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Research Article

Exploitation of indigenous fluorescent pseudomonads against stem and pod rot of groundnut caused by *Sclerotium rolfsii*

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ABSTRACT: Stem and pod rot of groundnut is an economically important soil borne disease caused by *Sclerotium rolfsii* Sacc. The present study aimed to evaluate the indigenous bacterial bio control agent *Pseudomonas fluorescens* strains having multiple traits related to bio control and plant growth promoting activity. Healthy rhizospheric soil from groundnut field was collected from different North Karnataka districts. Nineteen strains of *P. fluorescens* were isolated by serial dilution technique on King's B medium. The antagonistic potential of nineteen strains was tested against *S. rolfsii* by using dual culture technique. The per cent inhibition was highly variable among the nineteen strains of *P. fluorescens* ranging from 2.59 to 75.18 per cent. Maximum mycelial inhibition of *S. rolfsii* was recorded by the strain PF-2 (75.18%) followed by PF-3 (72.96%), PF-6 (69.62%) and least inhibition was recorded by PF-13 (2.59%). Five superior strains of *P. fluorescens* showed fluorescens under UV light, yellowish green pigmentation, rod shaped cells under microscope. Five superior strains were subjected for various biochemical tests and all the isolates were positive for biochemical characterization such as Gram staining, endospore production, catalase, starch hydrolysis, urease test, casein hydrolysis and gelatin liquefy action and negative for KOH and in dole test. Further, these strains were subjected for plant growth promoting traits such as HCN production, IAA production, siderophore production and volatile compounds production. Among these, the strains*viz*., PF-2 and PF-3 were scored as strong with respect to antagonism and growth promotion. The strainsPF-6, PF-7 and PF-10 were scored as moderate with light brown colour. Among the tested strains of *P. fluorescens*, the isolates PF-2 and PF-3 were recorded higher production of siderophore, isolates PF-6, PF-7 and PF-10 showed moderate production of siderophore. The strain PF-2 of *P. fluorescens* showed highest per cent of mycelial inhibition of *S. rolfsii* indicating higher production of volatile compounds, followed by PF-3 and PF-6. Whereas, the least mycelial inhibition was recorded by the isolate PF-10 indicating less production of volatile compounds.

KEYWORDS: Groundnut, management, *Pseudomonas*, sclerotium rot

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INTRODUCTION

In India, groundnut is a significant oilseed crop that ranks first in terms of area and second in terms of production after soybean. With 17.57 million tonnes produced, China leads the globe in groundnut production, followed by India with 6.73 million tonnes, or 36.01 and 13.79% of the 48.80 million tonnes produced globally overall. Groundnut production was estimated to be 82.54 lakh tonnes (kharif) in 2021–2022, down from 85.56 million tonnes (kharif) in 2020–2021. (kharif) (Anon, 2022).

Groundnut growers are extremely concerned about disease prevention since diseases pose a severe threat to the crop each year. Roots, stems, and pods are attacked by fungi that are transmitted through soil and seeds; this can lead to losses in terms of quantity and quality as well as an increase in soil infection. Due to the difficulty in recognising them and the similarity of their symptoms, diseases caused by soilborne pathogens pose a particular threat to the production of groundnut. In addition to direct losses, controlling soilborne infections results in higher input costs. Peanut is susceptible to losses brought on by illnesses carried by the soil because of the close contact of the pods with the soil. One of the fungus that causes the stem and pod rot of groundnuts is *Sclerotium rolfsii* Sacc.

The stem and pod rot disease affects all states that cultivate groundnut, but it is especially severe in Maharashtra, the Saurashtra region of Gujarat, and the Raichur district of Karnataka.

Pathogen-induced yield loss averages 25% but can occasionally reach 80–90 % (Grichar and Bosweel, 1987).

Similarly, Mayee and Datar (1998) have observed yield losses of nearly 25%.

A variety of chemicals have been proposed as treatments for Sclerotium rot; however, using chemicals has disadvantages, such as residual side effects that may be damaging to health and the emergence of pathogen resistance through repeated use of the same drugs. To treat this sickness, however, utilising bio-control agents offers a helpful option. Biological control is one of the most reliable and efficient methods for protecting plants. Antagonistic microorganisms are alternatives to fungicides.

Pseudomonas is gram negative bacteria belong to the phylum Proteobacteria, class Proteobacteria and family Pseudomonadaceae which includes fluorescent pseudomonads as well as few non-fluorescent species. Fluorescent Pseudomonads group consists of phytopathogenic and non-phytopathogenic strains. Among these, non phytopathogenic strains such as *Pseudomonas fluorescens*, *P. putida*, *P. chlororaphis*, *P. aureofaciens* and *P. aeruginosa* type species are present. As the name indicates, it secretes a soluble greenish fluorescent pigment called as fluorescin, particularly under low iron availability. It is an obligate aerobe, motile by means of multiple polar flagella. It has simple nutritional requirements and grows well in mineral salts media supplemented with any of a large number of carbon sources. Fluorescent pseudomonads species play an active role in suppression of pathogenic microorganisms by several mechanisms which include production of antibiotics as secondary metabolites at low concentration, competition, HCN, siderophore production and induced systemic resistance. Utilization of these microbial antagonists against plant pathogens in agricultural crops has been proposed as an alternate to chemical pesticides. The goal of the current study was to screen fluorescent pseudomonads for a variety of features related to bio-control and growth promotion.

MATERIALS AND METHODS

Isolation and maintenance of the pathogen (*Sclerotium rolfsii*)

Groundnut plants showing typical symptoms of stem and pod rot were used for isolation of *S. rolfsii*. From the infected stem portion of each diseased sample, the adhering soil particles and other debris were removed by thorough washing under running tap water. After that, infected stem portions were cut into small bits of 1 cm size and these were surface sterilized by immersing in 0.1 per cent mercuric chloride for 30 seconds. The stem bits were washed in three changes of sterile distilled water to remove the traces of mercuric chloride and blotted dry on clean, sterile tissue papers. Later, these bits were aseptically transferred to Petriplates containing PDA medium in a laminar air flow chamber and incubated at 27 ± 2 °C for 3 to 4 days. Mycelium from the diseased stem bits was transferred directly onto the medium with the help of sterile needle. Pure cultures of the pathogen *S. rolfsii* were maintained on PDA by periodical transfers.

Isolation and antagonistic activity of *Pseudomonas fluorescens* against *S. rolfsii* causing stem and pod rot in groundnut

Isolation of *Pseudomonas fluorescens*

Healthy rhizosphere soil from groundnut field was collected by sampling the soil adhering to the root system of about five plants was selected randomly. About six spots were selected randomly and the soil sample at the depth of 10-20 cm was collected. The collected samples were thoroughly mixed to obtain a representative sample of the area under examination. Collected samples were packed in a polythene bag and labeled properly then they were carried to Plant Pathology Lab, College of Agriculture, University of Agricultural Sciences, Raichur. The plant growth promoting rhizobacteria, *Pseudomona sfluorescens* was isolated by using King's B medium (KBM). Ten grams of soil was taken and suspended in 90 ml of sterilized distilled water and stirred well with vertex mixture to get 1:10 dilution (10-1). One ml from this was transferred to test tube containing 9 ml of sterilized distilled water to get 1:100 (10-2) dilution. Likewise, the dilution of the sample was prepared up to 10-7 *P. fluorescens.* One ml of a final dilution of each sample was pipette out into each sterile Petri plate separately to which a quantity of 15-20 ml of sterilized and molten medium was poured and gently rotated for uniform mixing, the plates were incubated at 28 ± 1 °C for 1-2 days for the isolation of *P. fluorescens* They were kept under observation daily for the appearance of green colonies by *P. fluorescens*.

Antagonistic potential of *Pseudomonas fluorescens* against *Sclerotium rolfsii*

Ninteen strains of *P. fluorescens* were screened for potential antagonistic activity against pathogenic fungus *S. rolfsii* by dual culture technique. Mycelial disc (5 mm dia.) was cut from an actively growing *S. rolfsii* culture and placed on the surface of fresh PDA medium at middle of the Petri plates. A loop full of actively growing bacterial culture was streaked parallel on either side of fungal disc and Petri plates without bacterial cultures were maintained as control and each treatment was replicated thrice. The plates were incubated at room temperature for 10 days. The degree of antagonism was determined by measuring the radial growth of pathogen with bacterial culture and control. The per cent inhibition over the control was calculated by using the formula (Vincent, 1927).

$$I = \frac{(C-T)}{C} \times 100$$

Where;

I = Per cent inhibition of mycelium

C = Growth of fungal mycelium in control.

T = Growth of fungal mycelium in treatment.

The efficient isolates were selected based on degree of inhibition (> 50 % inhibition). The source and designation of different isolates of *P. fluorescens* are presented in Table 1.

Table 1. Designation of indigenous strains of *Pseudomonas* spp. collected from healthy rhizosphere of groundnut from northern Karnataka

Sl. No.	District	Taluk	Isolate code
1	Bagalkot	Bagalkot	PF-1
2		Mudhol	PF -2
3		Hunagunda	PF -3
4	Yadgir	Shorapur	PF -4
5		Shahapur	PF -5
6	Koppal	Koppal	PF -6
7		Yalburga	PF -7
8		Gangavati	PF -8
9		Kushtagi	PF -9
10	Raichur	Raichur	PF -10
11		Manvi	PF -11
12		Deodurga	PF -12
13		Lingasugur	PF -13
14	Ballari	Ballari	PF -14
15		HuvinaHadagali	PF -15
16		H. B. Halli	PF -16
17		Kudligi	PF -17
18		Hospet	PF -18
19		Shirguppa	PF -19

Biochemical characterization of effective strains of *Pseudomonas fluorescens*

Gram's staining

For Gram staining, 24 h old culture of *P. fluorescens* was used. A loop of bacterial culture was taken and smeared on to clean glass slide and then air dried. Further, it was exposed to flame for two minutes and then covered with crystal violet for one minute. The slide was washed with distilled water and covered with iodine solution for one minute. Thereafter, it was washed with 95 per cent ethyl alcohol and subsequently with distilled water, drained and counter stained (safranin) for one minute. Then it was washed with distilled water, dried with tissue paper, air dried and then subjected for microscopic examination. The violet colour cells indicate Gram positive bacterium and pink colour cells indicate Gram negative bacterium.

KOH test

A loopful of bacterial culture from well grown colony was taken with loop on clean glass slide in a drop of 3 per cent aqueous KOH solution. The loop was raised a few centimetres above the slide and observed for the formation of a mucoid thread. The formation of strands of viscoid material represents the bacterium as Gram negative. Gram positive bacteria normally do not produce viscoid thread (Sallam *et al*., 2013).

Catalase test

The production of catalase is evidenced by the fact that catalase enzyme breaks hydrogen peroxide (H_2O_2) into H_2O and O_2. A loopful of single well isolated colony was placed on a clean microscope slide and added with 3 per cent H_2O_2. A positive result of catalase production was characterized by the rapid evolution of O_2 which is evidenced by bubble formation (Yunting *et al*., 2013).

Starch hydrolysis

The test culture was spot inoculated on to the starch agar plates and incubated at 28 ± 2 °C so as to allow the organism to grow. After the incubation period is over, the inoculated plates were flooded with Lugol's iodine and left few minutes for its action. Formation of clear zone around the colony was taken as positive for the test (Eckford,1927).

Urease test

The young cultures were inoculated to the test tubes containing sterilized ureabroth and incubated for 24-48 h at 28 ± 2 °C. The development of pink color was taken as positive for the test.

Casein hydrolysis

Young cultures of *P. fluorescens* were spotted on to the skimmed milk agar plates and incubated at 28 ± 2 °C for 24 h. The production of clear halo around the colony was taken as positive for the test (Seeley and Vandemark, 1970).

Gelatin liquefaction

The test cultures of *P. fluorescens* were inoculated to the presterilized nutrient gelatin deep tubes, and were incubated at 28 ± 2 °C for 24 h. The tubes were laterkept in a refrigerator at 4 °C for 30 minutes. The tubes with cultures that remained liquefied were taken as positive and those that solidified on refrigeration were taken as negative for the test (Blazevic and Ederer, 1975).

Indole production

The test cultures were inoculated to the pre-sterilized SIM agar tubes. The tubesthen incubated for 48 h at 28 ± 2 °C. After incubation each tube were added with 10 drops of Kovac's reagent. The production of cherry red colour was taken as positive for the indole production.(Ref)

Hydrogen sulfide (H_2S) production

Pre-sterilized tubes containing SIM agarwere stabbed with the test cultures alla long the walls of the test tubes. Inoculated tubes then incubated for 48 h at 28 ± 2 °C. After incubation, the development of black color along the line of the stab was noted as positive for the test (Cappuccino and Sherman, 1992).

Plant growth promoting activities of *P. fluorescens*

HCN production

For the production of HCN, *P. fluorescens* was streaked into nutrient agar plates amended with Glycine (4.4g/l). Later plates were inverted and a piece of What man filter paper no. 1 impregnated with 0.5 per cent picric acid and 2 per cent of sodium carbonate was placed on the lid. Petri plates were sealed with para film and incubated at 28 ± 2°C for 96 h. Change in colour of the filter paper from orange to brown after incubation was considered as production of HCN. A change of colour from yellow to light brown, brown or reddish-brown was recorded as weak (+), moderate (++) or strong (+++) reaction respectively.

IAA production

IAA production was measured by the method described by Patten and Glick (2002) with slight modification. The strains of *P. fluorescens* were cultured bysupple menting with tryptophan 0.5 mM for 48 h. The detection of IAA was determined by the development of pink color.

Siderophore production

Siderophore production was assayed by a plate method using the Chromoazurolsulphonate (CAS) agar method. The CAS agar medium was prepared according toprocedure given by Neilands and Schwayn (1987).

Solutions for CAS medium

1. **Fe CAS indicator solution:** 10 ml of 1mM $FeCl_3.6H_2O$ (in 10Mm HCl) was mixed with 50 ml of an acqueous solution of CAS (1.21mg/ml). The resultant dark puplemixture was added to 40 ml of an acqueous solution of HDTMA (Hexa decyl trimethy lammonium bromide, 1.82 mg/ml). The resulting dark blue solution was autoclaved and cooled to 50 °C.
2. **Buffer solution:** 30.24 g of PIPES (piperazine-N, N'-bis (2-ethane sulfonic acid) was mixed in 750 ml of salt solution containing 0.3 g KH_2PO_4, 0.5 g NaCl and 1.0 g NH_4Cl. The pH was adjusted to 6.8 with 50 per cent KOH and water was added to bring the volume to 800 ml, then 15 g agar was added and the solution was autoclaved and cooled to 50 °C. The 24 h old bacterial culture was spotted into the centre of a CAS agar plate and incubated at 28 ± 2 °C for 5 days, orange halos around the colonies on blue were indicative for siderophore production.

Volatile compound production

The *in vitro* volatile antimicrobial activity was assessed using paired plate method, where two lids of separate Petri plates were taken and poured with 20 ml of PDA for one plate and 20 ml of KB media on another plate. PDA plates were inoculated with the test pathogen *S. rolfsii* (5 mm disc) and upper lids amended with suitable media were inoculated with *P. fluorescens* cultures. The Petri plates were sealed mouth to mouth with para film. Control set consisted of only *S. rolfsii*on PDA inverted over un inoculated KB plates. Paired plates were incubated at 30°C for 7 days as triplicate. After incubation period colony diameter of the fungal pathogen was measured and compared with the control set.

RESULTS

Isolation and maintainance of native isolates of *P. fluorescens*

Isolation of native isolates of *P. fluorescens* from rhizosphere soil was carried out by serial dilution technique on King's B medium (KBM). Totally nineteen isolates were collected from rhizosphere soil representing different districts. All the isolates showed typical character of fluorescence under UV light after 48 h of incubation on KBM indicating that they are fluorescent pseudomonads. Pure culture of all the isolates was maintained at -80 °C by adding glycerol to a final concentration of 40 per cent (v/v).

In vitro* screening of native isolates of *P. fluorescens

Nineteen isolates of *P. fluorescens* isolated from healthy rhizosphere soil of groundnut were screened against *S. rolfsii* for mycelial inhibition by dual culture technique and results are presented in Table 4 and Plate 5. Radial mycelial growth of *S. rolfsii* was 90 mm in control plate as compared to the plates streaked with *P. fluorescens* culture. The per cent inhibition was highly variable among the nineteen isolates ranging from 2.59 to 75.18 per cent. Maximum mycelial inhibition of *S. rolfsii* was recorded in the PF-2 (75.18 %) followed by PF-3 (72.96 %), PF-6 (69.62 %) and least inhibition was recorded in PF-13 (2.59 %) as compared to the control (0.00 %). Later these efficient isolates were characterized morphologically

and biochemically using different tests (Table 2 and Figure 1).

Morphological, biochemical characterization and elucidation of PGPR traits of potential isolates of rhizobacteria

Five efficient strains of *P. fluorescens*, were evaluated for morphological, biochemical characters as well as PGPR traits based on in vitro analysis.

Morphological characterization of efficient *P. fluorescens* strains

The strains of *P. fluorescens* which shown significant inhibition of *S. rolfsii* under *in vitro* were grown on KBM and incubated at 28 ± 2 °C to study various morphological characters. Growth of all the isolates was observed 24 hours after incubation. All the tested isolates produced fluorescence under UV light, slimy, irregular colonies with yellowish green pigmentation. The cell shape was short rod in PF-10, medium rod in PF-6 and PF-7 and long rod in PF-2 and PF-3 (Table 3 and Figure 2).

Biochemical characterization of efficient isolates of rhizobacteria

Study on biochemical characters of efficient strains of *P. fluorescens* was carried out like gram staining, KOH test, catalase test, starch hydrolysis, urease test, casein hydrolysis, gelatin liquefaction, indole test and H_2S test.

Gram's staining

The *P. fluorescens* isolates tested for gram staining indicated that, all the five strains were Gram negative by taking counter stain (safranin) showing pink coloured cells

Table 2. Antagonistic activity of *P. fluorescens* isolates against *S. rolfsii*

Isolate number	Radial mycelial growth* (mm)	Per cent mycelial inhibition*
PF-1	63.66	29.25 (32.75)
PF-2	22.33	75.18 (60.12)
PF-3	. 24.33	72.96 (58.67)
PF-4	59.00	34.44 (35.94)
PF-5	46.00	48.88 (44.37)
PF-6	27.33	69.62 (56.56)
PF-7	30.00	66.66 (54.74)
PF-8	82.00	9.25 (17.72)
PF-9	63.00	30.00 (33.21)
PF-10	41.33	54.06 (47.34)
PF-11	79.00	11.85 (20.14)
PF-12	72.00	10.36 (18.79)
PF-13	80.66	2.59 (9.27)
PF-14	87.66	13.70 (11.10)
PF-15	86.66	29.25 (32.75)
PF-16	63.66	38.80(38.86)
PF-17	55.33	17.77 (24.94)
PF-18	72.00	20.00 (26.57)
PF-19	79.33	11.85 (20.14)
Control	90.00	-
S.Em.±		**0.81**
C. D. at 1%		**3.12**

*Mean of three replications
Figure in parenthesis are arcsine value

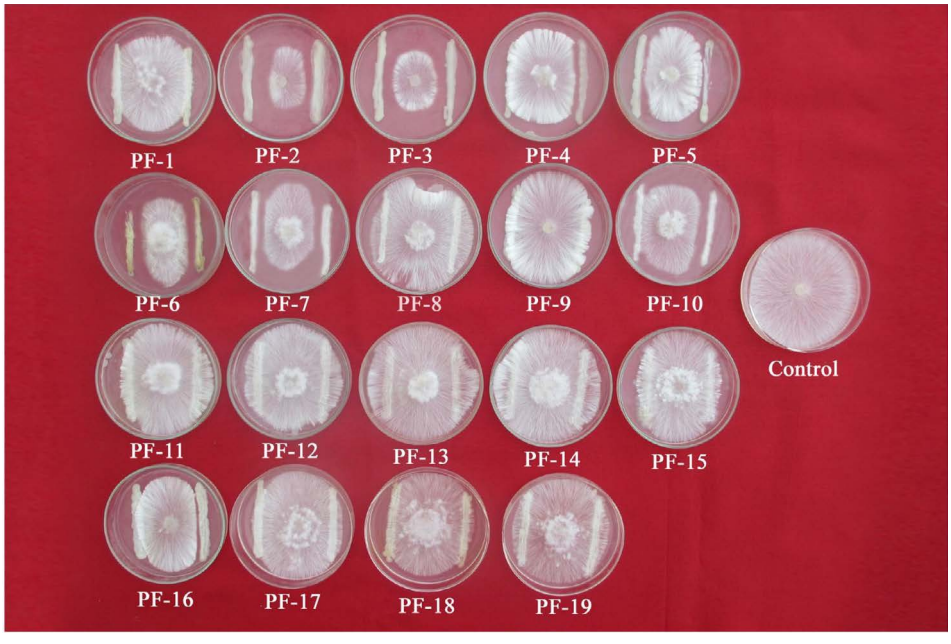

Figure 1. Inhibition of *S. rolfsii* by *P. fluorescens* strains.

when observed under microscope showing gram negative reaction (Figure 3).

Table 3. Morphological characterization of efficient strains of *P. fluorescens*

Isolates	Fluorescence under UV light	Yellow green pigmentation	Cell shape
PF-2	+	+	Long rod
PF-3	+	+	Long rod
PF-6	+	+	Medium rod
PF-7	+	+	Medium rod
PF-10	+	+	Short rod

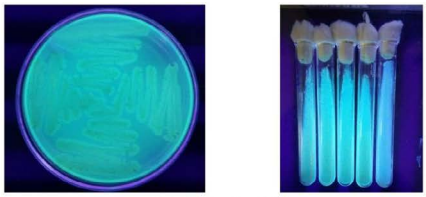

Figure 2. Morphological characterization of *P. fluorescensstrains.*

KOH test

A loopfull of bacterial culture from well grown colony was taken with loop on clean glass slide in a drop of 3 per cent aqueous KOH solution. Observations were made on formation of mucoid thread. If the cell lyses, the liberated cellular DNA makes the mixture viscous or stringy. The positive KOH test indicates a gram negative organism. It was recorded as positive for KOH test for all the strains of *P. fluorescens* by forming a mucoid thread (Figure 3).

Catalase test

A loopfull of single well isolated bacterial colony was placed on a clean glass slide and added with 3 per cent hydrogen peroxide (H2O2). A positive result of catalase production was recorded by producing gas bubbles. All the tested strains of *P. flurescens*, showed positive for the catalase test by forming gas bubbles (Figure 3).

Starch hydrolysis

After incubating the starch agar plates for 24 h which were inoculated with the efficient strains of *P. fluorescens*, flooded with Lugol's iodine and left few minutes for its action. The observations were made on formation of clear zone around the colony. All the efficient strains of *P. fluorescens*have shown clear zone around their colony, which indicates positive for the starch hydrolysis test (Figure 3).

Urease test

The test tubes containing sterilized urea broth were inoculated with the young bacterial culture of strains of *P. fluorescens*. After incubation, development of pink colour was recorded. Results revealed that all the tested strains of *P. fluorescens* developed pink colour which was taken as positive for urease test (Figure 3).

Casein hydrolysis

The skimmed milk agar plates were inoculated with young bacterial culture of strains of *P. fluorescens*. After incubation, the plates were observed for the formation of halo zone around the colony. The positive result indicates the formation of halo zone. The tested strains of *P. fluorescens*produced halo zone around the colony, indicating the positive result (Figure 3).

Gelatin liquefaction

The test cultures efficient strains of *P. fluorescens* were inoculated to the pre-sterilized nutrient gelatin deep tubes. After incubation, the tubes were later kept in a refrigerator at 4 °C for 30 minutes. The tubes with cultures that remained liquefied were taken as positive and those that solidified on refrigeration were taken as negative for the test. In this study, all the efficient strains of *P. fluorescens*have showed positive for the test *i.e*., the tubes remained liquefied even after refrigeration (Figure 3).

Indole test

The test cultures efficient strains of *P. fluorescens*were inoculated to the pre-sterilized SIM agar tubes. After incubation each tube was added with 10 drops of Kovac's reagent. The production of cherry red colour was taken as positive for the indole production. All the efficient strains of *P. fluorescens* showed positive for the test, which were showing formation of cherry red colour (Figure 3).

H_2S production

Pre-sterilized tubes containing SIM agar were stabbed with the test cultures all along the walls of the test tubes. After incubation, the efficient strains of *P. fluorescens* were observed for development of black colour along the line of the stab. The result was taken as positive if there is a formation of black colour along the line of stab. The results of H_2S test revealed that all the tested efficient strains of *P. fluorescens* (Figure 3).

Plant growth-promoting activities of efficient strains of *P. fluorescens*

Plant growth promoting activities like production of HCN, IAA, siderophore and volatile compounds by efficient strains of *P. fluorescens* were evaluated.

HCN production

HCN production by the efficient strains of *P. fluorescens* is known to induce systemic resistance in plants and all the

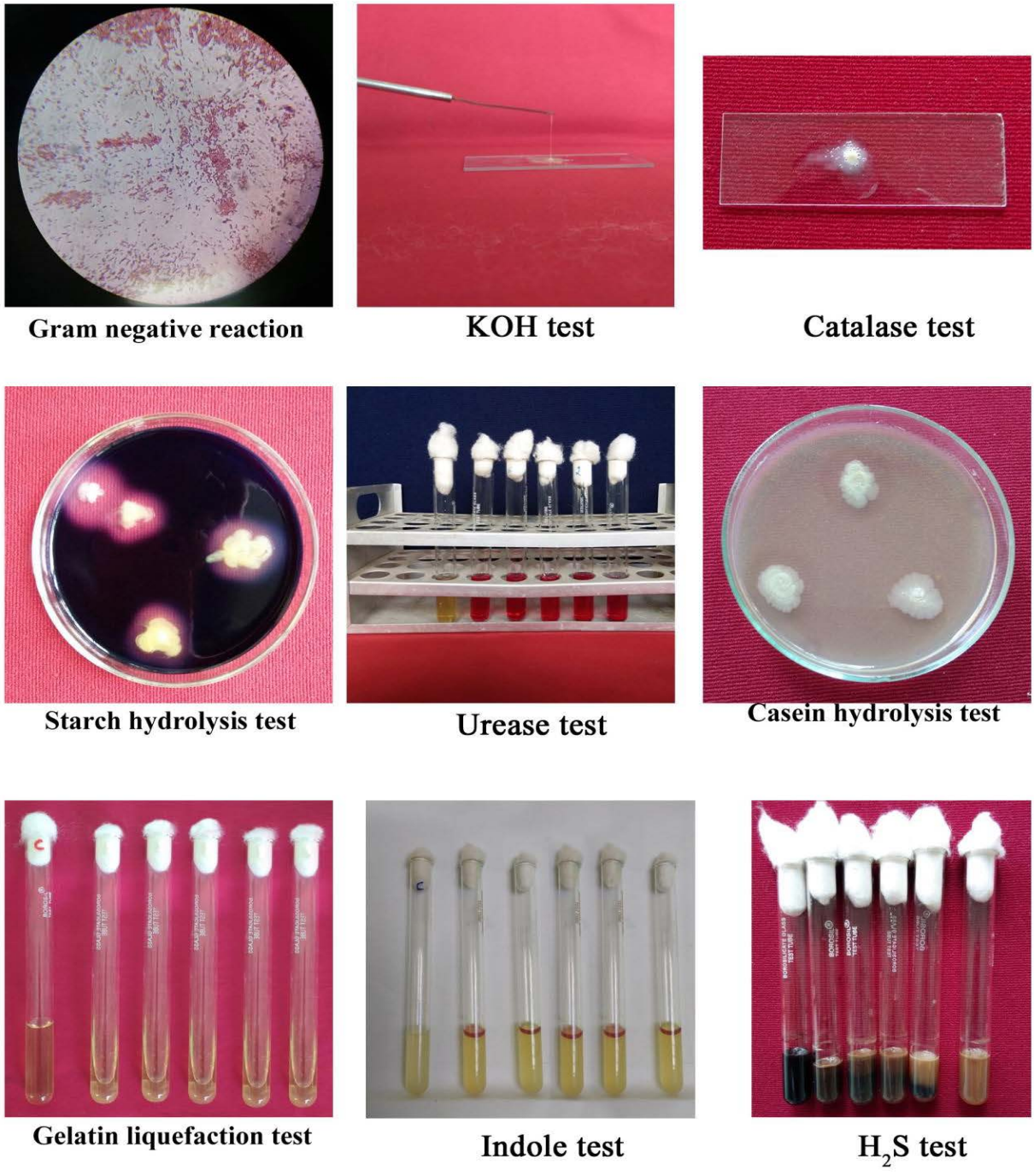

Figure 3. Biochemical characters of efficient *P. fluorescens* strains.

efficient strains of *P. fluorescens* tested were positive which was indicated by change in colour of the filter paper from orange to brown after incubation. Among the five efficient strains of *P. fluorescens*, the efficient strains PF-2 and PF-3 changed the yellow colour of the filter paper to brick red indicating higher level of HCN production compared to other isolates and were scored as strong (+++). The efficient strains PF-6, PF-7 and PF-10 were scored as moderate (++) with light brown colour (Figure 4).

IAA production

As a member of the group of phytohormones, IAA plays an important role in plant growth and is considered the most important native Auxin. In the present study all the efficient strains of *P. fluorescens* were qualitatively tested for the production of IAA and the results revealed that all the tested efficient strains of *P. fluorescens* showed positive for the IAA production (Figure 4).

Siderophore production

Production of siderophore by antagonistic microorganisms is believed to be a mechanism of pathogen suppression. Bacteria with the ability to produce siderophore can increase the availability of iron near the roots for plant uptake thus enhances plant growth. In the present study, all the isolates showed positive for siderophore production on Chrome Azurol S (CAS) agar medium which was indicated by production of yellow/orange coloured zone surrounding the bacterial growth.

Among the tested efficient strains of *P. fluorescens*, the efficient strains PF-2 and PF-3 recorded higher production of siderophore (4 mm orange colour zone), and efficient strains PF-6, PF-7 and PF-10 showed moderate production of siderophore (2-4 mm orange colour zone) (Figure 4).

Volatile compounds production

All the efficient strains of *P. fluorescens* were tested for volatile compound production assay in paired plate technique. The results revealed that the efficient strains of *P. fluorescens* produced considerable amount of volatile metabolites which varied with the isolates. The efficient strains of *P. fluorescens*, PF-2 showed highest per cent mycelial inhibition of *S. rolfsii* (55.55 %) indicating higher production of volatile compounds, followed by PF-3 (52.59 %), PF-6 (50.74 %) and PF-7 (47.33 %). Whereas, the least mycelial inhibition was recorded by the isolate PF-10 (42.22 %) indicating less production of volatile compounds (Figure 4).

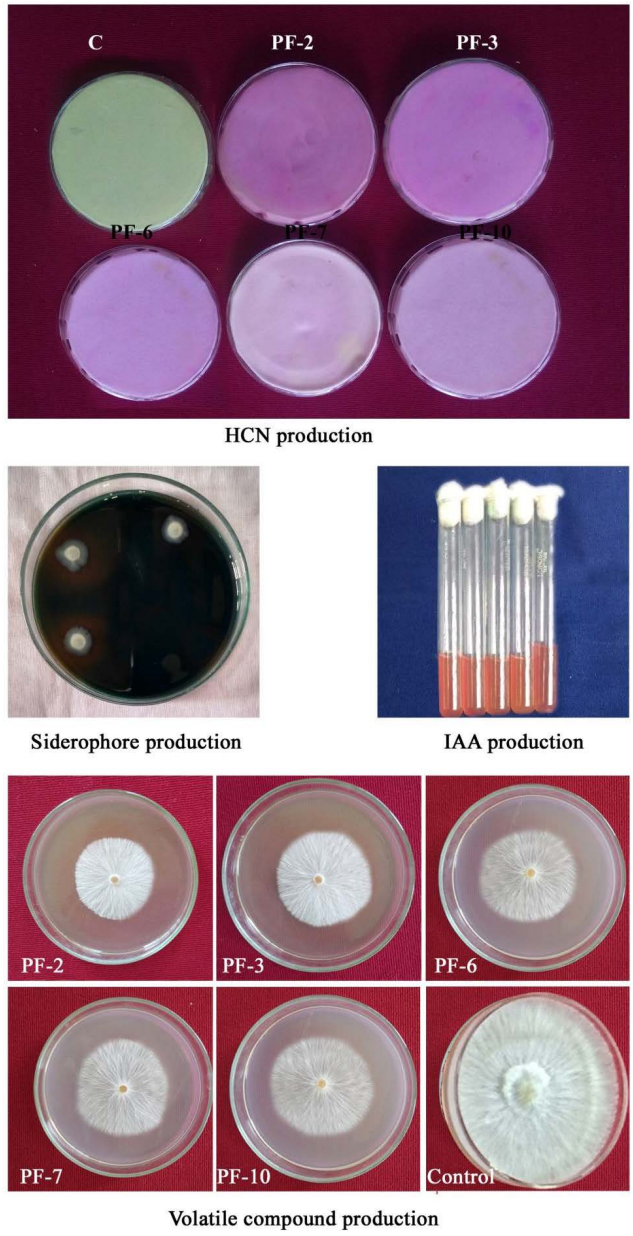

Figure 4. Plant growth promoting traits of efficient strains of *P. fluorescens.*

DISCUSSION

A total of nineteen strains of *P. fluorescence* isolated from the healthy rhizosphere soil of groundnut plant. Isolation of *P. fluorescence* was carried out by serial dilution technique on King's B medium. Shreedevi (2017) isolated *P. fluorescence* isolates from the healthy rhizosphere of groundnut plants on King's B medium. A total of 140 isolates of *P. fluorescence* were isolated from soil samples of healthy groundnut rhizosphere by spread plate technique on King's B agar (Charulatha *et al*., 2013). Nirmala and Reddy (2014) isolated fifty five isolates of *P. fluorescens* from the rhizosphere soil of groundnut fields. Similarly, Priyanka *et al.,* (2017) isolated 62 *P. fluorescence* isolates on King's B medium from the 37 rhizosphere soil samples collected from major soybean growing areas of Dharwad (15) and Belagavi (22) districts of Karnataka using serial dilution and spread plate technique.

All the nineteen isolates showed antagonism against *S. rolfsii*. The per cent inhibition of all the isolates was highly variable ranging from 2.59 to 75.18 per cent. Similar results were obtained with Roopa and Krishnaraj (2017), where they have tested one hundred and thirty four Pseudomonas isolates against *S. rolfsii*. Among them, nineteen isolates were highly inhibiting and appeared significantly different in their inhibition against *S. rolfsii*, 4 isolates showed high inhibition of *S. rolfsii*. The Pseudomonas isolate CA/RN was found as an efficient antagonist against *S. rolfsii* in dual culture technique showing the inhibition upto 94 per cent (Rakh *et al*., 2011). Several authors also reported that *P. fluorescens* inhibited *S. rolfsii* (Priyanka *et al*., 2017).

The results of the experiment revealed that all the five strains showed Gram negative reaction, produced fluorescens under UV light, produced slimy, irregular colonies with light yellowish green pigmentation and they were medium to long

rod shaped. For taxonomic identification of *Pseudomonas*, these characters are considered (Cartwright and Benson 1985). Results were in line with Charulatha *et al.,* (2013) where they identified the Pseudomonas sp. isolated from groundnut rhizosphere based on morphological tests. The bacterial antagonist VSMKU 2013 isolate was Gram negative, rod shaped, and produced yellowish green pigment on King's B medium. Fluorescent Pseudomonas spp. was characterized on the basis of morphological biochemical and physiological tests as prescribed in Bergey's manual of systematic bacteriology. The isolates JS-7, JS-16, JS-24, JS-31 and JS-52 were Gram negative, rod shaped and produced fluorescent pigment under UV light. Similar findings were reported by several workers (Shruthi, 2017).

All the five strains were Gram negative and showed positive for other biochemical characters such as KOH test, catalase test, starch hydrolysis, urease test, casein hydrolysis, gelatin liquefaction, indole test and H2S test. By these tests all these isolates were confirmed as *P. fluorescens* as reported by earlier workers (Mallesh, 2008). Charulatha *et al.,* (2013) reported biochemical characterization of the isolate VSMKU 2013 which showed positive result for gelatin liquefaction, arginine dihydrolase, oxidase, catalase, citrate utilization, protease, and sucrose-positive and hence the strain VSMKU 2013 was identified as *Pseudomonas* sp. Saravanan *et al.,* (2013) characterized *P. fluorescens* biochemically. Results showed that, all ten isolates were positive to catalase, amylase, gelatinase and siderophore production, while only three isolates (Pf5, Pf6 and Pf9) were oxidase positive.

Production of hydrogen cyanide as secondary metabolite is a common mechanism of fluorescent pseudomonads. Among the 5 isolates of *P. fluorescens* tested, the isolates PF-2 and PF-3 changed the yellow colour of the filter paper to brick red indicating higher level of HCN production compared to other isolates and were scored as strong. The isolates PF-6, PF-7 and PF-10 were scored as moderate with light brown colour. Similar findings were observed with Mallesh *et al.,* (2009), they tested the seven strains of rhizobacteria, all the strains tested were positive for hydrogen cynide (HCN) production. Among these HCN producing strains, RB13 and RB50 scored and strains RB10 and RB43 were graded as weak based on the colour development. All the tested isolates of *P. fluorescens* produced HCN, among these isolates, PFP-4 and PFP-11 showed higher level of HCN production, with brick red colour (Shruthi, 2017). Out of the 52 antagonistic isolates, 47 isolates produced HCN showing very good biocontrol potential against the phytopathogens (Priyanka *et al*., 2017).

CONCLUSION

All the tested strains of *P. fluorescens* have shown antagonistic activity against *S. rolfsii* and all these strains have shown plant growth promoting traits like HCN, IAA, siderophore and volatile compounds production. These efficient strains of *P. fluorescens* can be used as a component in integrated disease management for sclerotium rot in groundnut.

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