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BIOLOGICAL CONTROL OF *Sclerotium rolfsii*, CAUSING STEM ROT OF GROUNDNUT BY *Pseudomonas cf. monteilii 9*

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Abstract

In an attempt to develop effective biocontrol system for management of stem rot disease caused by *Sclerotium rolfsii* in groundnut, 11 *Pseudomonas spp*. isolated from rhizospheric soil, were evaluated for their antagonistic activity against *Sclerotium rolfsii*. A soil bacterium identified as, *Pseudomonas cf. monteilii 9*, showed highest antagonistic activity against *Sclerotium rolfsii*. In dual cultures, the *Pseudomonas cf. monteilii 9* inhibited the *Sclerotium rolfsii* up to 94 % in terms of dry weight. *Pseudomonas cf. monteilii 9* produced diffusible antibiotic, volatile metabolites, hydrogen cyanide and siderophore which affect *Sclerotium rolfsii* growth *in vitro*. This strain also produced a clear halo region on skim milk agar plates, indicating that it excretes protease which played vital role in inhibition of *S. rolfsii*. 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.

Keywords: *Sclerotium rolfsii*, *Pseudomonas cf. monteilii 9*, Groundnut

Introduction

Sclerotium rolfsii Sacc. [*Athelia rolfsii* (Curzi) Tu & Kimbrough] causes the disease known as southern blight in a wide variety of crops. *Sclerotium rolfsii* forms brownish sclerotia that can survive in soil for long periods, frequently tolerating biological and chemical degradation due to the presence of melanin in the outer membrane (Chet, 1975). Among the methods employed to manage *S. rolfsii* are the following: fungicide applications, solarization, use of antagonistic microorganisms, deep plowing, crop rotation, and incorporation of organic and inorganic residues (Punja, 1985).

Chemical fungicides are extensively used in current agriculture. However, excessive use of chemical fungicides in agriculture has led to deteriorating human health, environmental pollution, and development of pathogen resistance to fungicide. Because of the worsening problems in fungal disease control, a serious search is needed to identify alternative methods for plant protection, which are less dependent on chemicals and are more environmentally friendly. Microbial antagonists are widely used for the biocontrol of fungal plant diseases. Rhizospheric bacteria have proved as excellent agents to control soil-borne plant pathogens. Bacterial species like *Bacillus*, *Pseudomonas*, have been proved in controlling the fungal diseases. Bacteria identified as plant growth promoting rhizobacteria and biocontrol strains often belong to the following genera (i) *Bacillus* (Nair *et al.,* 2002), (ii) *Pseudomonas* (Mark *et al.,* 2006).

Pseudomonas spp received great attention as biocontrol agent because of their catabolic versatility, excellent root-colonizing abilities and production of broad range antifungal metabolites such as 2,4-diacetylphloroglucinoal (DAPG), pyoluteorin, pyrrolnitrin and phenazines (Chin-A-Woeng *et al.,* 2001; Raaijmaker *et al.,* 2002). The mechanisms through which *Pseudomonas* spp. control plant diseases involve (i) competition for niches and nutrients, (ii) antibiosis, (iii) predation, and (iv) induction of plant defense responses.

The objective of this study was to evaluate the effect of *Pseudomonas cf. monteilii* as a biocontrol of *S. rolfsii*, causing Stem rot of Groundnut.

Material and Methods

Isolation of *Sclerotium rolfsii*

The fungal pathogen *Sclerotium rolfsii* was isolated from infected groundnut part in Department of Microbiology, Dnyanopask College of Art's, Commerce and Science, Parbhani- 431401(India) on potato dextrose agar (PDA) plates and incubated at 28 °C for 4–6 days. Stock culture of *S. rolfsii* was maintained on PDA slants and stored at 4 °C.

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Isolation of *Pseudomonas spp*. from rhizospheric soil

Rhizospheric soil from different healthy plants such as soybean, neem, groundnut, tur etc. were collected in poly-ethylene bags and brought to the research laboratory. 1 gm of soil sample was inoculated into 100 ml nutrient broth and kept for incubation at room temperature for 24 hr. For isolation of *Pseudomonas* spp, 1ml of this nutrient broth was transferred to selective enrichment media, Cetrimide broth and kept for incubation at room temperature for 24 hr. From enriched Cetrimide broth, a loopful of culture was streaked on Cetrimide agar (Brown and Lowbury, 1965) and the plates were incubated at room temperature till colonies were observed (24 – 48 hr). 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.

Screening for Potential Biocontrol agents against phytopathogen

All the isolates were screened for potential antagonistic activity against pathogenic fungi *S.* on King's B agar (Ran, *et al.,* 2003) using dual culture technique (Rangeshwaran and Prasad, 2000). An agar disc (5 mm dia.) was cut from an actively growing (96 hr) phytopathogen, *S. rolfsii* culture and placed on the surface of fresh King's B agar medium at the one side of the Petri plates. A loopful of actively growing bacterial isolates (each) was placed opposite to the fungal disc. Plates inoculated with phytopathogen and without bacteria were used as control. Each experiment was carried out in triplicates. Plates were incubated at room temperature for 7 days. Degree of antagonism was determined by measuring the radial growth of pathogen with bacterial culture and control and percentage inhibition calculated by the following equation (Riungu *et al.,* 2008).

$$\text{Infection (\%)} = \frac{\text{Colony diameter of Pathogen alone (Control)} - \text{Colony diameter of Pathogen + Antagonist}}{\text{Colony diameter of Pathogen alone}} \times 100$$

Identification of Biocontrol agent

An efficient biocontrol agent obtained from screening was identified by 16S rDNA sequencing carried out at Agharkar Research Institute (ARI) Pune, Maharashtra.

In vitro Characterization of biocontrol features

To investigate the biocontrol mechanism, the efficient rhizospheric isolates were tested for the production of Non-volatile diffusible antibiotic, volatile metabolite, HCN production, siderophore and protease assay.

Detection of Non-volatile diffusible antibiotic

This effect was tested according to Montealegro *et al.,* (2003) with 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 antagonistic bacterial suspension. After incubation for 48 h at room temperature, the membrane with the grown bacterial isolate was removed, and the plate was inoculated in the middle with a 5 mm disk of a pure culture of fungal pathogen and plates were re-incubated at room temperature for 7 days and the growth of the pathogen was measured. Control 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.* Experiment was run in triplicates Results were expressed as means of % inhibition of fungal pathogens in the presence and absence of antagonistic bacterial isolate.

Detection of volatile metabolites

For detection of volatile antifungal metabolites production by the antagonistic bacteria, two half plates (sterile) were taken. The plates were poured with sterile molten and cooled Kings B Agar and allowed to solidify. Now the bacterial antagonist culture was inoculated on one of the half plate in centre and on the other half the four day old pure culture of fungal phytopathogen was placed. Both half plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension. The plates were sealed to isolate the inside atmosphere and to prevent loss of volatiles produced. Plates were incubated at room temperature for 6 days and the growth of the pathogen was measured and compared to control plates developed in the absence of the bioantagonist. Experiment was run in triplicate (Montealegro *et al.,* 2003). Results are expressed as means of inhibition (%) of the growth of fungal pathogens in the presence and absence of any bacterial isolate. Percent inhibition was calculated using the following formula (Montealegro *et al.,* 2003).

$$\text{Percent Inhibition} = \frac{100\ (C - T)}{C}$$

Where,

C = Radial growth of fungus in control plates (mm)

T = Radial growth of fungus on the plate inoculated with Antagonist (mm)

Detection of Hydrogen cyanide

Production of hydrogen cyanide was detected using the method of Reddy *et al*, (2008) with few modifications. Isolates were grown in test tubes containing King's B broth supplemented with 4.4 gm l-1 of glycine. Filter paper (Whatmann No. 1) strips of uniform size (10 cm long and 0.5 cm wide) soaked in alkaline picrate solution and placed in side the test tubes in hanging position and the test tubes were incubated at room temperature (28±2°C) for 48 h. After incubation, the sodium picrate present in the filter paper was reduced to reddish compound in proportion to the amount of hydrocyanic acid evolved. The color was eluted by placing the filter paper in a clean test tube containing 10 ml distilled water and the absorbance was measured at 625 nm. Three replications were maintained for each isolate.

Siderophore Analysis

Inoculum Preparation

The inoculum of *Pseudomonas spp* was prepared in King's B medium and incubated at 28 °C on rotatory shaking incubator (120 rpm) for 18-20 h.

Culture and Cultivation

Siderophore production was studied using modified Succinate medium of Meyer and Abdullah (1978) consisting following components (g/l) Succinic acid (4), K_2HPO_4 (6), KH_2PO_4 (3), $(NH_4)_2\ SO_4$ (1), $MgSO_4$ (0.2), and pH (7.0). 0.1ml of inoculum were separately inoculated in 250 ml Erlenmeyer flask containing Succinate medium and then incubated on rotatory shaker incubator for 48 h at 28°C. A supernatant was harvested by centrifuging the culture at 10,000 rpm in cooling centrifuge at 4°C for 10 minute.

Quantitative Detection of Siderophore

Quantitatively siderophores in culture filtrate were detected as per Payne, (1994) where 0.5 ml of culture filtrate was mixed with 0.5 ml of CAS solution. A reference was prepared using, uninoculated succinate medium (used for siderophore production by *Pseudomonas*). Both the test and reference were read at 630 nm and % siderophore units in the culture filtrate were calculated.

$$\%\ \text{Siderophore Units} = \frac{Ar - As}{Ar} \times 100$$

Where,

Ar = Absorbance of reference at 630 nm. As = Absorbance of test sample at 630 nm.

Cell wall degrading enzyme production

Production of protease

Production of extracellular protease was tested according to Maurhofer et al. (1995). Efficacy of the antagonistic bacteria isolates in production of protease was tested by streaking each bacterial isolate on Skim milk agar plate. Each bacterial isolate under study was spot inoculated on solidified plates of Skim-milk agar and incubate overnight for production of proteases. The experiment was performed in triplicate. The bacterial isolates that produced protease were identified by a halo zone around bacterial colony and were measured (Maurhofer et al., 1995).

Pot Assay for biocontrol of stem rot (*in vivo*)

To check the biocontrol ability of *Pseudomonas cf. monteilii* 9 *in vivo* against *Sclerotium rolfsii*, a pot assay was set up using two groundnut varieties SB XI and TAG-24. Mass multiplication of *S. rolfsii* was carried out in Potato Dextrose broth at room temperature for 3 weeks (Ordentlich *et al.,* 1988) and then the numbers of sclerotia produced were used for the preparation sick pots. This experiment was carried out in three sets of pots and triplicates. All pots were first disinfected with 5 % $CuSO_4$ solution. First set (Positive Control) of three pots, was filled with 150 gm sterilized soil sand (1:1) mixture with sclerotia of *S. rolfsii* artificially inoculated at 1 sclerotia/ gm soil (Fouzia Yaqub and Saleem Shahzad, 2005). The pots containing inoculum were incubated for 15 days at room temperature, frequently stirred and watered for colonization of fungus in the soil. Then 5 surface disinfected untreated seeds of groundnut varieties, TAG-24, and SB XI each were sown in pots. Second set (Negative Control) of three pots, was inoculated with 150 gm of sterilized soil sand mixture and 5 untreated seeds of SB XI and TAG-24 each were sown in pots. Third set (Test) of three pots, was filled with 150 gm of sterilized soil sand mixture artificially inoculated with sclerotia of *S. rolfsii* at rate of 1 sclerotia/ gm of soil. The pots containing inoculum were incubated for 15 days at room temperature, frequently stirred and watered for colonization of fungus in the soil and then 5 biocontrol agent's formulation (15 gm/ kg of seeds) treated seeds of groundnut varieties, SB XI and TAG-24 each were sown in pots. All these pots were kept at room temperature and watered regularly. Percent germination, shoot length, root length, and chlorophyll content were recorded after 15, 30, 45 and 60 days.

Vigour index was calculated by using the formula of Baki and Anderson (1973).

Vigour index = (Mean shoot length + mean root length) x Germination (%)

Per cent disease control (PDC) was calculated by the following formula after 60 days.

PDC= (% Disease in check) - (% Disease in Treatment)/ (% Disease in check) x 100

Result and Discussion

Isolation of *Pseudomonas spp*. from rhizospheric soil

During this research work, 11 *Pseudomonas spp* were isolated from rhizospheric soil of different healthy plants such as Soybean, Neem, Groundnut, Tur etc. All the rhizospheric isolates were tentatively named as in Table 1 and maintained on Nutrient Agar slants for further screening.

Screening for Potential Biocontrol agents against phytopathogen

It was observed that *Pseudomonas* isolate CA/RN found as an efficient antagonist against *S. rolfsii* in dual culture technique (Photo Plate 1) while none of the other *Pseudomonas* isolates found efficient (Table 1).

Photo Plate 1 Dual culture technique of CA/RN against *Sclerotium rolfsii* A) Control of *Sclerotium rolfsii* B) CA/RN Culture with *Sclerotium rolfsii*

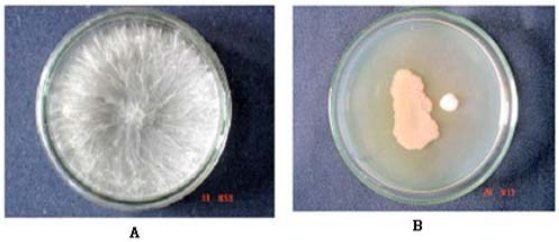


Table 2: *In vitro* screening for potential biocontrol agents against *Sclerotium rolfsii*

Strain	*Sclerotium rolfsii*
CA/RN	4
CA/RT	2
NM/S1/CA	0
NM/S2/CA	0
NM/S6/CA	0
NM/S8/CA	0
CA/RT	2
CA/RG	3
AVM 1	1
AVM 3	3
SBC-B	3

Each number is mean of three replicates. 0- none, 1= inhibition zone 1-25 %, 2= inhibition zone 26-50 %, 3= inhibition zone 51-75 %, 4= inhibition zone 76-100 %

It was revealed that culture CA/RN was able to inhibit *Sclerotium rolfsii* (94 %). Our results when compared with the results earlier reported by Kishore *et al.,* (2005), for control of *Sclerotium rolfsii* with *Pseudomonas aeruginosa* in dual culture. It was found that our results with *Pseudomonas* are far better than the above mentioned results because of the fact that there was only 32-74 % inhibition recorded where as 94 % inhibition was recorded for *S. rolfsii*.

Identification of Biocontrol agent

Analysis of the 16S rDNA gene sequences showed that CA/RN was closely related to *Pseudomonas cf. monteilii 9* (98% similarity) with GenBank database accession number AF181576.

In vitro Characterization of biocontrol features

To investigate the biocontrol mechanism of the selected strains namely, *Pseudomonas cf. monteilii 9* was tested for production of Non-volatile diffusible antibiotic, volatile metabolite, production of HCN, and siderophore. *Pseudomonas cf. monteilii 9* produced non-volatile diffusible antibiotic, volatile metabolite, HCN, and siderophore.

Detection of Non-volatile diffusible antibiotic

For detection of non-volatile diffusible antibiotic, results were recorded after 7 days of incubation.

Results show that the *Pseudomonas* strains was able to produce non-volatile diffusible metabolites and able to inhibits the *Sclerotium rolfsii* (100%) as shown in Photo Plate No.2

Photo Plate 2: Effect of non-volatile diffusible antibiotic on *S. rolfsii* produced by *Pseudomonas cf. monteilii 9*
A) *Pseudomonas cf. monteilii 9,* B) Control of *S. rolfsii*

A B

Several authors have reported involvement of antibiosis in biocontrol of plant pathogens e.g. the biocontrol agents, *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum f. sp. cubense* race (Getha and Vikineswary, 2002) and *Pantoea agglomerans* strain Eh252 on *Erwinia amylovora* (causal agent of fire blight in orchards) (Stockwell *et al.,* 2002) and the biocontrol of *Pythium* damping off of Pea by *Burkholderia cepacia* (Heungens and Parke, 2001) was attributed to antibiosis. These results are in accordance to the results recorded by us i.e. *Pseudomonas cf. monteilii 9* produced non-volatile diffusible antibiotic which inhibited *S. rolfsii*. All these results supported that the strains produced antibiotics which might include Phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (2,4-DAPG), Pyoluteorin (Plt), Pyrrolnitrin (Prn).

Detection of volatile metabolites

Pseudomonas cf. monteilii 9 produced volatile metabolites which inhibited *S. rolfsii* (100 %) as shown in Photo Plate 3. Our results when compared with the results earlier reported by Howell *et al.,* (1988), Diby *et al.,* (2005) for control of *Pythium ultimum* and *Phytophthora capsici* respectively. It was found that our results with *Pseudomonas* are far better than the above mentioned results because of the fact that there was only 2-23 % and 36-70 % inhibition recorded where as our was 100% inhibition for the tested pathogens. These results are promising for biocontrol of phytopathogen.

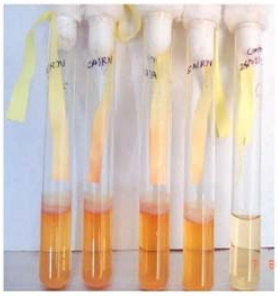
Photo Plate 3: Effect of volatile metabolites on *S. rolfsii* produced by *Pseudomonas cf. monteilii 9*

Detection of Hydrogen cyanide

In *Pseudomonad* species, hydrogen cyanide is released by the decarboxylation of glycine (Wissing, 1974). As described by Kloepper *et al.,* (1991) the HCN formed a brownish red compound with sodium picrate and the intensity of the colour increases with the amount of HCN. In the present study, *Pseudomonas cf. monteilii 9* produced HCN which turned yellow colored filter strips to reddish color whose absorbance was read at 625 nm (A625-0.02) as shown in Photo Plate 4. Defago *et al.,* (1990) has also demonstrated by mutational analysis and complementation that production of HCN by *Pseudomonas fluorescens* strain, CHAO accounted for about 60 % of the biocontrol activity.

A leaf colonizing *Pseudomonas* sp. constructed to overproduce HCN protected wheat from a leaf pathogen, *Septoria tritici* more effectively than the parent strain (Flaishman *et al.,* 1996). Hydrogen cyanide (HCN) effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations. However, producer microbes, mainly Pseudomonads, are reported to be resistant (Bashan and de-Bashan, 2005). The results obtained for production of HCN by *Pseudomonas cf. monteilii 9* are in accordance with the above results and played contributory role in inhibition of pathogens, *S. rolfsii.*

Photo Plate 4: HCN Production by *Pseudomonas cf. monteilii 9*



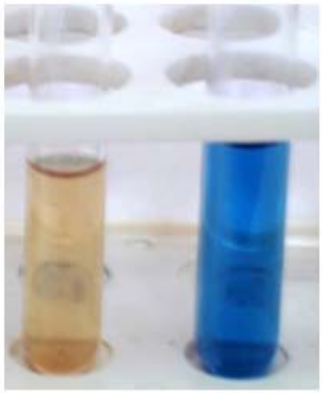
Siderophore Analysis

Inoculum of both cultures was inoculated in modified Succinate medium of Meyer and Abdullah (1978) and incubated on rotary shaking incubator at 28 ^{o}C for 48 h.

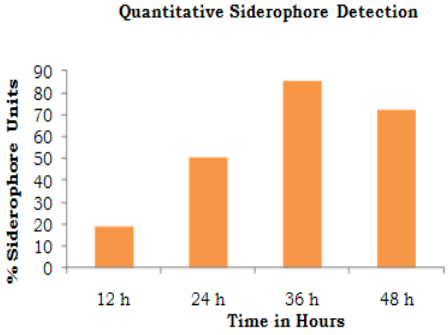
Quantitative Detection of Siderophore

A supernatant of the culture was harvested after 12, 24, 36 and 48 h by centrifuging the culture at 10,000 rpm in cooling centrifuge. Quantitatively siderophores in supernatant was detected as per Payne, (1994) where 0.5 ml of culture filtrate was mixed with 0.5 ml of CAS solution. A reference was prepared using, uninoculated Succinate medium. Absorbance of both the test and reference were read at 630 nm and % siderophore units in the culture filtrate were calculated. *Pseudomonas cf. monteilii 9* gave instant color change of CAS reagent from blue to classical golden orange (Photo Plate 5) and produced maximum siderophore whose absorbance was read at 630 nm. % siderophore unit of culture was calculated after 12, 24, 36 and 48 h (Graph 1).

Photo Plate 5: Liquid CAS assay for Siderophore Production



Graph 1: Siderophore productions by *Pseudomonas cf. monteilii* with respect to time



In the time course of siderophore production, maximum siderophore secretions by *Pseudomonas cf. monteilii 9* (85.51%) was recorded after 36 h. Siderophore being a secondary metabolite produced under iron stress condition hence maximum siderophore was recorded 36 h. thereafter, re-incubation showed decline in the % siderophore. Here for siderophore production the batch needs to be harvested after 36 h. for maximum recovery of siderophore.

Cell wall degrading enzyme production

Production of protease

Production of extra cellular protease was tested according to Maurhofer *et al.* (1995) on skim milk agar plates. *Pseudomonas cf. monteilii 9* strongly produced protease enzyme showing zone of clearance, of about 13 mm around the colony on skim milk agar (Photo Plate 6). Earlier Dunne *et al.,* (1997) demonstrated that biocontrol of *Pythium ultimum* in the rhizosphere of sugar beet by *Stenotrophomonas maltophila* (Hugh) Palleroni and Bradbury W81 was due to the production of extra cellular protease. Although, chitinolytic activity appears less essential for PGPB such as *S. plymutica* IC14 when used to suppress *S. sclerotiorum* and *B. cinerea*, synthesis of proteases are involved (Kamensky *et al.*, 2003).

Above facts support our result that culture *Pseudomonas cf. monteilii 9* produced protease enzymes which contributed for the biocontrol of *S. rolfsii.*

Photo Plate 6: Protease assay

Pot Assay for Biocontrol of Stem rot (*In Vivo*)

It is evident from Photoplate 7 and 8 that the vigour of groundnut in artificially infested soil (Positive control) was poor as compared to the negative control. Also it was observed that, *Pseudomonas cf. monteilii 9* treated groundnut seeds showed good over all vigor as compared to positive control which also corresponds to the results obtained *in vitro*. Treatment with the *Pseudomonas cf. monteilii 9* increased % seed germination, shoot length, root length No. of leaves and chlorophyll content of groundnut, as depicted in Table 2. It was also revealed that incidence of the disease symptoms in the sick pot (positive control) after 60 days of sowing was observed where as no symptoms were recorded in the test pots (bacterized seeds) even after 60 days.

The percent disease control due to *Pseudomonas cf. monteilii 9* treated seeds compared to the untreated check (Positive control), was found in range from 45.45 to 66.67%. These results were some what similar to that got by Kishore *et al.,* 2005 where groundnut seed endophytes *Pseudomonas aeruginosa GSE 18* and *GSE 19* reduced the seedling mortality by 54% and 58%, compared to the control.

Photoplate 7: Pot Assay with *Pseudomonas cf. monteilii 9* against *Sclerotium rolfsii* (TAG 24) after 60 days
A) Positive Control (TAG 24 Seeds + *S. rolfsii*)
B) Test (*Pseudomonas cf. monteilii 9* + TAG 24 Seeds + *S. rolfsii*)

A) B)

Photoplate 8: Pot Assay with *Pseudomonas cf. monteilii 9* against *Sclerotium rolfsii* (SB XI) after 60 days
A) Positive Control (SB XI Seeds + *S. rolfsii*)
B) Test (*Pseudomonas cf. monteilii 9* + SB XI Seeds + *S. rolfsii*)

A) B)

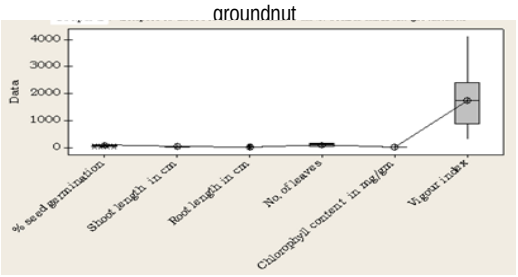
Table 2:- Influence of seed bacterization with *Pseudomonas cf. monteilii 9* on establishment of groundnut in sick pots with *Sclerotium rolfsii*

Groundnut varieties	Pots (Day incubation)	% seed germination	Shoot length in cm	Root length in cm	No. of leaves	Chlorophyll content in mg/g	Vigour index
TAG 24	Test $_{15}$	80	7.7	4.0	56	0.348	939
	Positive Con $_{15}$	40	5.0	2.4	19	0.280	296
	Negative Con $_{15}$	80	6.8	3.3	39	0.332	808
	Test $_{30}$	80	17.00	5	115	0.460	1760
	Positive Con $_{30}$	40	11.1	5	16	0.288	644
	Negative Con $_{30}$	80	16.8	5	112	0.477	1744
	Test $_{45}$	80	22.2	8.1	139	0.538	2424
	Positive Con $_{45}$	40	16.2	3.0	16	0.288	768
	Negative Con $_{45}$	80	21.0	8.0	135	0.529	2320
	Test $_{60}$	80	25.0	20.0	142	0.564	3600
	Positive Con $_{60}$	40	15.0	6.0	16	0.288	840
	Negative Con $_{60}$	80	24.0	5.0	139	0.555	2320
SB XI	Test $_{15}$	86	7.9	4.3	67	0.368	1049.2
	Positive Con $_{15}$	66.66	4.9	2.7	24	0.278	506.6
	Negative Con $_{15}$	80	7.2	4.0	47	0.355	896
	Test $_{30}$	86	17.5	7.6	122	0.446	2158.6

Positive Con 30	66.66	10.0	6.3	92	0.393	1086.5
Negative Con 30	80	16.8	6.3	115	0.439	1848
Test 45	86	23.0	15.0	140	0.516	3268
Positive Con 45	66.66	16.0	3.0	95	0.401	1266.5
Negative Con 45	80	21.0	13.3	136	0.511	2744
Test 60	86	27.0	21.0	147	0.541	4128
Positive Con 60	66.66	23.0	4.0	101	0.428	1799.8
Negative Con 60	80	23.0	13.0	142	0.535	2880
F-calculated value				64.97		
F- Table value at 5%				2.27		

The data obtained during this experiment was analyzed by One-Way (Unstacked) ANOVA using Minitab 15 and Statplus 2008 Statistical Software. It was revealed by data analysis that variations in values % germination, shoot length, root length No. of leaves and chlorophyll content of the treatment by using *Pseudomonas cf. monteilii 9* as biocontrol agents against *Sclerotium rolfsii,* causing stem rot disease in groundnut, was found to be significant at 5 % level because the calculated F values (64.97) was found to be more than the Table Value (2.27) which clearly indicates that the results obtained, are not mere by chance but due to the effective treatment. This statistical explaination is further strengthened by the boxplot.

Graph 2: Boxplot effect of *P. monteilii* 9 in *S. rolfsii* infested groundnut



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