Journal of Biological Control, 28(3): 151-159, 2014

Research Article

Management of wilt of *Arachis hypogea* (groundnut) caused by *Fusarium oxysporum* with *Trichoderma* spp. and *Pseudomonas fluorescens*

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ABSTRACT: *Fusarium oxysporum* the soil borne pathogen causes *Fusarium wilt* in more than hundred plant species. The fungus causes severe yield loss in ground nut of plants. *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescens* inhibited the growth of the *Fusarium oxysporum* infecting *Arachis hypogaea.* The treated plants showed enhanced Chlorophyll, Carotenoid, Total Sugars, Reducing sugars, Non-reducing sugars, Proteins, Amino acids, Nucleic acids (DNA and RNA) contents. In the present study among the three biocontrol agents, *Pseudomonas fluorescens* (2%) was the most efficient in controlling the growth of *Fusarium oxysporum* and it significantly enhanced the levels of chlorophyll and carotenoid content. In other biochemical parameters, Total Sugars, Reducing sugars, Non-reducing sugars, Proteins, Amino acids, Nucleic acids (DNA and RNA) *Trichoderma viride* (1%) inhibited the growth of *Fusarium oxysporum* causing *Fusarium wilt* on *Arachis hypogaea*. The study strongly suggests that *Pseudomonas fluorescens* (2%) and *Trichoderma viride* (1%) can be exploited for the biological control of *Fusarium wilt* disease at field level.

KEY WORDS: *Arachis hypogaea, Fusarium oxysporum, Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens*

(Article chronicle: Received: 08-09-2014; Revised: 15-09-2014; Accepted: 23-09-2014;)

INTRODUCTION

Arachis hypogaea is an important oilseed crop in many tropical and subtropical areas of the world. *Fusarium* is a genus of harmful fungi that cause vascular diseases in plants such as watermelon, cucumber, tomato, pepper, muskmelon, bean, cotton and groundnut. Armstrong and Armstrong 1981; Gordan *et al.,* 1997; (Mayee, 2005; Rasheed *et al.,* 2004). It invades roots and cause wilt through colonization of xylem tissue (Tjamos and Beckman, 1989). It causes complete loss in grain yield, if the disease occurs in the vegetative and reproductive stages of the crop. (Halila and Stange, 1996; Navas *et al.,* 2000). Biological control provides an alternative to the use of synthetic pesticides with the advantages of greater public acceptance and reduced environmental impact. The use of microorganisms as biological control agents seeks to restore the beneficial balance of natural ecosystem which is often lost in the crop situation (Cutler and Cutler, 1999). *Trichoderma* spp. are among the most frequently isolated soil fungi and are present in plant root ecosystem (Harman *et al*., 2004). *Trichoderma* spp. are the effective antagonistic microorganisms in controlling *Fusarium wilt* (Nikam *et al.,* 2007). *Trichoderma* species have long been recognized as agents for the control of plant pathogenic fungi and have the ability of promoting plant growth (Samuels, 2006). *Pseudomonas fluorescens* can be isolated from the root rhizosphere as PGPR they colonize plant roots and stimulate plant growth or reduce the incidence of plant disease (Kloepper and Schroth, 1978).

Chlorophyll is the key pigment playing an important role in the process of photosynthesis. The photosynthetic pigments are responsible for absorbing solar energy for the process of photosynthesis in the host plants. Any change in the pigment content would be reflected immediately on the photosynthetic efficacy of the plant and subsequently on its growth and yield. Hence the analysis of pigment content in the healthy, infected and infected treated plants becomes essential. Agrios (1997) reported that due to the growth of the pathogen water supply in the plant's vascular tissue is

affected which induces the stomata to close, the leaves wilt and the plant eventually dies. Sugars are precursors for the synthesis of phenolics, phytoalexins, lignin and callose. Hence, they play an important role in defense mechanism of plants against invading pathogens Sugars play a major role in disease resistance by suppressing the pectinolytic and cellulotytic enzymes essential for pathogenesis (Horsfall and Diamond, 1957). Infection by pathogens interferes with host nucleic acids and protein metabolism especially enzymes. The present study deals with biocontrol activity of *Fusarium wilt* of *Arachis hypogaea* with the application of *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas fluorescens* by focusing on their effect on important plant biochemical parameters such as chlorophyll, carotenoids, sugars, protein, nucleic acids.

MATERIALS AND METHODS

Trichoderma viride, *Trichoderma harzianum and Pseudomonas fluorescens* were obtained from Institute of Microbial Technology (IMTECH), Chandigarh and were used for the present study. The pathogen *Fusarium oxysporum* was obtained from the infected leaves of *Arachis hypogaea* and was purified by single conidium isolation method. The purified culture was stored in the slants of potato sucrose agar (PSA).

Medium and Growth

Fusarium oxysporum was grown on PSA for 30 days and further grown in Czapek's medium for 7 days and filtrate was taken. *Trichoderma viride and T. harzianum* were grown on Malt Extract agar and *Pseudomonas fluorescens* on King's B medium in conical flask. The cultures were centrifuged at 5800 rpm for 10 min and culture filtrate was taken.

In Vivo Studies

Four plants of *A. hypogaea* raised from the seeds (JLR-variety) were grown in each of six earthen pots (25cm diameter) upto 75 DAS and grouped into three sets. First set of two pots-Control plants. These plants was sprayed with distilled water on 30 DAS and left without any treatment. Second set of two pots-Infected plants. These plants was sprayed with culture of pathogen, *Fusarium oxysporum* at 30 DAS and left without any treatment. Infected-treated –third set was sprayed with pathogen on 30 DAS. These infected plants were sprayed with culture filtrates of antagonistic microorganisms, *Trichoderma viride* (1%), *Trichoderma harzianum* (1.5%) and *Pseudomonas fluorescens* at 40 DAS.

At 50 DAS, the leaves of control, infected and infected treated plants were collected for the estimation of biochemical parameters such as Chlorophyll, Carotenoid, Total sugars, Reducing sugars, Non-reducing sugars, Protein, Amino acid, DNA and RNA.

Chlorophyll content

The method suggested by Inskeep and Bloom (1985) was used for the estimation of chlorophyll. In a test tube 10 ml of N, N - dimethylformamide (DMF) was taken and 50 mg leaf bits was placed in the test tube and stored for 24h at 4°C. Using the Spectrophotometer the absorbance of the colored supernatant was read at 647 nm and 666 with DMF as blank. The total chlorophyll content was calculated using the following formula:

$$\text{Total chlorophyll (mg g}^{-1}\text{ fw)} = \frac{(17.9\times A_{647}) + (8.08\times A_{666})}{1000\times w\times a} \times V$$

Where,
A - Absorbance at specific wavelength (nm).
w - Fresh weight of the sample (mg).
V - Volume of the sample (ml).
a - Length of the light path in the cell (1 cm).

Carotenoid content

The carotenoid content was determined using the method of Ikan (1969).Absorbance values for the leaf extracts were determined at 480,647 and 666 nm.

$$\text{Corrected 0.D} = A_{480} + (0.114 \times A_{666}) - (0.638 \times A_{647})$$

$$\text{Total carotenoids (mg g}^{-1}\text{ fw)} = \text{0.D.} \times 1 \div 100 \times 1 \div w$$

Total sugars

The total sugars were estimated by the method proposed by Dubois *et al.* (1956). Fresh leaves were oven dried (80°C, 48h) and powdered using a mortar and pestle. 50 mg dried leaf powder was boiled in a water bath for 10 min with 10 ml of 80% ethyl alcohol. The homogenate was first cooled and then centrifuged at 600 rpm for 15 min. The supernatant was saved and made up to 20 ml with 80% ethyl alcohol. This extract was used for quantitative estimation of carbohydrates.

Cold anthrone reagent (4ml) was added to **1** ml of ethanolic extract. This mixture was shaken vigorously and boiled for 10 min in a boiling water bath. After cooling in running tap water, the absorbance was read at 620 nm in Systronics Spectrophotometer. A **st**andard curve was prepared with known concentration of glucose.

Reducing sugars

The reducing sugars were estimated by the Nelson's modification of Somogyi's method Nelson (1944). To 1 ml of ethanolic extract, 1 ml of fresh copper reagent prepared by mixing copper tartarate and copper sulphate solution (25:1 v/v) was added. The mixture was heated for 20 min in a boiling water-bath and cooled. One ml of arsenomolybdate reagent was added and the contents incubated for 15 min. The solution was then diluted to 25 ml with distilled water and the color intensity was read at 500 nm in Spectrophotometer. The content of the reducing sugar was calculated using the standard graph for glucose.

Non-Reducing sugars

The amount of non-reducing sugars was determined by the formula suggested by Loomis and Shull (1937).

Non-reducing sugars = Total sugars - free reducing sugars × 0.95

Protein

Protein was estimated by the modified method of Lowry Furlong e*t al.* (1973).

Extraction of Protein

2 g of the lamina was weighed. To this 10 ml of cold 10% Trichloro Acetic Acid (TCA) was added in ice. This was centrifuged at 2500 rpm for 10 min. The supernatant was discarded. The above step was repeated twice with 5 ml of 10% TCA followed by centrifugation. The pellet was washed twice with 5 ml of absolute ethanol and centrifuged again. To the precipitate, 2.5 ml of 10% TCA was then added, mixed and again centrifuged at 2500 rpm for 10 min. The supernatant was stored. To the precipitate, 5 ml of 5% TCA was added, mixed and heated in a boiling water-bath for 15 min. The tubes were cooled and centrifuged at 3000 rpm for 15 min. This step was repeated and supernatant obtained each time was pooled with the first one. The residual pellet was dissolved in 5ml of 0.1N NaOH and centrifuged at 3000g for 10min. The supernatant was used for protein estimation.

To 0.5 ml of the protein extract, 2.5 ml of reagent C was added and the mixture was incubated for 5-10 min in dark and then 0.25 ml of reagent D was added. The mixture was incubated for another 20-30 min. The color developed was read at a wavelength of 610 nm. The protein standard was estimated by using BSA as a stock solution. Protein content was expressed in mg of protein/g of fresh leaf tissue.

Amino Acid

The amino acid content was estimated by the method of Moore and Stein (1954). To 0.1 ml of alcoholic extract one ml of freshly prepared ninhydrin solution was added. To this, 0.9 ml of distilled water was added .It was heated in a boiling water bath for 20 min and cooled under running tap water. Five ml of diluant solution was added to the above mixture and kept for 15 min. The color developed was read at 570 nm in spectrophotometer against a reagent blank. The amino acid contents of the sample were determined with the help of a standard curve prepared for glycine.

Nucleic acids

Extraction Method Schneider (1945)

2 g of the lamina was weighed. To this 10 ml of cold 10% Trichloro Acetic Acid (TCA) was added in ice. This was centrifuged at 2500 rpm for 10 min. The supernatant was discarded. The above step was repeated twice with 5 ml of 10% TCA followed by centrifugation. The pellet was washed twice with 5 ml of absolute ethanol and centrifuged again. To the precipitate, 2.5 ml of 10% TCA was then added, mixed and again centrifuged at 2500 rpm for 10 min. The supernatant was stored. To the precipitate, 5 ml of 5% TCA was added, mixed and heated in a boiling water-bath for 15 min. The tubes were cooled and centrifuged at 3000 rpm for 15 min. This step was repeated and supernatant obtained each time was pooled with the first one. The supernatant containing nucleic acids was used for the estimation of DNA and RNA.

DNA

Method of Burton (1956) was used for the DNA estimation. To 1.5 ml of TCA extract, 3 ml of diphenylamine reagent was added. The tubes were kept in a water bath and maintained at 70°C for 20 min and then cooled. The colour development was read at 600 nm on a Spectrophotometer. A Standard calibration curve was prepared by using known concentration of calf thymus DNA. The DNA content was expressed in mg DNA/g fresh weight of the leaf tissue.

RNA

RNA was estimated by the method of Rawal *et al.* (1977). To the 0.5 ml nucleic acid fractions, 3 ml of orcinol reagent was added and heated on a water bath for 20 min at 90°C and then cooled. The color development was read at 665 nm. A standard calibration curve was prepared by using known concentration of purified RNA. The RNA content was expressed in mg RNA/ g fresh weight of the leaf tissue.

RESULTS AND DISCUSSION

It was observed that control plants were healthy (Fig. 1A) while those that were inoculated with culture filtrate of *Fusarium oxysporum* showed infection at 30 DAS (Fig. 1B) and the leaves became shrunken and wilted by 55 DAS (Fig. 1C). The infection was controlled to the maximum in the infected plants which were sprayed with culture filtrate of antagonistic microorganisms, *viz. Trichoderma viride* (1%) (Fig. 2A)*, Trichoderma harzianum* (1.5%) (Fig. 2B), and *Pseudomonas fluorescens* (2%) (Fig. 2C).

Table 1. Total Chlorophyll and Carotenoid content

Treatments	Concentration (%)	Total chlorophyll content (mg/g.f.w.)	Carotenoid content (mg/g.f.w)
Control Plants	-	5.34±0.19	0.16±0.01
Plants infected with *Fusarium oxysporum*	-	1.39 ± 0.11[a]	0.05 ± 0.003[a]
Infected plants treated with *T. viride*	1	3.29 ± 0.15[a]	0.11 ±0.005[a]
Infected plants treated with *T. harzianum*	1.5	2.17 ± 0.13[a]	0.10 ±0.004[a]
Infected plants treated with *P. fluorescens*	2	3.69 ± 0.17[a]	0.12±0.006[a]

[a]p< 0.001 as compared to control. The values within a column followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n = 3).

The results obtained revealed that maximum amount of total chlorophyll was noticed in the leaves of control plants (5.34 mg/g). Low chlorophyll content was noticed in the plants infected with *F. oxysporum* (1.39 mg/g). *P. fluorescens* - sprayed plants showed the highest values among the treatments *(*3.69 mg/g). This was followed by that of *T. viride* (3.29 mg/g) and *T. harzianum* (2.17 mg/g). The carotenoid content was found to be higher in control plants (0.16 mg/g). Low carotenoid content was recorded in *F. oxysporum* infected plants (0.05 mg/g). Among the treatments the plants sprayed with *P. fluorescens* culture filtrate (0.12 mg/g), showed highest carotenoid content, followed by those sprayed with *T. viride* (0.11 mg/g), and *T. harzianum* (0.10 mg/g) (Table 1).

Maximum reduction in the total chlorophyll content (73.97%) and carotenoid content (68.75%) were recorded in *F. oxysporum* infected plants compared to that of control ones. Among the treatments, minimum reduction in the total chlorophyll and carotenoid (30.89, 25%) was seen in *P. fluorescens*-sprayed plants. The next least reduction in the total chlorophyll and carotenoid was recorded in the *T. viride* sprayed plants (38.38%, 31.25%) followed by *T. harzianum* - sprayed plants (59.36%, 37.50%).

Table 2. Total sugar, reducing sugar and non-reducing sugarcontent

Treatments	Total sugar (mg/g.f.w)	Reducing sugar (mg/g.f.w)	Non-reducing sugar (mg/g.f.w)
Control plants	22.05 ± 0.26	12.56 ± 0.18	9.01 ± 0.09
Plants infected with F. *oxysporum*	10.72 ± 0.12[a]	6.00 ± 0.11[a]	4.46 ± 0.005[a]
Infected plants treated with *T. viride*	16.68 ± 0.18[a]	9.60 ± 0.17[a]	6.72 ± 0.034[a]
Infected plants treated with *T. harzianum*	14.29 ± 0.15[a]	8.38 ± 0.14[a]	5.60 ± 0.032[a]
Infected plants treated with *P. fluorescens*.	13.09 ± 014[a]	7.19 ± 0.12[a]	5.60 ± 0.032[a]

[a]p< 0.001 as compared to control. The values within a column followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n = 3)
ap< 0.001 as compared to control.

The leaves of the control plants recorded highest amount of total sugar (22.05mg/g). The lowest amount was noticed in the infected plants (10.72 mg/g). Among the treatments higher quantity of total sugar was noticed in the plants sprayed with *T. viride* (16.68 mg/g) followed by those sprayed with *T. harzianum* (14.29 mg/g) and *P. fluorescens* (13.09 mg/g). The leaves of control plants recorded highest amount of reducing sugar (12.56 mg/g). The lowest amount was recorded in the infected plants (6.00 mg/g). Among the treatments highest quantity of reducing sugar was recorded in the plants sprayed with sprayed with *T. viride* (9.60 mg/g), followed by those sprayed with *T. harzianum* (8.38 mg/g) and *P. fluorescens* (7.19 mg/g). Maximum amount of non-reducing sugar was found in the leaves of control plants (9.01mg/g). The lowest amount was observed in the infected plants (4.46 mg/g). Among the treatments higher quantity of non-reducing sugar was observed in the plants sprayed with *T. viride* (6.72 mg/g) followed by those sprayed with *T. harzianum* (5.60 mg/g) and *P. fluorescens* (5.60 mg/g). (Table 2).

The leaves of infected plants showed the highest reduction in the total sugar by 51.38%; reducing sugar by 52.22%; non-reducing sugar by 50.49% compared to control plants. The least reduction was noticed in *T. viride* - (1%) sprayed plants (24.35%, 23.56%, 25.41%) followed by *T. harzianum* - sprayed plants (35.19%, 33.28%, 37.84%), and *P. fluorescens* - sprayed plants (40.63%, 42.75%, 37.84%).

Table 3. Protein and amino acid content

Treatments	Protein (mg/g.f.w)	Aminoacid (mg/g.d.w)
Control plants	24.90 ± 0.70	18.42 ± 0.90
Plants infected with F. *oxysporum*	9.43 ± 0.34[a]	9.01 ± 0.34[b]
Infected plants treated with *T. viride*	19.21 ± 0.51[a]	16.66 ± 0.89
Infected plants treated with *T. harzianum*	14.91 ± 0.45[a]	15.88 ± 0.59[a]
Infected plants treated with *P. fluorescens.*	11.80 ± 0.39[a]	13.52 ± 0.58[b]

[a]p< 0.001 as compared to control [a]p< 0.01 [b]p<0.001 as compared to control. The values within a column followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n = 3).

The leaves of the control plants recorded highest protein content (24.90 mg/g) and the lowest protein content was recorded in the infected leaves (9.43 mg/g) which was (62.12%) less than that of control ones. Among the treatments the plants sprayed with *T. viride* were found to show the higher quantity of protein content (19.21mg/g), followed by those sprayed with *T. harzianum* (14.91 mg/g) and *P. fluorescens* (11.80 mg/g) The leaves of control plants recorded highest amino acid content (18.42 mg/g) and the lowest amino acid content was recorded in the infected leaves (9.01 mg/g). Among the treatments the plants sprayed with *T. viride* were found to show the highest amino acid content (16.66 mg/g), followed by those sprayed with *T. harzianum* (15.88 mg/g) and *P. fluorescens* (13.52 mg/g) (Table 3).

The protein content (62.12%), amino acid content - (51.08%) was reduced heavily in *Fusarium oxysporum* infected plants compared to those of control plants. The least reduction of protein and amino acid was found (22.85%, 9.88%) in the T. *viride* - sprayed plants followed by *T. harzianum* - sprayed plants (40.12%, 13.78%) and *P. fluorescens* - sprayed plants (54.58%, 26.60%).

Table 4. DNA and RNA content

Treatments	DNA (mg/g.f.w)	RNA (mg/g.f.w)
Control plants	28.51± 0.91	37.34 ± 0.92
Plants infected with *F. oxysporum*	12.44 ± 0.35[a]	11.64 ± 0.34[a]
Infected plants treated with *T. viride*	21.48 ± 0.69[a]	22.84 ± 0.54[a]
Infected plants treated with *T. harzianum*	18.71 ± 0.67[a]	19.13 ± 0.45[a]
Infected plants treated with *P. fluorescens*	17.46 ± 0.60[a]	18.57 ± 0.43[a]

[a]p<0.001 as compared to control. The values within a column followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n = 3).

The leaves of control plants recorded highest DNA content (28.51 mg/g). The lowest amount was noticed in the infected plants (12.44 mg/g) which was (56.36%) less than that of control ones. Among the treatments the higher amount of DNA was noticed in the plants sprayed with *T. viride* (21.48 mg/g), followed by those sprayed with *T. harzianum* (18.71mg/g) and *P. fluorescens* (17.46 mg/g). The leaves of control plants recorded the highest RNA content (37.34 mg/g). The lowest amount was recorded in the infected plants (11.64 mg/g) which was (68.82%) less than that of control ones. Among the treatments the higher amount of RNA was recorded in the plants sprayed with *T. viride* (22.84 mg/g), followed by those sprayed with *T. harzianum* (19.13mg/g) and *P. fluorescens* (18.57mg/g) (Table 4).

Maximum reduction in the nucleic acid contents (DNA-56.36%), (RNA-68.82%) was noticed in the *Fusarium oxysporum* infected plants compared to those of control plants. Of all the treatments, that of *T. viride* - sprayed plants (1%) showed least reduction (24.65%) in DNA and in RNA content (38.83%) followed by *T. harzianum* - sprayed plants (34.37%, 48.76%) and *P. fluorescens* - sprayed plants (38.75%, 50.26%).

Singh and Joshi (1997) reported the reduction of photosynthetic activity due to less chlorophyll content in the groundnut and consequently leading to death of the plant. Agrios (1997) reported that due to the growth of the pathogen *Fusarium oxysporum* water supply in the plant's vascular tissue is affected which induces the stomata to close, the leaves wilt and the plant eventually dies. The plants treated with *Pseudomonas fluorescens* showed inhibition of the pathogen by increasing the levels of chlorophyll content in treated plants. *P. fluorescens* produces the broad spectrum antibiotic 2,4 diacetyl phloroglucinol Keel *et al*., (1996) that inhibits mycelial growth of *Fusarium oxysporum* Schouten *et al.* (2004). Nagabhushana (2006) observed that the highest decrease in total sugars was recorded in wilt susceptible GS-1 genotype inoculated with the pathogens *F. udum* and *Heterodera cajani* over healthy pigeon pea plants. Sindhan and Parashar (1996) reported that polyphenol content was increased due to infection by early and late leaf spot pathogens of groundnut and the concentration of total sugars, reducing sugars, and non-reducing sugars was decreased. Due to infection by seed borne fungi, the concentration of total sugars, reducing sugars, non-reducing sugars and proteins were decreased in cowpea seeds (Ushamalini *et al*., 1998). The least reduction in the bioagents-sprayed leaves may be due to the ability of the plants to utilize the carbohydrates for the biosynthesis of phenolic compounds which help in induced

systemic resistance. This finding substantiates the works of Neish (1964) and Rajavel (2000) in *Pseudomonas*- and *Trichoderma*-treated *Capsicum* fruits infected with *Colletotrichum capsici*. *Trichoderma* spp. are considered as potential biocontrol and growth promoting agents for many crop plants. It can easily colonize in plant rhizosphere and help to promote the plant growth (Verma *et al*., 2007; Harman *et al*., 2004). Vinale *et al.* (2008) suggested that the secondary metabolites such as auxin - like compounds or auxin inducing substances by *Trichoderma* plant interaction might be a reason for the improved growth. Chakrabarti and Basuchaudhary (1979) found that the pathogen secretes protease which is involved in the breakdown of protein leading to the maximum reduction in the protein and amino acid content in safflower wilt caused by *Fusarium* sp. Decrease in protein content by fungal proteolytic enzymes might be due to hydrolysis of protein by fungal proteolytic enzymes (Jamaluddin *et al*., 1977). Charitha Devi and Radha (2012) found that in *Cucurbita pepo* leaves infected with Cucumber Mosaic Virus (CMV) the concentration of DNA was decreased. Asha and Kannabiran (2001a) observed that the DNA was decreased in the chilli leaves infected with *C. capsici*. This is due to the fact that the pathogen's enzyme interfered with the cell division process thereby reducing the rate of cell division. Siddharamaiah *et al*. (1979) observed that DNA content was reduced in groundnut leaves due to infection resistant isolate of *Puccinia arachidis*. Badar and Qureshi (2012) established that *Trichoderma hamatum* in combination with *Rhizobium* spp. was found significantly effective in increasing the amount of protein content of *Vigna mungo* seeds.

Though inhibition of *F. oxysporum* was effected by the spray with culture filtrates of three antagonistic microorganisms, the maximum inhibitory concentration of culture filtrate of individual microorganism varies from one another. This might be due to the nature and concentration of antifungal compounds of the concerned species. The higher quantity of chlorophyll and carotenoid contents was found in the infected plants treated with *Pseudomonas fluorescens*. Among the treatments *Trichoderma viride* sprayed plants showed highest value in Total sugar, reducing sugar, non-reducing sugar, protein, Amino acids, DNA and RNA content than the plants infected with *Fusarium oxysporum*.

All these biocontrol agents *(T. viride, T. harzianum* and *P. fluorescens)* are effective in the control of *Fusarium wilt* of groundnut with slight variations in each biochemical parameters. In the present study, *P. fluorescens* proved to be an effective biocontrol agent by enhancing the chlorophyll and carotenoid biochemical parameters. It also increased other biochemical parameters such as sugars, protein, amino acid, Nucleic acids, Pectinolytic, Cellulolytic, Antioxidant enzymes, and Phenols.

Fig. 1A. Control - Plants - 45th day

Fig. 1B. Plants infected with pathogen - 45th day

Fig. 1C. Plants infected with pathogen-55th day

Fig 1. Effect of the pathogen, *Fusarium oxysporum* on the growth and morphology of *Arachis hypogaea*

Fig. 2A. Treatment with *Trichoderma viride* - (1%)

Fig. 2B. Treatment with *Trichoderma harzianum* - (1.5%)

Fig. 2C. Treatment with *Pseudomonas fluorescens* - (2%)

Fig. 2. Effect of culture filtrate of biocontrol agents on the growth and morphology of *Arachis hypogaea* infected with *Fusarium oxysporum*

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