordance with the results earlier reported by Rakh *et al.* [36], Ganesan and Sekar [35]. Our result with *Pseudomonas aeruginosa* AL98 looks in accordance with the above-mentioned result. These results be real promising for biocontrol of phytopathogenic fungi of groundnut.

Table 5 Influence of seed bacterization with *Pseudomonas aeruginosa* AL98 on establishment of TAG24 groundnut in sick pots with *Sclerotium rolfsii*

Pots (Day incubation)	% Seed germination	Shoot length in cm	Root length in cm	No. of leaves	Chlorophyll content in mg/g	Vigour index
T $_{Pseudomonas\ 30}$	86	17.1	14.6	123	0.457	2726.2
Control $_{30}$	40	11.1	5.0	16	0.288	644
T $_{Pseudomonas\ 60}$	86	24.0	8.0	145	0.568	2752
Control $_{60}$	40	15.0	6.0	16	0.288	840

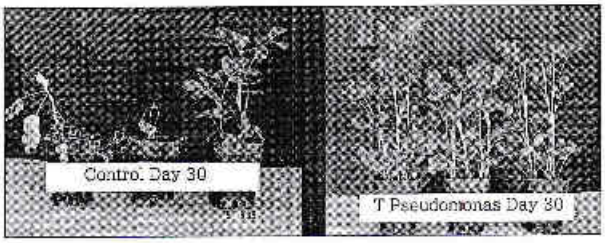

Plate 4 Influence of volatile metabolites on the growth of *S. rolfsii*

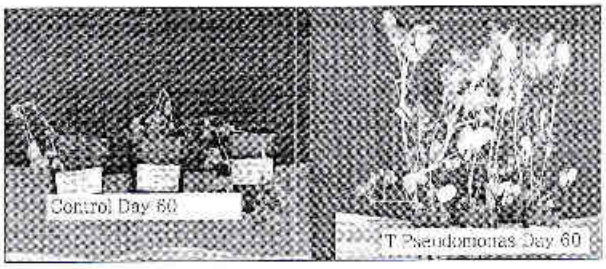

Plate 5 Effect of *Pseudomonas aeruginosa* AL98 treatment on the incidence of Stem rot in groundnut TAG24

Pot assay for biocontrol of stem rot disease (In vivo assay)

Initial results of *in vitro* experiments revealed that, *Pseudomonas aeruginosa AL 98* gifted to efficiently control tested pathogens, *S. rolfsii*. Hence, to further confirm and cross check the results, *In vivo*, experiment in pots were undertaken by artificially infested soil (sick soil) with phytopathogens, *Sclerotium rolfsii*, causing stem rot. It was evident from *Pseudomonads* treated groundnut seeds showed good over all vigor as compared to control (Plate 5). The effect of *Pseudomonas aeruginosa* AL 98 treatment on percent seed germination, shoot length, root length, no. of leaves, chlorophyll content and vigour index were studied and the results were presented (Table 5). The percent decrease in stem rot disease incidence in groundnut TAG24 due to *Pseudomonas* treatment compared to the untreated check (control), ranged from 40.16 to 54.54%. ANOVA Data analysis of this treatment by Microsoft Excel 365 reveal that the *Pseudomonas* treatment found significant at 5% level.

In pot culture experiment, pathogen alone in control showed typical stem rot symptom. Biocontrol agent *Pseudomonas aeruginosa* AL 98 treated plants showed reduced disease incident and showed vigorous growth and healthy looking compared with control plants. The length of the root and shoot, percent seed germination, chlorophyll content, and vigour index also increase at different level over control plant.

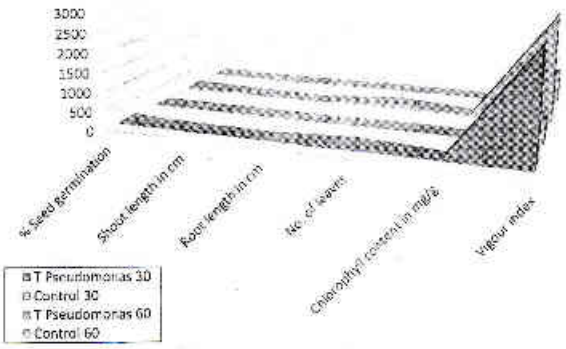

Fig 1 Effect of *P. aeruginosa* AL98 treatment on incidence of Stem rot in groundnut

Similar observation was recorded by Ganesan and Sekar [35], where *P. fluorescence* treated plants showed reduced disease incident of stem rot in groundnut crop with increase in the length of the root and shoot, and fresh and dry weight of the

shoot and root also increases at different level over control. Rakh *et al.* [36] recorded that in pot assay for control of *Sclerotium rolfsii*, *Pseudomonas cf. monteilii 9* treated seeds showed decrease in incidence of disease up to 45.45 to 66.67% in comparison to untreated seeds. Also, Rajendraprasad *et al.* [29] found that the combination of seed treatment with *Pseudomonas fluorescens*-3 + soil application of *Trichoderma harzianum* -1 (T_6) was effective in promoting seed germination and in reducing pre and post emergence tomato damping off caused by *S. rolfsii*. These findings were in accordance with our experimental findings.

CONCLUSION

This biocontrol agent would be exploited as good agent for management of stem rot disease of groundnut. Also serves as good alternative for chemical fungicides which was used for control of fungal phytopathogen.

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