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Biocontrol potential of *Trichoderma viride, Pseudomonas fluorescens* for *Fusarium* wilt of cowpea (*Vigna unguiculata* L.)

Ramasamy, P. and Sundaram, L.[*]

Department of Botany, School of Life Sciences, Periyar University, Salem. Tamil Nadu, India.

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Abstract *Trichoderma viride (*MH333256), *Pseudomonas fluorescens* (Ps01), *Fusarium oxysporum* (Fu04) were morphological identified and biochemical characterization was determined under compound microscope. All isolates were phylogenetic confirmed by genetic DNA isolation and PCR analysis. The healthy seeds of Cowpea (*Vigna unguiculata* L.) were used in experiment. Results described in treatments of control, Ps01, Tr02, Fu04, Ps01+ Fu04, Tr02+ Fu04, Ps01+ Fu04+Tr02, after inoculation to the seeds sown in the pots. In pot experiment, the bio-inoculated plant (Tr02) significantly enhanced the plant biomass, chlorophyll, nitrogen, NPK content and soil microbial population after 45 DAI. Bioinoculant (Tr02, Ps01) highly inhibited plant pathogen (Fu04) and increased in plant fresh weight (13.4 g) when compared to the control (9 g) and Fu04 (6 g).

Keywords: *Trichoderma viride, Pseudomonas fluorescens, Fusarium oxysporum,* Siderophore, Phosphate solublization

Introduction

Soil-borne pathogens cause the diseases leading to low quality and yield of the crops. Fungi is the most important soil-borne pathogens that damaged the several major crops. *Pythium, Botrytis, Rhizoctonia* and *Fusarium* are found to be the main pathogenic fungi (Djonovic *et al*., 2007). The rhizosphere is related to a diverse range of microorganisms, especially bacteria that colonizing the roots are called rhizobacteria (Munees Ahemad and Mulugeta Kibret, 2014). Rhizobacteria is free-living soil bacteria named plant growth promoting rhizobacteria (PGPR) that reported to control soil-borne pathogens and enhanced the plant growth regulators, induced root exudation and enhanced availability of plant nutrients (Akhtar *et al*., 2012). *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthobacter, Burkholderia, Bacillus, Gluconoacetobacter, Paenibacillus, Enterobacteria, Saccharomyces, Actinobacteria, Trichoderma* and *Serratia* including to be PGPR that reported

[*] **Corresponding Author:** Sundaram, L.**; Email:** pavirambot@gmail.com

to promote plant growth. Phosphate solubilizing microorganisms provided insoluble phosphate to plants after added or existed in the soil through acidification, chelation and iron- exchange reactions (Coutinho *et al*., 2012). *Trichoderma* spp. are important biological agents to control plant pathogens, and many seed-borne fungi viz. *Fusarium moniliforme, Fusarium oxyysporum, Rhizoctonia solani, Fusarium solani, Botryodiplodia theobromae* and *Alternaria alternata* in dual culture assay. *Trichoderma* spp. reported to produce 6-peptyl pyrone (6-pp) 'coconut aroma' which is volatile compound that can be played a role of biocontrol perspective (Vinale, *et al*., 2008). *Pseudomonas fluorescens* produced amylase for biofilm removal and degradation of extracellular polymeric substances (Phyllis Molobela, *et al*., 2010).*Trichoderma* spp, *Pseudomonas fluorescens*, *Bacillus subtilis* are reported to be the most important biocontrol agents against several plant pathogens under a variety of environmental conditions (Chet, 1987). Recently, the combination of biocontrol agents reported to decrease disease incidence in many plants (Latha *et al*., 2011). A synergistic interaction between strains of *Trichoderma* spp. and antagonistic bacteria such as *Pseudomonas* spp. has been positive reported for their combination to control plant pathogens (Whipps, 1997). The mechanisms of PGPR to promote the plant growth are N2 fixation, solubilization of mineral phosphates and other nutrients, ability to produce phytohormones, vitamins, enzyme, siderophore as well as against plant pathogens (Ahmad *et al*., 2008). The objective was to evaluate the potential biological control of *Trichoderma viride, Pseudomonas fluorescens* against *Fusarium* wilt of cowpea (*Vigna unguiculata* L.).

Materials and Method

Isolation of soil microbes

The experiment was conducted at Botanical Garden, Department of Botany, Periyar University, Salem, India. Soil sample was collected at Salem district, Eastern Ghats, Tamil Nadu, India and brought to the laboratory in sterile polyethylene bags for isolation bacteria by serial dilution technique.

Biochemical tests

All bacterial isolates were tested by gram reaction. Culture was smeared on clean slide and flooded the smear with Gram's iodine for 2 min, decolorized the stain using ethyl alcohol (95%), then few drops of safranine for 2-4 min, washed out with tap water and mounted in glycerin (Cappuccino and

Sherman, 2002). Indole testing was done by 2 ml of peptone solution added to 5 ml of bacterial culture and incubated for 48 hrs. A 0.5 ml of Kovac's reagent was added, shacked well and examined after 1 minute. A presence of red colour in the reagent layer indicated indole activity. If there is no formation of red colour that signed as negative indole (Morello *et al*., 2002). Methyl red (MR) and Voges-Proskauer (VP) test was done as follows:- MRVP broth was added 5 ml bacterial suspension and incubated at 28° C for 48 hrs, 5 drops of MR indicator added to each test tube which including the control, and observed the colour change, followed by ten drops of VP-I and 2-3 drops of VP-II reagent. The colour change was observed and compared with control (Morello *et al*., 2002). Citrate utilization test was done by culturing the bacterial strain in test tube containing Simon's citrate medium and incubated for 24-72 hours, then incubated and observed the deep blue colour that indicated as positive result (Ruchhoft *et al.,* 1931). Catalase activity was done by transferring the bacterial strain to yeast extract mannitol agar slant and incubated at 28°C for 3 days, then added 1 ml of 1% hydrogen peroxide, and observed for catalase activity. (Taylor and Achanzar, 1972). Oxidase production was done inoculated bacterial strain in yeast extract mannitol agar slant and incubated at 28°C for 3 days, then placed on oxidase disc (N,N-Tertra methyl- para-phenylediamine dihydrochloriode). Development of blue or purple coloour indicated a positive for oxidase production (Gaby and Hadley, 1957). Urease activity was done by inoculating the bacterial strain in Christensen's urea agar slants and incubated for at 28°C for 3 days, then observed the formation of colour at 6 hrs, and every 24 hrs until 6 days. The positive result indicated a bright pink colour (Mac Faddin, 2000). Phosphate solubilization test was done by inoculating the bacterial strain to Pikovsakaya's agar medium, incubated at 28±2 ℃ for 24 hours. The presence of clearing zones around the bacterial colonials indicated positive for phosphate solubilization (Chen *et al*., 2006). Protein estimation was done by estimating total cell protein following the procedure of Drews (1965). Fe Cl_3 test was done using 0.5 ml of 2% aqueous ferric chloride solution. The appearance of reddish brown /orange color indicated a positive for siderophore production.

Microbial siderophore production was done by qualitative and quantitative methods. Qualitative detection of siderophore (plate assay) was done by qualitative detection of siderophore plate assay followed the method of Schwyn and Neilands (1987). The qualitative assay chrome azurol sulfonate (CAS) agar was used. The cultures were inoculated on blue agar and incubated at 37▫C for 24-48 hours. Observation was based on the color change due to transfer ferric iron from blue complex to siderophore. The sizes of yellow-orange haloes was measured around the growth that indicated total siderophores

activity. Quantitative spectrophotometric assay for siderophore production (liquid assay) was done by the bacterial culture grown in a minimal medium (T medium) at 37▫C for 24 hours under static and shaking conditions (100 rpm) at 37▫C for 24 hours. The cells were removed by centrifugation at 3000 rpm for 15 min. A 0.5 ml of the culture supernatant was added with 0.5 ml CAS solution, and 10 µl shuttling solution (sulfosalicyclic acid). The formation was observed for color change after 20 min, then determined using the spectrophotometer at 630 nm. The minimal medium and reference solution (minimal medium +CAS dye+ shuttle solution) were used during the determination.

Isolation of Trichoderma viride

Trichoderma spp. was isolated from soil sample collected in Salem district, Eastern Ghats, Tamil Nadu, India by pour plate techniques (Johnson and Curl 1972), the fungal colonies were transferred to PDA medium and pure culture maintained in PDA slants for further studies.

Isolation and identification of Fusarium oxysporum

The fungal pathogen, *Fusarium oxysporum* causing wilt and rot diseases in cowpea was isolated from naturally infected cucumber plants using standard isolation techniques (Riker and Riker, 1936). The infected plant materials were collected and brought to the laboratory. The sterilized bits were then placed in sterile petri dishes containing oatmeal agar medium, and incubated at 28 ^{0}C. Hyphal tip was transferred to Potato sucrose Agar (PSA) and Potato dextrose agar (PDA) slants and pure cultures were maintained for further studies (Riker and Riker, 1936). The pathogen associated with cowpea wilt and rot diseases was identified as cultural and morphological characters. Cultural characters of pathogen such as rate of growth, growth pattern etc. in the potato dextrose media were studied. Morphological characters of the pathogen like length of sporangia, L/B ratio, stalk length etc. were studied by slide culture technique using lactophenol cotton blue staining.

DNA isolation and 16s rRNA amplification

The isolates was inoculated in 50 ml of culture medium and incubated until it reached the 600nm. The cells were harvested by centrifuging at 1250rpm at ambient temperature. The cell pellet resuspend in 467 µl TE buffer and further incubated with 33 µl of lysing buffer (30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K) for 1 hrs at 37°C. The lysate extracted with an equal

volume of 25:24:1 phenol: chloroform: isoamyl alcohol. The aqueous phase transferred to a 1.5 micro centrifuge tube and added with 1/10 volume of 3M sodium acetate and incubated at -20 ℃ for 30 min. after the incubation 0.6 volumes of isopropanol added and mixed gently and centrifuged at 14000rpm for 20 min at 4 ℃. DNA pellet washed with 1 ml of 70 % ethanol twice by spinning at 14000rpm for 20 min at 4℃. The DNA pellet was resuspended in 100 µl TE buffer and stored at 4℃, overnight. The quality and quantity of the DNA was estimated by OD ratio of 260/280,260/230 nm through 8 port nanodrop (Thermo Fisher Scientifics, USA) and 1% agarose gel respectively. The 16S rRNA gene sequence of bacteria was amplified with the extracted DNA using universal primers 8 F 5'-AGA GTT TGA TCC TGG CTC A and 1492 R 5'-GGT TAC CTT GTT ACG ACG ACT T (Edwards *et al*., 1989). The PCR reaction mix consisted 50 mM KCl 10 mM Tris,1.5 mM MgCl2, 0.2 mM of each deoxynucleoside triphosphate, 1 µM of each primer, 50 ng of extracted DNA and 1 U of Taq polymerase (NEB,UK) in a volume of 50 µl. amplification was carried out in a thermal cycler (gene Amp 2700, Applied biosystems, USA) using following temperature program: initial denaturation at 94℃ for 5 min, 30 cycles of 94℃for 30 s, 55℃ for 30 s and 72℃ for 1.5 min, and final extension of 5 mg/l ethidium bromide and the amplicons compared with standard 1kb marker ladder (NEB, UK). Sequencing were carried out with same universal primers on ABI 3100 automated DNA sequence by standard BigDye® Terminator v3.1 Cycle Sequencing Kit (Life technologies, USA) cyclic amplification method at PAR life sciences and Research Pvt Ltd, Trichy.

Antagonistic characteristics of Trichoderma against pathogenic fungi in dual culture plate technique

The antagonistic effect of *Trichoderma* against *Fusarium oxysporum* was tested by dual culture plate technique outlined by Skidmore and Dickinson (1976). Plant pathogen from 7 days old culture grown on PDA was transferred aseptically. The petri dishes containing PDA and placed at 28 ± 2^{o}C for 24 hours, then *Trichoderma* isolates were trnsferred in the same petri dishes, away from the pathogen and incubated for 120 hours. Three replications were done in each isolate. Pathogen grew only in PDA served as control. Data collected as growth rate.

Antagonistic activity of Pseudomonas against Fusarium oxysporum

Pseudomonas isolates against *Fusarium oxysporum* was tested by dual culture follwed the method of Skidmore and Dickinson (1976). The selected

Pseudomonas from each location were used to compare with commercial culture *Pseudomonas*. The 7 days old culture of plant grown on PDA was transferred to PDA and incubated at 28 ± 2oC for 24 hours. Then, *Pseudomonas* was moved to the same petri dish, apart way from pathogen, incubated for 5 days. Three replicated experiment were done in each isolate. Pathogen grew only PDA served as control. Datacwere collected as growth rate.

Antibiotic susceptibility test

The isolates were cultured for 68 hours at 28℃ and suspended with sterile saline approximately 10^7 cells/ml were transfered King's B agar slant, and suspended in sterile saline. Antibiotiic test was used Streptomycin, Chloromphencol and Chloromphencol applied and incubated at 28℃. Then, observation for resistant, intermediate or sensitive to antibiotics according to the work of Baure *et al*. (1966).

Greenhouse experiment

The experiment was done pots to evaluate the potential of *Pseudomonas* and *Trichoderma* spp. against *F. oxysporum.* Potting mixture was a red soil: sand at the ration of 3:1 w/w, autoclaved for 1 hour for 3 alternative days and. Seeds of cow pea were surface disinfected with $HgCl_2$ 0.05 % for 20 seconds and washed. *Trichoderma* and *Fusarium*) were mixed in soil of 100 g mixture (10 g *Trichoderma* formulation, 10 g *Fusarium* and 80 g soil) before sowing seeds for 2 days. *Pseudomonas* was applied as seed treatment by soaking seeds in suspension. Treatments were *Pseudomonas* (Ps01), *Trichoderma* (Tr02), *Fusarium* (Fuo4), Ps01+ Fuo4, Tr02+ Fuo4, Ps01+ Fuo4+ Tr02. Data were collected as growth parameters at 45 DAI and subjected to analysis of variance. Chlorophyll content and microbial population were determined according to the work of Rajagopalan and Raju (1972).

The photosynthetic pigments (mg/g) was estimated as chlorophyll a and b contents according to the method of Arnon (1949) and carotenoids content was determined according to the method of Kirk and Allen (1965). Chlorophyll content calculated was used the formula of Arnon (1949). The total nitrogen content was done using micro-Kjeldahl method (Umbriet *et al.,* 1972). The catalyst was prepared according to Humphries (1956). Soil pH and electrical conductivity and Soil NPK were done NPK as described by Subbiah and Asija 1956. Soil phosphorus was estimated by the method by Olsen *et al.,* 1954. Potassium content was estimated by the method of Jackson, 1973.

Results

Isolation of soil microbes

The fungal pathogen isolated from cucumber named *F. oxysporum* Fu04 (Figure 1-Plate 3). *P. fluorescens* was isolated and identified morphological characters under compound microscope (Figure 1-Plate 1). *T. viride* and *F. oxysporum* were morphological identified under compound microscope (Figure1-Plate 3, Table 1).

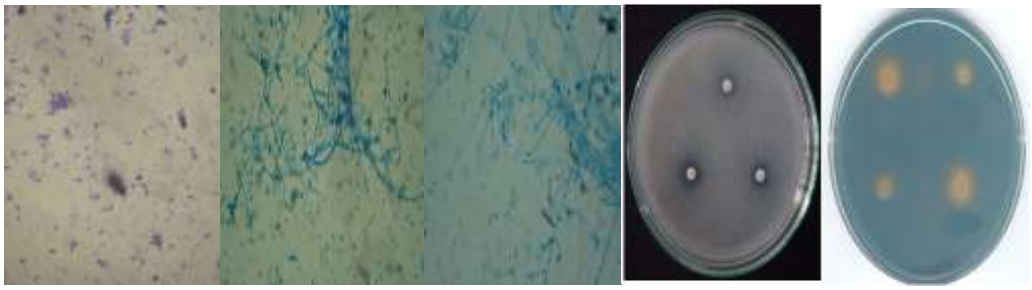

Plate 1 **Plate 2** **Plate 3** **Plate 4** **Plate 5**

Figure 1. Morphological characters under compound microscope and phosphate solubilization test, Siderophore test: Plate 1= *Pseudomonas* (10x × 40x); Plate 2= *Trichoderma*- Spore (10x ×40x); Plate 3= *Fusarium*- Spore (10x × 40x); Plate 4= phosphate solubilization test; Plate 5= Siderophore test

Table 1. Macroscopic features of fungal isolates

S.no	Colony color on PDA	Colony reverse color on PDA	Growth pattern	Identified fungal isolates
1	Dark Green color	Yellowish green color	Fast growing: wooly becoming compact in time	*Trichoderma* spp.
2	White Color	Pink color	Fast growing: cottony aerial mycelium	*Fusarium* spp.

Biochemical characterization of Pseudomonas isolate

Ps01 was gram negative, rod, motile and maximum growth at 37^0C and identified as *Pseudomonas fluorescens* (Table 2). The citrate test was reported the utilize citrate as carbon and energy source which resulted to be citrate positive (Table 2). To conducted and check the potential of bacterial isolates to produce indole test. Ps01 proved to be an indole positive that confirmed as *Pseudomonas* (Table 2). *Pseudomonas fluorescens* was methyl red positive and

showed the extreme acidity in acid fermentation and resulted the methyl red was positive (Table 2).

Table 2. Biochemical characterization of *Pseudomonas* spp.

S.no	Test	Ps01
1	Gram staning	Gram negative
	Shape	Rod
2	Motility	+
3	**IMViC test**	
	Indole production	+
	Methyl red test	+
	Voges –Proskauer	+
	Citrate utilization	+
4	Acid production test	+
	Extra –cellular enzymes	
5	Catalase activity	+
6	Oxidase production	+
7	Nitrate reductase activity	-
8	Urease activity	+
9	Phosphate solution	+
10	Siderophore production	+
	$FeCl_3$ test	+

(+) positive, (-)negative

Extra cellular enzyme activity of Pseudomonas fluorescens

Ps01 was immediately turned to dark purple which indicated oxidase positive. The catalase in (Ps01) was proved to produce the bubbles due to the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen (Table 2). The bacteria had ability to degrade urea into ammonia and carbon dioxide. It produced pink colour that indicated asurease positive (Table 2). Phosphate solubilizing ability was shown in pikovskaya agar. *Pseudomonas* (Ps01) isolate expressed the clear zone (Figure 1-Plate 4)**.** Siderophore was produced in CAS agar showing the yellow-orange haloes. Ps01 was conformed to produce hydroxamate producing siderophores (Figure 1-Plate 5). Ps01 and Tr02 were confirmed species by DNA sequencing and PCR analysis (Figure 2 and 3).

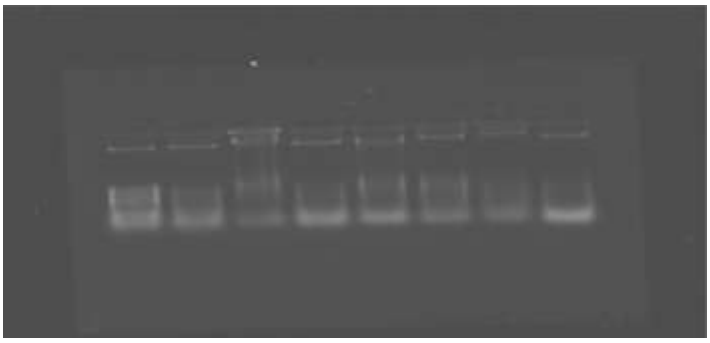

Figure 2. *Pseudomonas fluorescens* (PCR)

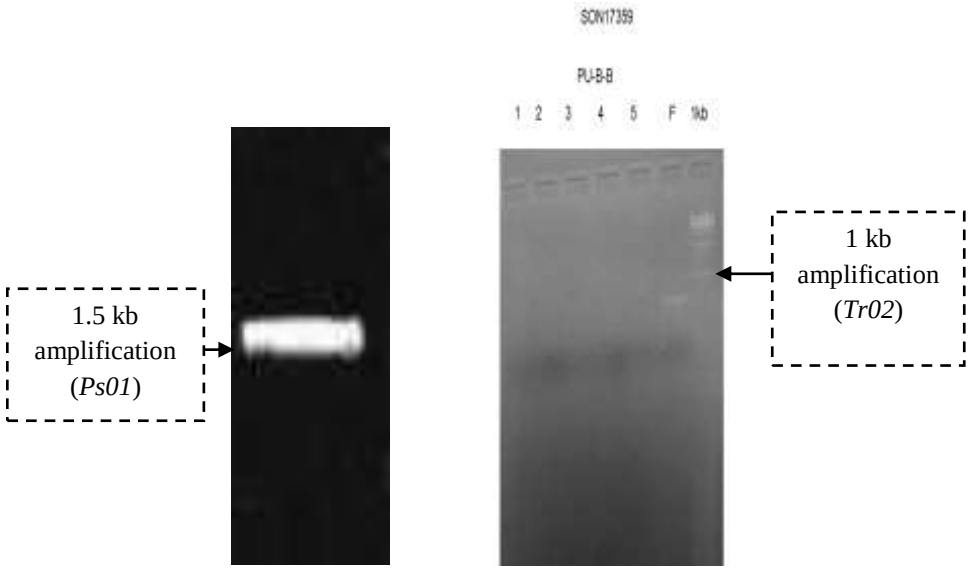

Figure 3. *Pseudomonas fluorescens* (1.5 Kb), *Trichoderma viride* (1 Kb) 16s rRNA amplification

Antibiotic susceptibility test

Pseudomonas fluorescens was grown in agar plates with different antibiotics. It proved an antibiotic resistance to Streptomycin, Chlorompheneco and Chloromphenecol (Table 3, Figure 4-Plate 1).

Table 3. Antibiotic Sensitivity Test of *Pseudomonas fluorescens*

S.no.	Commercial antibiotic	Inhibition zone diameter (mm)
1.	Streptomycin^{-25}	17.5 mm
2.	Chloromphecol^{-25}	20.3mm
3.	Chloromphecol^{-50}	21.8mm

Plate 1 **Plate 2** **Plate3**

Figure 4. Antibiotic and antagonistic test: Plate 1= Antibiotic sensitivity test; Plate 2: Antagonistic activity of Fu04 and Tr02; Plate 3= Antagonistic activity of Fu04 and Ps01

Antagonistic activity test

Pseudomonas fluorescens and *Trichoderma viride* isolates were proved to antagonize *Fusarium oxysporum* in dual culture (Figure 4-Plate 2&3).

Pot experiment

After 45 DAI, Ps01 (*Pseudomonas fluorescens*) and Tr02 (*Trichoderma viride*) inhibited the Fu04 (*Fusarium oxysporum*) and increased the growth of *Vigna unguiculata* L. Ps01 and Tr02 inoculated plants showed better growth parameter (36.1cm) than control (24.2cm) and *Fusarium* (22.4 cm). Plant pathogen was highly inhibited by bioinoculants which highly enhanced the plant length (Figure 5)**.** Ps01 and Tr02 inoculated plant showed better fresh weight (2.3g) than the control (1.20g) and *Fusarium* (0.60g) (Figure 6). The fresh and dry weight of plants treated *Pseudomonas, Trichoderma* showed better dry matter than the control and *Fusarium*. Ps01 and Tr02 inoculated plant showed better dry weight (1.4g) than the control (0.50gm) and *Fusarium* (0.20gm) (Figure 7).

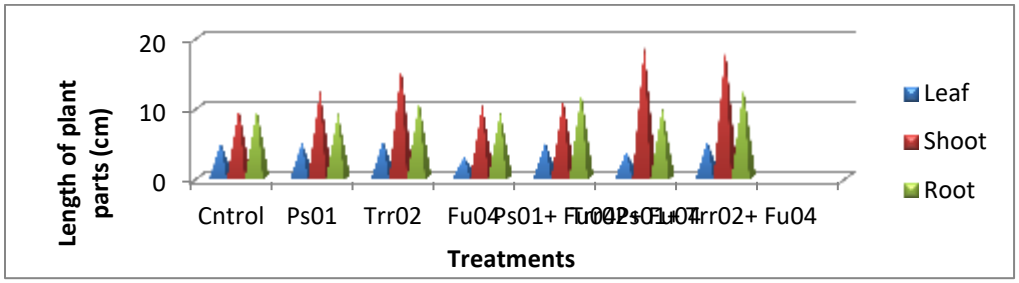

Figure 5. Growth characters of *Vigna unguiculata* L. leaf stem and root length at 45 DAI (cm/plant)

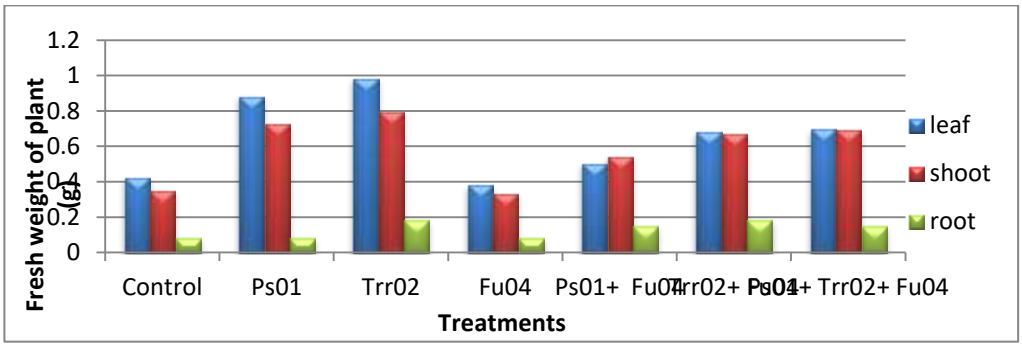

Figure 6. Growth characters of *Vigna unguiculata* L. fresh weight at 45 DAI (g/plant)

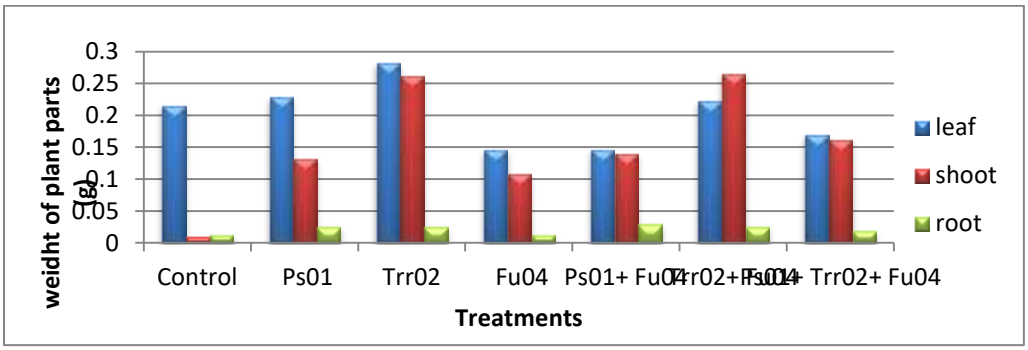

Figure 7. Growth characters of *Vigna unguiculata* L. dry weight at 45 DAI (g/plant)

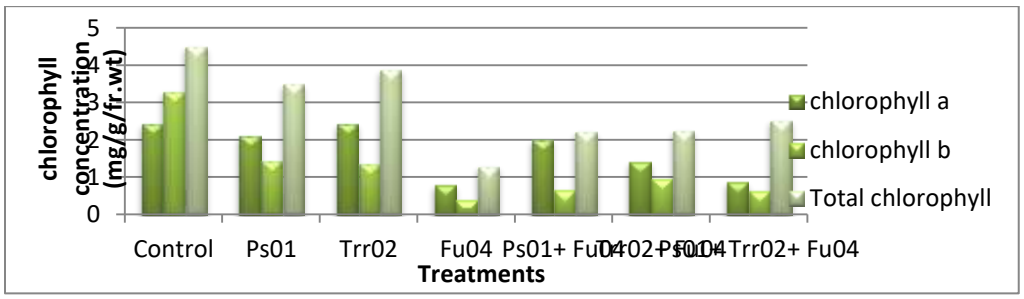

Figure 8. Analysis of Total chlorophyll content in *Vigna unguiculata* at 45 DAI (mg/g/fr.wt)

The results showed the leaf chlorophyll content of *Vigna unguiculata* that reduced when compared to the control. The result showed high chlorophyll accumulation in control, *Pseudomonas* and *Trichoderma* treatnents. Ps01 and Tr02 inoculated plants had better total chlorophyll content (7.40 mg/g/fr.wt) than the control (4.30 mg/g) and *Fusarium* (3.40 mg/g/fr.wt) as seen in Figure 8.

There was a statistically significant affected in total nitrogen content (g/plant) of *Vigna unguiculata.* Bioinoculants (Ps01+ Tr02) was recorded higher content (1.13 mg N/g dry plant) (Table 4).

Table 4. Effect of bio inoculants on total nitrogen content in *Vigna unguiculata* at 45 DAI (mg N/g dry plant)

S.no	Treatments	Total nitrogen content (mg N/g dry plant)
1	Control	1.08
2	Ps01	1.23
3	Tr02	1.06
4	Fu04	0.96
5	Ps01+ Fu04	1.02
6	Tr02+ Fu04	1.14
7	Ps01+ Tr02+ Fu04	1.13

Result showed in uninoculated soil had less NPK content, and Bioinoculants had high level of NPK content (Table 5). The microbial population in soil sample, control and microbial inoculated plants sown in the pots as shown in Table 6 and Figure 9. It was significantly higher in bio inoculants in the entire soil as compared to uninoculated control. Microbial analysis showed that the high total bacterial population was 3.1 $X10^7$ CFU/g soil. The soil had a fungal population of 12.6 $X10^7$ CFU/g soil and pathogen showed soil microbial population was 3.2 $X10^7$ CFU/g soil.

Table 5. Characterization of soils (mg/kg soil)

S.no	Treatments	PH	EC	Macro nutrient			Micro nutrient			
				N	P	K	Fe	Mn	Zn	Cu
1	Control	7.8	0.20	88.2	4.5	462.5	6.49	11.18	0.59	2.01
2	Ps01	6.3	0.54	165	7.3	343.3	4.29	13.21	2.52	2.94
3	Tr02	4.2	0.76	234	10.6	335.4	4.45	09.61	2.65	3.65
4	Fu04	5.2	0.54	132	5.8	208.1	2.38	02.28	0.94	1.94
5	Ps01+ Fu04	8.3	0.47	267	8.5	324.0	3.12	13.18	2.89	2.16
6	Tr02+ Fu04	7.5	0.54	312	7.2	345.0	4.43	12.68	1.75	2.38
7	Ps01+Tr02+Fu04	6.9	0.59	270	8.5	486.1	3.07	14.92	2.08	3.36

EC -Electrical Conductivity; **N** - Available Nitrogen; **P**- Available Phosphorus **K** -Available Potassium ;

Nitrogen: 0 to 46 mg/Kg soil-low; 47 to 113 mg/kg soil-medium; 113 above mg/kg soil-High;

Potassium: 0 to 113 mg/kg soil-low; 113 to 181 mg/kg soil-medium; 181 above mg/kg soil-high;

Phosphorus: 0.0 to 4.5 mg/kg soil – low; 4.6 to 9.0 mg/kg soil – medium; 9.0 above mg/kg soil – high.

Table 6. Impact of after inoculated soil Counting of Microbial population at 45 DAI (CFU/g soil)

S.no	Treatments	Total bacterial population in soil (CFU/g soil)	Total fungal population in soil (CFU / g soil)
1	Control	1.3 $X10^7$	1.1 X 10^5
2	Ps01	3.1 $X10^7$	1.6 X 10^5
3	Tr02	2.2 $X10^7$	12 X 10^5
4	Fu04	3.2 $X10^7$	6 X 10^5
5	Ps01+ Fu04	7.5$X10^7$	1.8 X 10^5
6	Tr02+ Fu04	1.8 $X10^7$	5.3 X 10^5
7	Ps01+ Tr02+ Fu04	12.6 $X10^7$	11.6 X 10^5

CFU – Colony forming unit

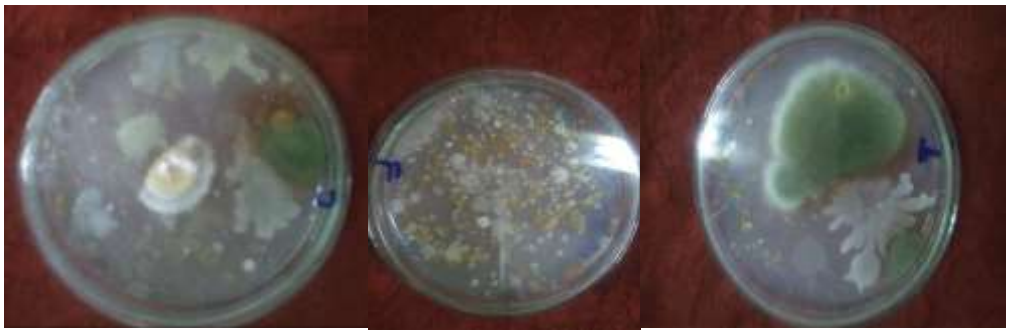

Figure 9. Isolation of microbial population

Discussion

The present study was carried out in soil sample collected from Salem district, Tamil Nadu. Fungal PGPF and plant pathogen was collected from plant leaves in Salem. Culturing on different agar medium revealed presence of *Pseudomonas* bacteria. The classification of the *Pseudomonas* genus by specific physiological and biochemical characteristics is helpful but is not enough to distinguish all *Pseudomonas* species. Results confirmed through identification and characterization of the *Pseudomonas fluorescens* can only be achieved by combining cultural, biochemical tests. In Gram's staining, the isolated *Pseudomonas* strains showed Gram-negative, pink colour, motile, rod shaped in appearance. These findings agreed with the findings reported by earlier researchers (Tripathi *et al*., 2011). The similar result found in the isolates which gave positive reaction for Catalase activity, Oxidase activity, citrate utilization, arginine hydrolysis and negative reaction for nitrate reductase activity (Bhojiya and Joshi, 2015). Similarly, it reported that qualitative

estimation of siderophores by *P. fluorescens* showed that its powerful producer of siderophores under limited iron on King's B medium. *P. fluorescens* can be used as a biocontrol against soil borne phytopathogens. (Ramyasmruthi *et al*., 2012). Similar experiments for the identification of microbes through morphological and microscopic (Singh *et al*., 2006). The microbial isolates (Ps01, Tr02) were confirmed through genetic DNA and PCR analysis. The isolate showed maximum similarity with *P. fluorescens* based on 16S rRNA analysis (Anita *et al*., 2006). Most of the soil borne fungi play an important role to cause several diseases. Cowpea is attacked by fungi and bacteria causing diseases leading to crop losses. *Fusarium oxysporum* is a major disease const of Cowpea (Baysal *et al*., 2013).

The present study that revealed bacterial and fungal inoculations were found significantly increased in plant growth parameters. Result was observed that microbial inoculations can be promoted the growth of cowpea. Seed bacterization resulted to increase seed germination, plant biomass, chlorophyll and nitrogen content (Almas Zaidi *et al*., 2015). *P. fluorescens* was proved for antibiotic resistance. Similar result was observed in *P.s fluorescens* which sensitive to kanamycin, gentamicin, streptomycin, ampicillin, penicillin, and carbenicillin (Slininger *et al*., 2003). Bioinoculants inoculated plants enhanced the growth parameters and inhibited the pathogen growth was also found by Hesamedin and Gholamreza (2014).

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