Original Research Article

Integrated Approach for the Management of Soil Borne Disease
_Fusarium oxysporum_ in _vitro_ in Groundnut

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**Abstract**

All the three bicontrol agents were found to be compatible with each other. Among the various oilcake extracts tested, mahua cake (10%) inhibited the mycelial growth of _F. oxysporum_ to an extent of 60.77 per cent. The crude antibiotic extracted from Pf1 showed maximum growth inhibition of _F. oxysporum_ up to 63.33 per cent. The diffusible non-volatile metabolite of Tv1 was effective in retarding the growth of the pathogen up to 91.44 per cent. The volatiles released by Tv1 reduced the growth of the pathogen by 87.55 per cent. Production of HCN was observed to be in higher quantity in Pf1 and Pf2 while isolates of _B. subtilis_ did not produce the same. Two isolates of _P. fluorescens_ (Pf1, Pf2) and _T. viride_ (Tv1 and Tv2) produced siderophore whereas the isolates of _B. subtilis_ (Bs2, Bs10) were negative for the same. Pf1 and Pf2 produced more SA than Bs2 and Bs10.

**Keywords**

Oilcake extract, Crude antibiotic, pathogen

**Introduction**

Groundnut plants affected with wilt exhibited greyish green discolouration and flaccidity of leaves followed by yellowing of foliage and wilting. Vascular browning of internal tissues was also noticed. In the pathogenicity tests carried out _in vitro_ as well as _in vivo_, plants inoculated _F. oxysporum_ produced the same symptoms as observed in the field. The findings corroborate with that of Jofee (1973) who observed bleaching of foliage, drying of canopy with vascular browning of tap roots in wilt of groundnut caused by _F. oxysporum_. In cotton infection caused by _F. oxysporum_ f.sp. _vasinfectum_ lead to loss of leaf turgidity, leaf yellowing and withering. Wilting was either partial or complete.

Tap roots were stunted with browning and blackening of vascular tissues (Prakasam et al., 1993). In gingelly symptoms of Fusarium wilt include partial or total wilting of plants at flowering and podding, with a purple band.
extending from the base upwards. When the main stem or primary branches were split browning or blackening of internal tissue was noticed (Correll, 2005).

**Materials and Methods**

**Preparation of aqueous extracts from oil cakes**

Required quantity of each oil cake was weighed and powdered separately. The powder was soaked in sterile distilled water @ one g in 1.25 ml of water and kept overnight. The material was ground using a pestle and mortar filtered, through a muslin cloth and the filtrate was centrifuged at 10,000 rpm for 15 min. The supernatant served as the standard extract solution (100%) (Dubey and Patel, 2000).

**Testing the antifungal activity of oil cake extracts against *F. oxysporum* in vitro**

The efficacy of oil cake extracts was tested against *F. oxysporum* by poisoned food technique (Schmitz, 1930). Ten ml of aqueous extract of oil cake was mixed with 90 ml of PDA to obtain 10 per cent concentration and sterilized. The sterilized medium (15 ml per Petri dish) was poured in sterilize Petri dish and allowed to solidify. A five- mm mycelial disc of *F. oxysporum* was cut from actively growing culture and placed at the centre of each Petri dish and incubated at room temperature. PDA medium without the extract of oil cake served as control. The radial growth of *F. oxysporum* was recorded 7 DAI.

**Screening of fungicides in vitro**

The inhibitory effect of six fungicides *viz.*, carbenzadim, Kocide 1011 (35% metallic copper), copper oxychloride, benomyl, Saff (carbenzadim + mancozeb) and Curzate (cymoxanil + mancozeb) each at four concentrations (0.05%, 0.1%, 0.15%, 0.2%) on the growth of *F. oxysporum* were evaluated by poisoned food technique (Schmitz, 1930). Each treatment was replicated three times with proper control. The fungal growth was measured after seven days and per cent inhibition was calculated.

**Biochemical characterization of *P. fluorescens* and *B. subtilis***

The effective bacterial antagonists screened against *F. oxysporum* were identified and characterized based on the diagnostic tests detailed in the laboratory guide of Schaad (1992).

**Diagnostic tests for *P. fluorescens* (Table 3)**

**Gram staining**

Gram staining was carried out to differentiate the bacteria as Gram-positive or negative.

**KOH test**

A drop of bacterial suspension was thoroughly mixed with a drop of 3% KOH on a glass slide. Gram negative bacteria became gummy upon mixing due to separation of chromosomes as thin strands.

**Growth at 45°C and 4°C**

Bacterial cultures were inoculated in KB broth and incubated at 45°C and 4°C and those with turbidity after 24 to 48 h were recorded as positive.

**Fluorescent pigment production**

Cultures were streaked on KB medium and incubated at room temperature. After 48 h the colonies were examined for fluorescence under ultraviolet rays.
Leaven formation

The 24-h-old cultures were streaked on NA medium with 5% sucrose (w/v) and incubated at room temperature.

The presence or absence of convex, white mucoid colonies was observed after 3-5 days.

Arginine dihydrolase reaction

Cultures were stabbed into a tube of Fhornley's medium and over laid with sterile mineral oil. The tubes were incubated at 28 ± 2°C. The faint pink colour of the medium turning to red (alkaline) in four days indicated positive reaction.

Gelatin liquefaction

Cultures were streaked on NA with 0.4% gelatin in a Petri plate and incubated at 28 ± 2°C for three days. The surface of the medium was flooded with 10 ml of acidified mercuric chloride solution (HgCl₂ - 12 g, distilled water - 80 ml, conc. HCl - 16 ml).

Clear zone around the colonies indicated a positive reaction.

Diagnostic tests for B. subtilis (Table 4)

Utilization of citrate

The slants with Simmons citrate agar were streaked with cultures and incubated at room temperature (28 ± 2°C). When the inoculated green colour of the medium turned blue after 24 - 48 h, it indicated the utilization of citrate by the bacteria.

Growth in 7% NaCl

Cultures were inoculated in nutrient broth with 0.5 glucose and 7% NaCl and the growth was observed daily up to seven days.

Anaerobic growth in glucose broth

Glucose broth was inoculated with test cultures, overlaid with sterile mineral oil and incubated at 28 ± 2°C. The growth of the cultures was observed after 48 h of incubation.

Starch hydrolysis

Petri dishes containing starch agar were streaked with test cultures. After five days of incubation, the plates were flooded with Lugol's iodine. Clear, colourless zone around the colonies indicated positive reaction for starch hydrolysis.

Catalase test

Bacterial cultures were inoculated on to NA slants. After 24 h, one ml of 3% H₂O₂ was allowed to flow over the surface of the culture and the production of bubbles of gas indicated positive reaction.

Mode of action of biocontrol agents

Antibiotic production - bacterial antagonists

Extraction of crude antibiotic metabolites

The bacterial biocontrol agents viz., Bs₁, Bs₁₀, Pf₁ and Pf₂ grown for five days in pigment production broth were centrifuged at 5000 rpm for 30 min. The supernatant was adjusted to pH 2.0 with concentrated HCl and extracted with equal volume of benzene. The benzene layer was evaporated in a water bath and the residue was resuspended in 0.1 N NaOH (Rossales et al., 1995).

Effect of bacterial antibiotics on the growth of F. oxysporum

The effect of antibiotics extracted from bacterial antagonists was tested against the
growth of *F. oxysporum* by filter paper disc assay (Lam and Ng, 2001). Three sterile filter paper discs were placed on solidified PDA in Petri dishes. The crude antibiotic extracted was pipetted on filter paper @150 µl/disc. A five-mm- mycelial disc of the fungus was placed at the centre of the plate and incubated at 28 ± 2°C. Filter paper without antibiotic served as control. Surface area of inhibition was measured by tracing the area of inhibition in a trace paper, plotting it on a graph sheet and comparing with control.

**Effect of non-volatile metabolites of *T. viride on the growth of *F. oxysporum***

The effect of non-volatile, diffusible metabolites of *T. viride* on the growth of *F. oxysporum* was studied by the method of Dennis and Webster (1971). Sterilized cellophane disc of 90-mm-dia was layered on top of the PDA in Petri plates. Five-mm-disc of *Trichoderma* spp. was placed at the centre of the cellophane disc and plates were incubated at 28°C for three days. The cellophane disc along with the growth of *Trichoderma* was gently and aseptically removed on 3rd day and five-mm-disc of pathogen from actively growing culture was placed at the centre of PDA and the plates were incubated again. The diameter of the fungal growth was measured when the control plate attained the full growth.

**Effect of volatiles of biocontrol agents on the growth of *F. oxysporum***

The inhibitory effect of volatiles produced by biocontrol agents on the growth *F. oxysporum* was estimated by paired Petri-plate technique (Laha *et al.*, 1996). The isolates of *P. fluorescens* and *B. subtilis* were streaked on KB and NA respectively. For *T. viridea* five mm-mycelial disc of the fungus was placed at the centre of PDA plate. The plates with PDA inoculated with pathogen at the centre were inverted over the plates having the antagonists and both the plates were sealed together with Parafilm and incubated at 28 ± 2°C. PDA plates with the pathogen inverted over the plates without antagonist served as control. The inhibitory effect of volatiles produced by biocontrol agents was assessed based on the mycelial growth of the pathogen when compared to control.

**Hydrogen cyanide (HCN) production**

**Qualitative assay**

HCN production of fungal and bacterial biocontrol agents was tested qualitatively following the method of Bakker and Schipper (1987). The antagonistic bacteria were streaked on KB medium amended with glycine at 4.4g/ l. In case of *Trichoderma*, a five- mm mycelial disc of the fungus was placed at the centre of plate containing PDA amended with glycine.

Sterile filter paper saturated with picric acid solution (2.5 g of picric acid; 12.5 g of Na₂CO₃, 1000 ml of distilled water) was placed in the upper lid of the Petri plate. The dishes were sealed with Parafilm and incubated at 28°C for 48 h. A change of colour of the filter paper from yellow to light brown, brown or reddish-brown was recorded as weak (+), moderate (+++) or strong (++++) reaction respectively.

**Quantitative assay**

Antagonistic bacteria were grown in KB broth amended with glycine ( 4.4g/ l) while *T. viridewas* cultured on potato dextrose broth containing glycine. Uniform strips of filter paper (10 x 0.5 cm²) were soaked in alkaline picrate solution and kept hanging inside the conical flask. After incubation at 28 ± 2°C for 48 h the sodium picrate in the filter paper was reduced to a reddish compound in proportion
to the amount of HCN evolved. The colour was eluted by placing the filter paper in a test tube containing 10 ml of distilled water and its absorbance was read at 625 nm (Sadasivam and Manickam, 1992).

**Siderophore production**

**Qualitative assay**

Forty eight-h-old bacterial cultures were streaked on succinate medium amended with indicator dye. In case of *Trichoderma*, a five mm-mycelial disc of the fungus was placed at the centre of the plate containing the same medium. The tertiary complex chrome azural S (CAS) / Fe$^{3+}$ / hexadecyl trimethyl ammonium bromide served as an indicator. Change of blue colour of the medium surrounding the growth of the culture to fluorescent yellow indicated the production of siderophore. The reaction of each bacterial strain was scored either positive or negative to the assay (Schwyn and Neilands, 1987).

**Nature of siderophore**

The bacterial isolates of *P. fluorescens* and *B. subtilis* were inoculated in 10 ml of KB broth and nutrient broth respectively. The cultures were incubated in a rotary shaker at 120 rpm for 48 h. The bacteria multiplied in the broths were used as the sample for the determination of the nature of siderophore.

**Hydroxymate type**

It was examined by tetrazolium salt test. Instant appearance of a deep red colour by addition of tetrazolium salt to the culture under alkaline conditions indicated the presence of hydroxymate type of siderophore production (Snow, 1984).

**Carboxylate type**

The assay was conducted by Vogeli’s chemical test where the disappearance of pink colour on addition of phenolphthalein to the culture under alkaline condition indicated carboxylate nature of siderophore (Vogeli *et al.*, 1998).

**Quantitative assay**

*P. fluorescens* and *B. subtilis* were grown on KB and nutrient broth respectively. After three days, the cultures were centrifuged at 10,000 rpm for 20 minutes. The pH of the cell free culture filtrate was adjusted to 2.0 with HCl and equal quantity of ethyl acetate was added, mixed well in a separating funnel and the ethyl acetate fraction was collected. The process was repeated three times to bring the entire quantity of siderophore from the supernatant.

The ethyl acetate fractions were pooled, air dried and dissolved in five ml of 50% ethanol. Five ml of the fraction was mixed with five ml of Hathway reagent (1.0 ml of 0.1 M FeCl$_3$ in 0.1 N HCl to 100 ml of distilled water with 1.0 ml of potassium ferricyanide). The absorbance of dihydroxy phenol was measured at 700 nm (Reeves *et al.*, 1983). The quantity of siderophore produced was calculated using a standard graph of dihydroxy benzoic acid (Dileep *et al.*, 1998) and expressed as µg ml$^{-1}$ of culture filtrate.

**Salicylic acid (SA) production**

The antagonistic bacteria were grown in succinate broth for 48 h at 28 ± 2° C. Cultures were centrifuged at 10,000 rpm for 20 min. and the supernatant was used to quantify the SA production. Four ml of the supernatant was acidified with 1 N HCl to pH 2.0 and extracted with equal volume of chloroform.
Four ml of water and 5 µl of 2 M FeCl₃ were added to the pooled chloroform phase. The absorbance of the purple iron-salicylic acid complex, which developed in the aqueous phase was measured at 527 nm (Meyer et al., 1992). Production of SA by biocontrol agents was calculated using a standard graph with SA dissolved in succinate medium and the results were expressed as µg ml⁻¹ of culture filtrate.

Compatibility between biocontrol agents

The compatibility of different antagonistic bacteria among themselves was tested by streaking the antagonistic test bacterium vertically on one side and streaking the other bacterium perpendicularly up to the test bacterium. The growth of both the bacteria were observed and recorded as positive or negative. The compatibility between T.viride and bacterial isolates was also tested.

Results and Discussion

Organic amendments and F. oxysporum

In the present study, the extract of mahua cake was found exhibit higher inhibitory effect on F.oxysporum. Srivastava et al., (1991) reported that the lowest mycelial growth of F. solani was observed in mahua cake incorporated medium. Pandey et al., (1996) and Mukthar, (2007) The aqueous extract of neem cake inhibited the growth of F. o. f. sp. ciceri. Neem cake has been found to completely control F. solani infection in 40 day-old soybean plants (Ali, 1997). Bhonde et al., (1999) reported that the extracts of neem cake at 10 per cent were found to be effective against F.solani and caused 80.4 per cent mycelial growth inhibition followed by mahua cake (75.1%). Padmodaya and Reddy (1999) noticed that neem cake extract was highly inhibitory to F. oxysporum. f. sp. lycopersici. Yelname et al., (2010) reported that the extract of neem cake showed excellent inhibitory effect against the chilli wilt pathogen F. solani. The radial growth of F. o. f. sp. psidii was significantly less in neem leaf extract incorporated medium (Srivastava et al., 2011) (Table 1).

Fungicides and F. oxysporum

In the present study, carbenazim, benomyl and Saff at 0.05 per cent completely inhibited the growth of F. oxysproum. Guo et al., (1993) obtained best results when carbenazim was used as a basal compound against Fusarium sp. on cotton. Yunusov et al., (1980) used benomyl to manage Fusarium wilt of cotton. Gupta et al., (1997) reported that carbenazim (100 mg/ml) was highly effective in inhibiting the mycelial growth of F. o. f. sp. Ciceri under in vitro. Christian et al., (2007) observed that the highest inhibition of 28 isolates of pathogenic fungi was obtained with carbenazim, benomyl and captan under in vitro. Carbenazim was observed to reduce the mycelial growth of F.o.f.sp.ciceri to the in vitro while metalaxyl was least effective (Subhani et al., 2011) (Table 2).

Compatibility among biocontrol agents

Compatibility of Trichoderma spp. with P.fluorescens was assessed by comparing the biocontrol agents applied alone or in combination to suppress take all disease of wheat (Duffy et al., 1996). The present study revealed that there was absence of growth inhibition between the isolates of P. fluorescens, B. subtilis and T. viride so that they were compatible among themselves. The interaction between strains of Pseudomonas was studied in vitro and the growth of various combinations of the bacterium in sugar beet spermsphere was found to correlate with their inhibitory behaviours on culture media (Fukui et al., 1994). Thilagavathy (2005) reported that strains of P. fluorescens (Pf₁ and Pf₁5) were compatible in vitro with B. subtilis
(Bs10) and T. viride. Latha (2006) observed that P. fluorescens (Pf1) and B. subtilis (Bs16) were compatible.

**Mode of action**

**Antibiosis**

In the present investigation, antibiotic of Pf1 exhibited more inhibitory effect on F. oxysporum followed by Pf2, BS10 and BS2. Several strains of Pseudomonas spp. and Bacillus spp. produce wide array of antibiotics which include lacton, 2-4 diacetylphloroglucinol (2-4 DAPG), HCN, oligomycin, oomycin A, phenazine, pyroline, pyocyanin, surfactin and several uncharacterized molecules (Kim et al., 1989; Keel and Defago, 1997; Whipps, 1997; Nielson et al., 1998).

Florescent pseudomonads in the plant rhizosphere have been found to improve the plant growth and suppression of plant disease by the production of antibiotics, siderophores, hydrolytic enzymes and HCN (Ahmad and Khan, 2001). John Bainton et al., (2002) reported that the naturally occurring florescent pseudomonads produced the antibiotic, 2-4 DAPG. Bacillus spp produced different inhibitory agents which have been categorized in peptide derivative family (Stein, 2005; Tamehiro et al., 2002). Bacilsynocin, a novel and broad spectrum phospholipid antibiotic was purified from B. subtilis strain 168 (Tamehiro et al., 2002). Srivastava and Salini (2009) reported that P. fluorescens produced secondary metabolites such as siderophore, HCN and protease which showed antagonistic activity against Fusarium spp.

**Volatile and disease suppression**

In the present investigation, the inhibitory effect of volatiles released by Tv1 was more pronounced than that of Pf1 and BS10.

Diffusible volatile compounds produced by T. viride and T. harzianum inhibited the germination and mycelial growth of F. oxysporum (Michrina et al., 1995; Pandey et al., 1997).

The volatile metabolite furanone produced by P. aureofaciens showed antifungal activity against F. solani, F. oxysporum, P. ultimum and Thielaviopsis basicola (Paulitz et al., 2000). Paramasivam (2006) reported the involvement of volatile and nonvolatile antibiotic compounds released by Trichoderma spp. against the sugar beet root rot pathogen S. rolfsii. Bacterial strains of P. fluorescens inhibited the mycelial growth of F. o. f. sp. dianthi by production of volatile metabolites under laboratory condition (Karimi et al., 2007). Retarded radial growth of F. oxysporum infecting groundnut was due to the volatile and non-volatile metabolites produced by Trichoderma spp. (Rajeswari and Kannabiran, 2011).

The isolate Bs10 was found to release volatiles that was inhibitory to F. oxysporum. Bacillus spp are ubiquitous in the environment (Nicholson, 2004) and found associated with antifungal activity by producing volatile compounds as well as non-volatile substances (Ryder et al., 1999; Bhaskar et al., 2005).

**HCN and disease suppression**

Production of HCN by certain strains of fluorescent pseudomonads has been involved in the suppression of soil-borne pathogens (Voisard et al., 1989). In our study, production of HCN was very strong in Pf1 than in Pf2. Role of HCN in disease suppression has been demonstrated by several scientists in various crops (Stutz et al., 1986; Voisard et al., 1989; Defago et al., 1990). HCN is the common secondary metabolite produced by rhizosphere pseudomonads (Schippers et al., 1990). Meena et al., (2001) compared the HCN production of several
strains of *P. fluorescens* and their efficacy in controlling root rot of groundnut caused by *M. phaseolina*. Pseudomonads releasing HCN were reported in the rhizosphere of tobacco in soils suppressive to *T. baccicola*, casual agent of black root rot of tobacco (Ramette et al., 2006).

**Siderophores and disease suppression**

Pseudomonads generally produce fluorescent, yellow-green, water soluble siderophores. The siderophores are either pyoveridins or pseudobactins. Production of the siderophores has been linked to the disease suppressive potential of certain florescent pseudomonads. In the present study, the strain Pf₁ produced more quantity of siderophore than Pf₂ and the siderophore was hydroxymate type. The fungal strain Tv₁ also produced siderophore.

Siderophore of *Pseudomonas* spp inhibited the chlamydospore germination of *F. s. f. sp. lini*, *F. oxysporum* f.sp. *cucurbitae* and *F. o. f. sp. cucumerinum* (Sneh et al., 1984; Wong et al., 1984). Klopper et al., (1988) documented the production of fluorescent siderophore by *P. fluorescens* which was attributed to its antagonistic action. Ake et al., (1991) reported that under iron deficiency, the culture filtrate of all strains of *Trichoderma* contained coprogen, coprogen B and ferricrocin as siderophore. *T. longi* and *T. pseudokoningii* produced furigen type of siderophore. The hydroxymate type of siderophore was ferribactin produced by *P. fluorescens* (Linget et al., 1992). Lim et al., (1999) showed that the siderophore of *P. fluorescens* GL20 inhibited spore germination and hyphal growth of *F. solani in vitro* and reduced the disease incidence with enhanced plant growth. Siderophore of *P. fluorescens* was inhibitory to the growth of *M. phaseolina in vitro* (Meena et al., 2001). *B. subtilis* (BSCBE4), *P. chlororaphis* (PA23) and *P. fluorescens* produced both hydroxymate and carboxylate type of siderophores (Mathiyazhagan et al., 2004). Ahmed et al., (2008) reported that siderophore production and antifungal activity was exhibited by 10 to 12.7 per cent of pseudomonas isolates.

**Table.1** Effect of oil cake extracts against *F. oxysporum in vitro*

| S.No. | Treatments          | Mycelial growth(cm) | Per cent reduction over control |
|-------|---------------------|---------------------|---------------------------------|
| 2     | Castor cake (10%)   | 6.10                | 32.22                           |
| 3     | Gingelly cake (10%) | 4.38                | 51.33                           |
| 4     | Mahua cake (10%)    | 3.53                | 60.77                           |
| 5     | Coconut cake (10%)  | 7.48                | 16.88                           |
| 6     | Cotton cake (10%)   | 7.20                | 20.00                           |
| 7     | Control             | 9.00                | -                               |
| CD    |                      | 0.34                |                                 |
### Table 2: Efficacy of fungicides against the growth of *F. oxysporum*

| Fungicides                  | Mycelial growth (cm) / Reduction over control (%) | Mean | 
|-----------------------------|--------------------------------------------------|------|
|                             | 7 DAI*                                            |      |
|                             | Concentration (ppm)                              |      |
|                             | 500       | 1000    | 1500    | 2000    |      |
| Carbendazim                 |          |         |         |         | 0.70 |
|                            | 0.00     | 0.00    | 0.00    | 0.00    |      |
|                            | (0.70)   | (0.70)  | (0.70)  | (0.70)  |      |
|                            | 100.00   | 100.00  | 100.00  | 100.00  |      |
| Copper oxychloride          |          |         |         |         | 1.58 |
|                            | 5.8      | 3.5     | 1.1     | 1.1     |      |
|                            | (2.4)    | (1.87)  | (1.04)  | (1.04)  |      |
|                            | 35.55    | 61.11   | 87.77   | 87.77   |      |
| Kocide                      |          |         |         |         | 1.96 |
|                            | 7.2      | 6.0     | 1.8     | 1.8     |      |
|                            | (2.68)   | (2.45)  | (1.35)  | (1.36)  |      |
|                            | 20.00    | 33.33   | 80.00   | 80.00   |      |
| Benomyl                     |          |         |         |         | 0.70 |
|                            | 0.00     | 0.00    | 0.00    | 0.00    |      |
|                            | (0.70)   | (0.70)  | (0.70)  | (0.70)  |      |
|                            | 100.00   | 100.00  | 100.00  | 100.00  |      |
| Saff (carbendazim12% + mancozeb 64%) | 0.00 | 0.00 | 0.00 | 0.00 |
|                             | (0.70)   | (0.70)  | (0.70)  | (0.70)  |      |
|                             | 100.00   | 100.00  | 100.00  | 100.00  |      |
| Curzate (cymoxanil 8% + mancozeb 64%) | 8.0 | 8.1   | 4.2    | 4.8    |
|                             | (2.82)   | (2.83)  | (2.05)  | (2.19)  |      |
|                             | 11.11    | 10.00   | 53.33   | 46.66   |      |
| Control                     |          |         |         |         | 3.00 |
|                            | 9.00     | 9.00    | 9.00    | 9.00    |      |
|                            | (3.00)   | (3.00)  | (3.00)  | (3.00)  |      |
| Mean                        | 1.85     | 1.75    | 1.36    | 1.38    | -    |

Figures in parentheses are square root transformed values;  *DAI - days after inoculation*

### Table 3: Characterization of *B. subtilis*

| Sl.No. | Diagnostic tests     | Bs₁₀ | Bs₂ |
|--------|----------------------|------|-----|
| 1.     | Gram reaction        | +    | +   |
| 2.     | KOH test             | -    | -   |
| 3.     | Growth at 45°C       | +    | +   |
| 4.     | Growth in 7% NaCl    | +    | +   |
| 5.     | Citrate utilization  | +    | +   |
| 6.     | Anaerobic growth     | -    | -   |
| 7.     | Starch hydrolysis    | +    | +   |
| 8.     | Catalase test        | +    | +   |
Table 4 Characterization of *P. fluorescens*

| Sl.No. | Diagnostic tests                                      | Pf1 | Pf2 |
|-------|-------------------------------------------------------|-----|-----|
| 1.    | Gram reaction                                         | -   | -   |
| 2.    | KOH test                                              | +   | +   |
| 3.    | Pigment production in King’s B medium                  | +   | +   |
| 4.    | Growth at 4°C                                          | +   | +   |
| 5.    | Growth at 41°C                                         | -   | -   |
| 6.    | Arginine dihydrolase                                   | +   | +   |
| 7.    | Gelatin liquefaction                                   | +   | +   |
| 8.    | Levan formation                                        | -   | -   |

**Salicylic acid**

Maurhofer *et al.*, (1994) observed that certain PGPR strains are capable of producing SA and are responsible for the induction of ISR in plants. In the current study, SA production was observed to be more in Pf1 while it was less in Bs10. Role of SA producing *P. aeruginosa* in disease suppression was studied by Buysens *et al.*, (1996). Inoculation of roots of chickpea with *P. fluorescens* strain H92 (or) with synthetic 0-acetyl salicylic acid induced systemic resistance against the charcoal rot fungus, *M. phaseolina* (Srivastava *et al.*, 2000).

Meena *et al.*, (2001) reported that production of SA was maximum in *P. fluorescens* strain Pf1 followed by ALR-7 and Pf MDU 2 isolates. There was a significant relationship between inhibitory activity of *P. fluorescens* strains *in vitro* and their level of SA production. SA production has been observed for several bacterial strains and exogenously applied SA induces resistance in plant species (Bakker *et al.*, 2003).

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