

**EFFECT OF PLANT GROWTH PROMOTING FUNGAL INOCULANT ON THE GROWTH OF *ARACHIS HYPOGAEA* (L.) AND IT'S ROLE ON THE INDUCTION OF SYSTEMIC RESISTANCE AGAINST *RHIZOCTONIA SOLANI*****J. Sudha Lakshmi\*<sup>1</sup>, T. Kuberan<sup>2</sup>, J. Anburaj<sup>4</sup> C. Sundaravadivelan<sup>3</sup>, P. Kumar<sup>3</sup>, and Manorama Dhanaseeli<sup>1</sup>**<sup>1</sup>\*Department of botany and Department of Microbiology, Lady Doak college, Madurai.<sup>2</sup>Post Graduate Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi - 626 124 <sup>3</sup>PostGraduate Department of Zoology, Ayya Nadar Janaki Ammal College, Sivakasi - 626 124 <sup>4</sup>Post

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**ABSTRACT:** Beneficial plant microbe interactions in the rhizosphere are primary determinants of plant health and soil fertility. Plant growth promoting fungi have great effect towards the growth of plant crops. Soil borne pathogenic fungi cause heavy crop losses all over the world. As the use of chemicals for disease control and fertilization causes environmental problems, there is a need for alternative control measures. The most important and economically cultivated plant pea-nut was selected to test the growth promotion by antagonistic microorganisms with or with our pathogen *Rhizoctonia solani*. This necessitates a study on Plant Growth Promoting Fungi (PGPF) as adequate work has gone on rhizobacteria. The present investigation was conducted to study the effect of PGPF on the growth of *Arachis hypogea* (L.) and its role of induction of systemic resistance against *Rhizoctonia solani*. Forty five rhizosphere fungal isolates were obtained from 12 different cultivated field crops and were screened for their potential to promote growth in *Arachis hypogea* (L.). The isolate (Cc<sub>2</sub>) obtained from *Cucumis sativus* (L.). Duch.ex. poir was identified as the potential plant growth promoting fungus. The effect of soil inoculation with the selected isolate Cc<sub>2</sub> on the growth of healthy plants of *Arachis hypogea* (L.) and those challenged with *Rhizoctonia solani* was studied by pot culture experiment. The overall vegetative growth of plant (Root and Shoot development, Dry matter accumulation) and reproductive growth (Pod and Seed development) were studied. The fungal inoculants improved effectively the growth both in plants challenged and unchallenged with *Rhizoctonia solani*. The soil inoculation of Cc<sub>2</sub> has improved the chlorophyll, carotenoid, anthocyanins, total soluble sugar and protein content compare to the untreated plants (T<sub>0</sub>) and plants infected by *Rhizoctonia solani* (T<sub>1</sub>). The phenol and proline contents were found to be higher in plants challenged with *Rhizoctonia solani*. Growth hormone production ability of the selected isolate was determined. Results revealed that the selected isolate could produce Indole Acetic Acid and Gibberellic Acid. The in vitro study by dual culture method revealed that there was a negative interaction (Antibiosis) between the plant growth promoting fungal inoculant (Cc<sub>2</sub>) and the pathogen *Rhizoctonia solani*. It could be concluded that the selected isolate Cc<sub>2</sub> proved to be a potential fungus in promoting plant growth and yield in *Arachis hypogea* (L.) and in inducing systemic resistance in *Arachis hypogea* (L.) against *Rhizoctonia solani*. Finally Cc<sub>2</sub> was identified as *Rhizopus* sp., in generic level.

**Key Words:** Plant Growth Promoting Fungi (PGPF), phytopathogens, carotenoid, antagonistic, rhizobacteria.

**INTRODUCTION**

Environmental concerns have led to the need for sustainable use of natural resources. The conventional agriculture has caused considerable negative impacts on soils and water. It is important to change certain agricultural managements to environmental cleaner techniques. The sustainable agriculture has pointed many approaches and techniques to reduce negative effects of conventional agricultural practices on environment. One of these strategies is the utilization of soil micro biota for the promotion of plant growth and control of plant diseases. (Botelho and Hagler, 2006). Plant diseases play a direct role in the destruction of natural resources in agriculture. In particular, soil borne pathogens cause greater losses, fungi being more aggressive.

The distribution of several pathogenic fungi such as *Pythium*, *Phytophthora*, *Botrytis*, *Rhizoctonia* and *Fusarium* has spread during the last few years due to changes introduced in farming with determined effects on crops of economic importance. (Chet et al., 1997).

*Rhizoctonia solani* can cause significant plant damage, impaired plant growth and crop failure. It can affect plant emergence and cause stem canker symptoms characterized by brown and black sunken lesions on the stems. Misshapen tubers with an uneven size distribution also result. It can cause diseases such as root rot, stem rot, seed decay, damping off and foliar blight in crops. Groundnut is a rich source of protein and it is one of the principle oil seed crops of India. Peanut was grown mainly for its edible oil. *Arachis hypogea* (L.) has a mutually beneficial symbiotic relationship with nitrogen fixing bacteria. Efforts have been continually concentrated towards increasing its yield. The potential of *Trichoderma* against plant diseases was recognized in the control of *Corticium solani* (Aluko and Hering, 1970), pea damping off (Lifshitz et al, 1986), *Pythium ultimum* (Migheli et al., 1988), *Fusarium wilt* (Zhang et al, 1996) and *Botrytis cinerea* (Elad and Kapat, 1999). Simon (1989) stated that plant growth promoting microbial inoculants play an important role in maintaining sustainable agricultural production. Hormones are naturally synthesized natural organic compounds which influence the growth and development living organisms. Auxins are naturally occurring substances with Indole possessing growth promoting activity. Auxin accumulation may be attributed to rapid synthesis by the infected plant, synthesis by the pathogen and operation of new pathway of IAA synthesis such as release of IAA from IAA protein complex (Mahadevan, 1984). Gibberellins induce elongation of internodes and the growth of meristems (or) buds. They usually inhibit adventitious root as well as shoot formation. The fungus produces many types of Gibberellins in the culture media. As the exploitation of microbial metabolites has gained status of a new trust area in plant protection and growth promotion, the present investigation was undertaken to study the effect of plant growth promoting fungi on the growth of *Arachis hypogea* (L.) and its role on the induction of systemic resistance against *Rhizoctonia solani*.

## MATERIALS AND METHODOLOGY

### Isolation and maintenance of the pathogen

The present study was carried out with *Rhizoctonia solani* isolated from the infected plants of ground nut collected from in and around Madurai District, Tamil Nadu, India. 20 ml of sterile and warmed PDA media were poured into sterile Petri plate and allowed to solidify. The fungi culture was inoculated the centre of the Petri plate by placing a 10 mm disc of 7 days old PDA culture. The plates were incubated at room temperature (28°C) for four days.

### Mass multiplication of pathogen

The inoculum of *R. solani* was developed by inoculating the pathogens on PDB medium and incubated at room temperature (28°C) for 10 days. The pathogen was thoroughly mixed.

### Isolation of Beneficial fungi

Soil samples were collected from the rhizosphere of 13 different cultivated field crops listed below.

### Crops selected for sample collection

Isolation of fungi from the collected soil samples was done by serial dilution technique. 1gm of soil sample was suspended in 9ml of sterile distilled water and 10-fold dilutions were prepared up to  $10^{-3}$ . 1 ml of diluted suspension was inoculated into each sterile Petri plate and 20 ml of Czapekdox agar was poured. The plates were rotated for uniform distribution and were incubated at room temperature for 4 days. The fungi developed on the medium were then isolated, pure cultured and maintained in Czapekdox agar slants. 45 fungal isolates were obtained and designed by numbers as follows:

Source of Rhizosphere fungi	Isolates
<i>Abelmoschus esculentus</i> (L.) Moench.	Ca1, Ca2, Ca3
<i>Allium cepa</i> (L.)	Cb1, Cb2, Cb3, Cb4, Cb5
<i>Cucumis soticus</i> (L.) Dueh. ex. poir	Cc1, Cc2, Cc3, Cc4
<i>Cyamopsis tetragonoloba</i> (L.) Taub.	Cd1, Cd2, Cd3, Cd4, Cd5
<i>Eleusine coracana</i> (L.) Gaertn.	Ce1, Ce2
<i>Gossypium</i> sp.,	Cf1, Cf2, Cf3, Cf4
<i>Helianthus annuus</i> (L.)	Cg1, Cg2, Cg3
<i>Murraya koenigii</i> (L.)	Ch1, Ch2, Ch3, Ch4
<i>Musa paradisiacal</i> (L.)	Ci1, Ci2, Ci3
<i>Pennisetum americanum</i> (L.) Schum	Cj1, Cj2, Cj3
<i>Polianthes tuberosa</i> (L.)	Ck1, Ck2, Ck3
<i>Saccharum officinarum</i> (L.)	Cl1, Cl2, Cl3
<i>Solanum melongena</i> (L.)	Cm1, Cm2, Cm3

### Screening for potential plant growth promoting fungus

Small plastic containers (12×8 cm) provided with drainage hole were filled with steam sterilized soil mixture containing garden soil and sand (3:1). Soils in each container was inoculated separately with three 1mm agar disc containing the mycelium of all the 45 isolates and sprinkled with sterile water and then covered with plastic sheet which was punctured with a pin to promote aeration. The inoculants were allowed to establish for a week. The plastic sheet was removed and the soil was thoroughly mixed with the inoculum. Healthy seeds of *Arachis hypogea* (L.) were soaked in water for 2 hours and sown (3 seeds/container). The soil was watered regularly and the effect of the fungal inoculants on the growth of seedlings was determined after 15 days in terms of seedling height and number of roots per seedlings.

### Identification of potential fungal isolate

The colony characteristics on czepeckdox agar were observed. The hyphae were stained with Lacto phenol cotton blue stain and microscopically analyzed for their characteristics. The isolate was identified to the generic level using the Manual of Soil- Fungi.

### Mass multiplication of beneficial microorganism

The inoculum of *Rhizoctonia solani* plant pathogen was developed by inoculating on beneficial microorganism PDB medium and incubated at room temperature (28°C) for 15 days. The beneficial microorganism was thoroughly mixed and the inoculum was used for the seed treatment experiments.

### *In vivo* study on the effect of plant growth promoting fungal inoculant on the growth of healthy seeds of *Arachis hypogea* (L) and that challenged with pathogen *Rhizoctonia solani*

A pot culture study was carried out to determine the effect of selected plant growth promoting fungal inoculant (Cc<sub>2</sub>) on the growth of healthy and infested plants of *Arachis hypogea* (L.) and those challenged with *Rhizoctonia solani*. Earthen pots (21×20cm) were filled with steam sterilized soil mixture containing garden soil and sand in the ratio of 3:1.

### Treatments

- ❖ T0 - Control (sterile soil only)
- ❖ T1 - Pathogen (*Rhizoctonia solani*)
- ❖ T2 - Plant Growth Promoting Fungal Inoculant (Cc<sub>2</sub>)
- ❖ T3 - Pathogen (*Rhizoctonia solani*) + Plant Growth Promoting Fungal Inoculant (Cc<sub>2</sub>)

The pots in T<sub>1</sub> and T<sub>3</sub> were inoculated with three 5 mm agar discs containing mycelium of *Rhizoctonia solani* and allowed to established for a week then Plant Growth Promoting Fungal Inoculant Cc<sub>2</sub> (three 5 mm discs of inoculum/pot) pots of T<sub>2</sub>, T<sub>3</sub> treatments. Sterile water was sprinkled after the inoculation and the pots were covered with polyethylene sheets to avoid the air borne pathogens contaminations. The inoculated organisms were allowed to infest the soil for a week.

The polyethylene sheets were removed and growth medium was thoroughly mixed with the inoculum. Certified seeds of *Arachis hypogea* (L.) were soaked overnight in sterile distilled water and seeds were sown in the pots (6 seeds / pot). Regular watering was done throughout the investigation. Duplicates were maintained for all the treatments. The plants were uprooted 60 days after germination and the following parameters were analyzed.

#### (a) Morphometric Analysis

- ❖ Number of roots
- ❖ Root length
- ❖ Shoot length
- ❖ Number of leaves
- ❖ Number of branches
- ❖ Number of Pods / Plant
- ❖ Leaf Area
- ❖ Fresh weight of the Pods/Plant
- ❖ Dry weight of the Plant

#### Determination of dry matter yield

The plants from each treatment were cut into pieces and kept in an oven at 100°C for 2 days. The dried sample was weighed and the dry matter yield was recorded.

#### Biochemical analysis

##### Determination of Chlorophyll

The chlorophyll content was estimated by Arnon's method . 100 mg leaf sample was homogenized with 10 ml of 80 % prechilled acetone. The extract was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and absorbance was read at 645 nm and 663 nm. Total Chlorophyll content was calculated by using the formula, Total chl =  $20.2 (A_{645}) + 8.02 (A_{663}) \times V/1000 \times W \times 10$  (Where, A - OD at specific nm, V- Final volume of plant extract in 80 % acetone, W - Fresh weight of leaf tissue used).

##### Determination of Anthocyanins

The anthocyanins content was determined by Swain and Hillis (1959). 100 mg fresh leaves were taken and ground with 10 ml of ethanol and filtered through what man No.1 filter paper. One ml of extract along with methanolic HCl was added to 1 ml of peroxide reagent and kept in dark for 15 minutes and the absorbance was read at 525 nm. Anthocyanin content was represented by O.D value ( $A_{525}$ )/gram of leaf tissue.

##### Determination of Protein

The protein content was determined by the procedure described by Lowry *et al.*, (1951). One gram of leaf sample was ground with 5 ml of phosphate buffer (pH 7) and centrifuged at 3000 rpm for 20 minutes. Three ml of the extract was taken with three ml of 20 % trichloroacetic acid and kept in water bath for 20 minutes and again it was centrifuged at 3000 rpm for 20 minutes. Pellet was collected and washed with 6ml of acetone and centrifuged. Pellet was dissolved in five ml of 0.1 NaOH and mixed well. It was kept for 10 minutes and 0.5 ml of Folin was added. It was incubated for 30 minutes in dark and the absorbance was read at 660 nm. The amount of protein was estimated using Bovine serum albumin as the standard.

### Determination of Total Soluble Sugars

The amount of total soluble sugar present in the leaf extract was determined by Anthrone method (Dubois *et al.*, 1951). Leaf sample (100 mg) was ground with ten ml of distilled water and added with two ml of 10 % Trichloro acetic acid and centrifuged at 3000 rpm for five minutes. Four ml of Anthrone reagent was added to one ml of supernatant. The test tubes were kept in boiling water bath after covering their mouth with glass marbles for ten minutes. The content was then cooled and the absorbance was read at 625 nm. The amount of sugar was determined using glucose as the standard.

### Determination of Proline

Free proline from plant tissues may be selectively extracted in aqueous Sulphosalicylic acid and its concentration was measured using Ninhydrin method. 200 mg of leaf sample was taken and ground with ten ml Sulphosalicylic acid and filtered with What man 1 filter paper. two ml of the extract along with two ml acid Ninhydrin and two ml of glacial acetic acid was taken, mixed well and kept in boiling water bath (100°C) for one hour. It was cooled in an ice for five minutes and added with four ml of toluene. The tubes were agitated vigorously. The upper pink chromophore layer was separated and the absorbance was read at 520 nm. The amount of Proline was determined using Proline as the standard.

### Determination of Phenol

The phenolic content was estimated by Folin-Ciocalteu method. 100 mg fresh leaves were taken and ground with ten ml of ethanol and filtered through what man 1 filter paper. One ml of extract was added with two ml of 20 % sodium carbonate. It was shaken well and kept in boiling water bath for one minute and cooled. The blue solution obtained was diluted to 25 ml with water and the absorbance was read at 650 nm. The amount of phenol was determined using Catachol as the standard.

### Determination of plant hormones

The healthy seeds of *Arachis hypogea* (L.) was soaked overnight in one week, two weeks, and three weeks old culture filtrate (C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub>) of selected fungal inoculant (Cc<sub>2</sub>) and sown in the small containers containing sterile soil. The containers were regularly irrigated growth of the seedlings were determined. The culture filtrate that revealed the enhanced growth of seedling was selected to determine the production of auxins and gibberellins.

#### Indole acetic acid

The amount of IAA produced in the culture medium was determined by the method described by Mandal *et al.*, (2007). Two weeks old culture of Cc<sub>2</sub> was centrifuged at 1000 g for 10 minutes. Two ml of Salkowsky reagent was mixed with one ml of cell free culture filtrate and incubated at room temperature for 30 minutes. The absorbance was read at 530 nm. The commercial Indole Acetic Acid was used as the standard.

#### Gibberllic acid

The amount of GA produced by Cc<sub>2</sub> was estimated by the method described by Mahadevan, A (1984) . 15ml of cell free culture filtrate was taken in a test tube and mixed with 2ml of zinc acetate solution. After 2 minutes, 2ml of potassium Ferro cyanide was added and centrifuged at 2000g for 15 minutes. To 5ml of supernatant, 5ml of 30% HCl was added and incubated at 20°C for 75 minutes. The absorbance was read at 254nm. The commercial Gibberllic acid was used as the standard.

### **Invitro study on the interaction between plant growth promoting fungal inoculant (cc<sub>2</sub>) and *Rhizoctonia solani***

The interaction between the selected Plant Growth Promoting Fungal inoculant and *Rhizoctonia solani* was tested by dual culture method suggested by Morten and Strobe (1998) using Czepeckdox agar medium. One mm agar discs of the two fungi cut separately using a sterile cork borer from the edges of five days old culture were kept apart on Czepeckdox agar medium and the plates were incubated at 35°± 2 ° C for five days. The plate inoculated with *Rhizoctonia solani* alone served as the control. The growth of *Rhizoctonia solani* in dual culture was compared with that of the control plate.

### **RESULT AND DISSCUSION**

The effect of Plant Growth Promoting Fungal (PGPF) inoculant and its role on the induction of systemic resistance against *Rhizoctonia solani* was studied by *in vivo* studies using *Arachis hypogea* (L.).

#### **Isolation and screening of Rhizosphere fungi for the plant growth promotion in *Arachis hypogea* (L.)**

About 45 fungal isolates from soil collected from the rhizosphere of 13 different cultivated field crops. A preliminary study was conducted to evaluate their effect on seedling growth of *Arachis hypogea* (L.). The result presented in (Table 1A -B) revealed that 10 among the 45 isolates tested showed plant growth (Cb<sub>2</sub>, Cc<sub>2</sub>, Cd<sub>2</sub>, Cf<sub>1</sub>, Cf<sub>2</sub>, Cg<sub>1</sub>, Ch<sub>1</sub>, Ch<sub>3</sub>, and Ck<sub>1</sub>). The soil inoculation of 16 isolates resulted in reduced plant growth promoting ability (Ca<sub>2</sub>, Cb<sub>1</sub>, Cb<sub>4</sub>, Cb<sub>5</sub>, Cc<sub>1</sub>, Cc<sub>4</sub>, Cd<sub>3</sub>, Cd<sub>4</sub>, Cg<sub>5</sub>, Cf<sub>4</sub>, Cg<sub>2</sub>, Cg<sub>3</sub>, Ch<sub>4</sub>, Ch<sub>5</sub>, Cj<sub>1</sub> and Ck<sub>3</sub>) and 19 of the isolates inhibited seed germination. Among the 10 plant growth promoting fungal isolates, maximum growth was observed in *Arachis hypogea* (L.) grown in soil inoculated with the isolate Cc<sub>2</sub> isolated from rhizosphere soil of *Cucumis sativus*. Hence Cc<sub>2</sub> was selected as the potential fungus for further studies.

#### **Identification of Selected Isolate Cc<sub>2</sub>**

The colony of isolate Cc<sub>2</sub> showed pre fused cottony growth of white mycelium which filled the entire culture plate . Using the above mentioned characteristics the fungal isolate Cc<sub>2</sub> was identified to the generic level as *Rhizopus*. The vegetative hyphae are aseptate and branched with an abundant production of Sporangiohores bearing sporangia at their ends. Sporangiospores are spherical, unicellular and hyaline.

#### ***In vivo* study on the effect of plant growth promoting fungal inoculant on the growth of healthy seeds of *Arachis hypogea* (L.) and that challenged with pathogen *R. solani***

The efficiency of the selected growth promoting fungal inoculant (Cc<sub>2</sub>) reflected on the growth and biochemical characteristics of *Arachis hypogea* (L.) was studied in healthy plants and those challenged with *Rhizoctonia solani* by pot experiment. Soil inoculation of (PGPF) inoculant (T<sub>2</sub>) was found to enhance root development as well as shoot growth. There was an increase in the number of roots (108.3 %), root length (86.57 %), shoot height (134.3 %) in T<sub>2</sub> over the control (T<sub>0</sub>). There was a triple fold increase in the number of leaves in T<sub>2</sub>. There was greater reduction in number of roots (41.6 %), root length (36.4 %) and shoot height (42.9 %) in pathogen infested plants (T<sub>1</sub>) grown in the soil without PGPF inoculant. There was triple fold increase in the number of leaves in T<sub>3</sub> than T<sub>1</sub>. The soil inoculation of PGPF inoculant enormously increased the shoot growth which made the plant more bushy than the other plants (Plate 1). There was a double fold increase in the leaf area due to soil inoculation with Cc<sub>2</sub> (Plate 2). The development of pods and seeds was improved in T<sub>2</sub> by inoculation (Plate 3, Table 2). There was a greater inhibition of pod development in plants infected by *Rhizoctonia solani* (T<sub>1</sub>).

This might be due to unhealthy roots affected by root rot. The overall growth of the plant was also found to be greatly affected (Plate 4). The pod size was improved in T<sub>3</sub> compare to infected plants (T<sub>1</sub>) which might be due to the suppression of *Rhizoctonia solani* by the selected fungal isolate. The dry matter accumulation was greater in plants grown in soil inoculated with Cc<sub>2</sub>. The increase was found to be 3 fold over the control (T<sub>0</sub>). The infested plants in T<sub>1</sub> showed poor dry matter yield compared to all other treatments. Soil inoculation with Cc<sub>2</sub> had improved the dry matter yield in infected plants (T<sub>3</sub>).



Plate 1. Macroscopic comparison of the result



Plate 2. Leaf area



Plate 3. Pods

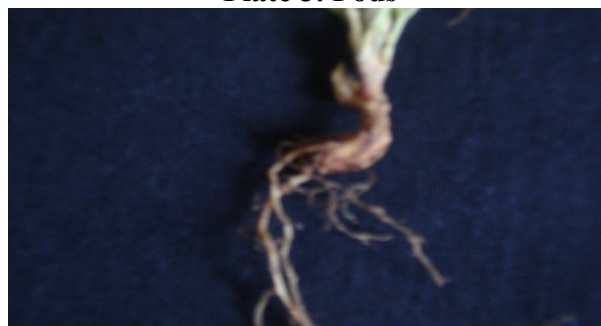


Plate 4. *Rhizoctonia solani* infected root

Table 1(A). Effect on Seedling Growth of *Arachis hypogea* (L.)

Treatment	No. of leaves	Shoot length (cm)	Fresh weight of the shoot (gm)	Dry weight of the shoot (gm)	No of branches
T0	8.33 ±1.15	5.83 ±0.57	1.89 ±0.03	0.06± 0.1	1.33± 0.57
T1	4.33±0.57	3.33±0.57	0.72±0.27	0.31 ±0.04	0.33± 0.57
T2	26.33± 3.51	13.66±1.52	11.79 ±1.4	3.06± 0.04	5.66± 0.57
T3	13.66±3.1	8 ±1	3.69 ±0.97	1.19± 0.11	3.66 ±0.57

Table 1(B). Effect on Seedling Growth of *Arachis hypogea* (L.)

Treatment	No. of roots (cm)	Root length (cm)	Fresh weight of the root (gm)	Dry weight of the root (gm)
T0	12± 2	22.33± 0.76	0.72± 0.1	0.37± 0.1
T1	7.33± 2	14.33 ± 0.57	0.14± 0.02	0.06± 0.03
T2	25.66± 2	41.66± 0.4	1.56 ±0.37	0.24± 0.14
T3	14.33 ±2	21.33 ±1.55	1.01 ±0.17	0.46± 0.03

### Effect of soil inoculation with PGPF inoculant (Cc2) on the selected biochemical parameters

The data collected for the selected biochemical parameters are presented in (Table 3).

#### Chlorophyll Content

The plants of T<sub>2</sub> had higher chlorophyll content than plants in other treatments (T<sub>0</sub>, T<sub>1</sub>, and T<sub>3</sub>). The lowest chlorophyll content was minimum in *R. solani* infected plants (T<sub>1</sub>) and the reduction found to be 31.5% over the control (T<sub>0</sub>). The presence of fungal inoculant in soil improved the chlorophyll content in plants infected by *R. solani* (T<sub>3</sub>) with an increase of 36.4% over T<sub>1</sub> (Table 3).

#### Carotenoids Content

The fungal inoculant increased carotenoids content to 90% over the control in T<sub>2</sub>. The carotenoids content was enhanced to 41.2% in infected plants due to the inoculation of PGPF inoculant (T<sub>3</sub>) (Table 3).

#### Anthocyanin Content

The results revealed that the anthocyanin content in infected plants (T<sub>1</sub>) was found to be minimum compared to all other treatments. Soil inoculation with the selected isolate Cc<sub>2</sub> had improved the anthocyanin content (22.7% in T<sub>2</sub> and 11.8% in T<sub>3</sub> increase over the control (T<sub>0</sub>) (Table 3).

#### Sugar Content

The plants infected by *R. solani* (T<sub>1</sub>) showed reduction in sugar level compared to all the other treatments. There was a slight increase in the T<sub>3</sub> which contain both the fungal inoculant and the pathogen *R. solani* in the soil (Table 3). Reduction in the level of sugar in the infected plants might be explained by reduction in Chlorophyll content which would have reduced the rate of photosynthesis.



### Protein Content

The infected plants (T<sub>1</sub>) had the minimum and the plants grown in soil inoculated with the fungal inoculant showed the maximum protein content the increase was very significant (300 % increase) over the control (Table 3).

### Proline Content

The results for proline content of plants in various treatments is presented in (Table 3). It is evident that proline accumulation was higher in plants grown in soil infested by *R. solani* (T<sub>1</sub>). The increase was found to be 96.2 % over the control (T<sub>0</sub>). The soil inoculant Cc<sub>2</sub> was reduced proline accumulation to 56.7 % though the plants were challenged with *Rhizoctonia solani* (T<sub>3</sub>). proline has been reported as biochemical indicates of resistance. The proline accumulation in *R. solani* infected plants in T<sub>1</sub> might be due to the induction of resistance against infection. Reduction of proline content in T<sub>3</sub> compared to T<sub>1</sub> might be due to minimization of the effect of *R. solani* by the added PGPF inoculant.

**Table 2. Development of pods and Seeds**

Treatment	Leaf area (cm <sup>2</sup> )	No. of pods / plant	Fresh weight of the pod (gm)	Dry weight of the pod (gm)
T0	6.5 ±0.5	4.66 ±0.57	0.29 ± 0.25	0.2 ± 0.11
T1	3.5 ±0.5	-	-	-
T2	20.5 ±0.5	8.33 ± 1.52	2.83 ± 0.25	1.2 ±0.61
T3	11 ± 1	5.66 ±0.57	2.05 ± 0.03	0.39 ± 0.25

**Table 3. Effect of Soil inoculation with PGPF inoculant (Cc2) on the selected biochemical parameters**

Treatment	Chlorophyll (mg/g) 663 & 645 nm	Carotenoid (mg/g) 480nm	Anthocyanin (mg/g) 525 nm	Protein (mg/g) 660 nm	Proline (mg/g) 520 nm	Phenol (mg/g) 650 nm	Soluble Sugars (mg/g) 625 nm
T0	0.85± 0.006	0.21 ±0.007	6.65 ± 0.35	76.89± 0.42	23.87± 1.08	22.1 ±0.31	35.94 ±0.03
T1	0.634± 0.199	0.175 ±0.003	5.3 ± 0.28	104.16	46.77± 0.41	24.08 ±0.13	32.57± 0.07
T2	1.515± 0.038	0.381 ±0.007	7.3 ± 0.45	205.34 ±0.43	13.84 ± 3.79	22.65± 0.13	39.41 ±0.01
T3	0.834 ±0.031	0.342 ±0.001	5.95± 0.07	153.26 ±0.63	29.83± 0.40	22.25± 0.09	37.83± 0.24

### Phenol Content

The plants not exposed to the soil inoculant and the pathogen had the minimum phenol accumulation. Plants infected by *R. solani* had maximum phenol content than all other plants. More accumulation of proline and phenol in infected plants (T<sub>1</sub>) might be due to induction of defense mechanisms in plants due to infection. Reduction in these parameters in T<sub>3</sub> might be due to suppression of pathogen by PGPF inoculants (Table 3).

### Production of hormones by the selected fungal inoculant (Cc<sub>2</sub>)

The ability of the selected isolate Cc<sub>2</sub> to produce growth hormones such as auxin and gibberellins in the culture broth was determined. The preliminary study made on the presence of hormones in different culture filtrate revealed that there was adequate production of hormones by selected fungal isolate were grown in Czapeckdox broth for 2 weeks which was reflected on the promotion of seedling growth (seedling height) in *Arachis hypogea* (L.). It was found that the isolate could produce Indole Acetic Acid (IAA) and Gibberellic Acid (GA) in the culture medium at the rate of 2.5mg/l and 4mg/l respectively. This ability could be attributed to the promotion of growth (vegetative and reproductive) in *Arachis hypogea* (L.). The GA produced by the fungus might be the reason for the enhancement of plant height which might be due to the promotion of longitudinal growth by GA. The growth promotion of rhizobacteria due to auxins and gibberellins had been already recorded (Basu and Ghosh, 2001, 2002; Roy and Basu, 2004 and Ahmed *et al.*, 2005). Gibberellins play an essential role in many aspects of plant growth and development mainly stems elongation and flower development. Greater stem growth in *Fuschia hybrida* and *Phasbitis nil* has also been reported (King *et al.*, 2000).

### Interaction between PGPF inoculant and *R. solani*

The interaction between Cc<sub>2</sub> and *R. solani* was studied by invitro studies by dual culture method. The result clearly revealed that Cc<sub>2</sub> could inhibit (or) suppress the growth of pathogen, *R. solani* very effectively. The antibiosis might be due to antagonistic activity against *R. solani*, production of inhibitory substances by Cc<sub>2</sub> are the direct hyphal interaction.

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