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Integrated Approach for the Management of Soil Borne Disease *Fusarium oxysporum in vitro* in Groundnut

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A B S T R A C T

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 Pf_1 showed maximum growth inhibition of *F. oxysporum* up to 63.33 per cent. The diffusible non-volatile metabolite of Tv_1 was effective in retarding the growth of the pathogen up to 91.44 per cent. The volatiles released by Tv_1 reduced the growth of the pathogen by 87.55 per cent. Production of HCN was observed to be in higher quantity in Pf_1 and Pf_2 while isolates of *B. subtilis* did not produce the same. Two isolates of *P. fluorescens* (Pf_1, Pf_2) and *T. viride* (Tv_1 and Tv_2) produced siderophore whereas the isolates of *B. subtilis* (Bs_2, Bs_{10}) were negative for the same.Pf_1 and Pf_2 produced more SA than Bs_2 and Bs_{10}

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 @ oneg 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*., carbendazim, Kocide 1011 (35% metallic copper), copper oxychloride, benomyl, Saff (carbendazim + 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^{o}C and 4^{o}C

Bacterial cultures were inoculated in KB broth and incubated at 45^{o}C and 4^{o}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 ($HgCl_2$ - 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, over laid 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% H_2O_2 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*., Bs_1, Bs_{10}, Pf_1 and Pf_2 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 the *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.viride*a 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 cenre 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 Na_2CO_3, 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.viride*was cultured on potato dextrose broth containing glycine. Uniform strips of filter paper (10 x 0.5 cm^2) 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.

Salicylicacid (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 $FeCl_3$ 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*. Yelmame *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, carbendazim, benomyl and Saff at 0.05 per cent completely inhibited the growth of *F. oxysproum*. Guo *et al.,* (1993) obtained best results when carbendazim 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 carbendazim (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 carbendazim, benomyl and captan under *in vitro***.** Carbendazim 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 spermosphere was found to correlate with their inhibitory behaviours on culture media (Fukui *et al.,* 1994). Thilagavathy (2005) reported that strains of *P. fluorescens* (Pf_1 and Pf_{15}) were compatible *in vitro* with *B. subtilis*

(Bs_{10}) and *T. viride*. Latha (2006) observed that *P. flourescens* (Pf_1) and *B. subtilis* (Bs_{16}) were compatible.

Mode of action

Antibiosis

In the present investigation, antibiotic of Pf_1 exhibited more inhibitory effect on *F. oxysporum* followed by Pf_2, BS_{10} and BS_2. 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, pyrolnitrin, pyocyanin, surfactin and several uncharacterized molecules (Kim *et al., 1*989; Keel and Defago, 1997; Whipps, 1997; Nielson *et al., 1*998).

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 fluorescent 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). Bacilysocin, 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.

Volatiles and disease suppression

In the present investigation, the inhibitory effect of volatiles released by Tv_1 was more pronounced than that of Pf_1 and BS_{10}. 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., 1*997).

The volatile metabolite furanone produced by *P. aureofaciens* showed antifungal activity against *F. solani, F. oxysporum*, *P. ultimum* and *Thielaviopsis bassicola* (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 Bs_{10} 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., 1*999; 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., 1*989). In our study, production of HCN was very strong in Pf_1 than in Pf_2. Role of HCN in disease suppression has been demonstrated by several scientists in various crops (Stutz *et al., 1*986; Voisard *et al., 1*989; Defago *et al., 1*990). HCN is the common secondary metabolite produced by rhizosphere pseudomonads (Schippers *et al., 1*990). 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. bassicola*, casual agent of black root rot of tobacco (Ramette *et al.,* 2006).

Siderophores and disease suppression

Pseudomonads generally produce fluorescent, yellow-green, water soluble siderophres. The siderophores are either pyoveridins or pseudobactins. Production of the siderophres has been linked to the disease suppressive potential of certain florescent pseudomonads. In the present study, the strain Pf_1 produced more quantity of siderophore than Pf_2 and the siderophore was hydroxymate type. The fungal strain Tv_1 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., 1*984; Wong *et al., 1*984). 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 fuigen 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. solaniin 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. oxysporumin 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. oxysporu*m

Fungicides	Mycelial growth (cm) / Reduction over control (%) 7 DAI*				Mean
	Concentration (ppm)				
	500	1000	1500	2000	
Carbendazim	0.00 (0.70) 100.00	0.00 (0.70) 100.00	0.00 (0.70) 100.00	0.00 (0.70) 100.00	0.70
Copper oxychloride	5.8 (2.4) 35.55	3.5 (1.87) 61.11	1.1 (1.04) 87.77	1.1 (1.04) 87.77	1.58
Kocide	7.2 (2.68) 20.00	6.0 (2.45) 33.33	1.8 (1.35) 80.00	1.8 (1.36) 80.00	1.96
Benomyl	0.00 (0.70) 100.00	0.00 (0.70) 100.00	0.00 (0.70) 100.00	0.00 (0.70) 100.00	0.70
Saff (carbendazim12% + mancozeb 64%	0.00 (0.70) 100.00	0.00 (0.70) 100.00	0.00 (0.70) 100.00	0.00 (0.70) 100.00	0.70
Curzate (cymoxanil 8% + mancozeb 64%)	8.0 (2.82) 11.11	8.1 (2.83) 10.00	4.2 (2.05) 53.33	4.8 (2.19) 46.66	2.47
Control	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (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

CD(P=0.05)		
Fungicide	=	0.39
Concentration	=	0.29
Fungicide × Concentration	=	0.77

Table.3 Characterization of *B.subtilis*

Sl.No.	**Diagnostic tests**	**Bs_{10}**	**Bs_2**
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	Pf_1	Pf_2
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 Pf_1 while it was less in Bs_{10}. 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. phasiolina* (Srivastava *et al.,* 2000).

Meena *et al.,* (2001) reported that production of SA was maximum in *P. fluorescens* strain Pf_1 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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