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BIO-CONTROL EFFICIENCY OF *PSEUDOMONAS FLUORESCENS* AGAINST STEM ROT OF TUBEROSE CAUSED BY *SCLEROTIUM ROLFSII*

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ABSTRACT

Tuberose is an important crop for cut flower and decorations which is a important raw material for the production of high grade perfumes. Last few year very severely affected several pest and diseases. Recent days it was affected by stem rot of tuberose is an important disease that affects the quality of flowers, being a soil borne plant pathogen. In India among the soil-borne fungal diseases of tuberose, stem rot caused by *S. rolfsii* is a potential threat to production and is of considerable economic significance for tuberose. This disease causes severe damage during any stage of crop growth, and yield losses. Chemical compounds have been used to control plant disease but it has adverse effect that creates health hazards for humans and other non-target organisms. The development of safer and environmentally feasible plant disease control alternative has become a top priority. In this context, biological control becomes an urgently needs for modern agriculture. Hence, an attempt was madebio efficacy of biocontrol against for the management of stem rot of tuberose bio-control agents are seems to better management alternative methods than chemical control. The results revealed that ten isolates *P. fluorescens*, Pf7 has recorded effective against *S. rolfsii*. These isolates are further subjected to morphological and biochemical characterization of plant growth promoting activity. All the native *P. fluorescens* isolates tested significantly inhibited the mycelial growth of *S. rolfsii* However, among the isolate Pf7 showed the maximum growth inhibition of *S. rolfsii* up to 75.94 per cent. This was followed by the isolates Pf9 and Pf5) in the decreasing order and the least growth inhibition of pathogen was exhibited by the isolates Pf6. The mycelial growth of *S. rolfsii* was found to be reduced with an increase in the conc. of culture filtrates of *P. fluorescens*, when compared to untreated control.

Keywords: Bio-efficiency, *Pseudomonas fluorescens*, stems rot, tuberose, *Sclerotium rofsi*i.

INTRODUCTION

Tuberose (*Polianthes tuberose* L.) is a commercially important ornamental bulbous plant cultivated in India for cut and loose flower trade. In India the commercial cultivation of tuberose is mainly confined to Bagnen, Kolaghat, Midnapore, Panskura, Ranaghat, Krishnanagar in West Bengal; Guwahati and Jorhat in Assam; Maharashtra and Pune, Nasik, Ahmednagar some parts of Rajasthan, and Satara in Gujrat, Punjab, Mysore, Devanhallitaluk in Karnataka; Guntur, Chitoor, Krishna, Dist. of Andhra Pradesh and Tamil Nadu. In Tamil Nadu the major growing areas are Coimbattore Dharmapuri, Dindugal, Krishnagiri, Tiruvannamalai, Salem, Vellore, Namakkal, Trichy, Sivagangai and Madurai, Thirunelveli and Erode are the leading districts taking up tuberose cultivation (Source – National horticultural board, NHB; Theradimani *et al.,* 2018). Tuberose is a multipurpose ornamental plant and, in the past, it was considered as pest and disease-free crop. Since last few years the crop is found badly infected with diseases like Stem rot, Leaf blight, Sclerotial wilt and alternaria leaf spot. In recent days the stem rot disease caused *Sclerotium rolfsii*, named by Saccardo, 1911, is found be more dangerous in tuberose cultivation. Under severe conditions, the losses due to stem rot disease may go up to 50-60 percent (Kakade, 2007). In Tamil Nadu the losses due to stem rot ranges from 25-40 percent (Theradimani *et al.,* 2018). The pathogen continues to plague the growers and causes economic losses. Thus, it is a severe threat to the tuberose cultivators, if steps are not initiated at the right stage. Besides that, the existing control efforts often met with limited successes.

By concerning the environmental safety and biological control are evolved to be the best alternate practises for the management of stem rot of tuberose Different species of bacteria *Bacillus subtilis*, *Pseudomonas fluorescens* are reported to be effective bio-control agents against several soil borne pathogens (Zape *et al.,* 2014; Asish Mahato and Bholanath Mondala, 2014). Several workers reported that successful application of antagonists can control *S. rolfsii* in various crops (Parmar *et al.,* 2015; Dwivedi *et al.,* 2016; Ramzan *et al.,* 2016). Using compatible strains of plant growth promoting and bio-control microorganisms such as *Pseudomonas* spp., in a is to maximize plant growth and biological control of phytopathogens. In addition, native isolates of certain bio control agents showed superiority over other isolates for the management of soil borne diseases (Dubey and Patel, 2001).

Their applicability as bio-control agents has drawn wide attention because of the production of secondary metabolites such as siderophore, antibiotics, volatile compounds, HCN, enzymes and phytohormones (Nagarajkumar *et al.,* 2004). In vitro antibiosis of *P. fluorescens* strains towards the rice sheath rot pathogen *S. oryzae* demonstrated that

Table 1. Cultural and biochemical characteristics of native *Pseudomonas fluorescens* isolates

S.No	Isolate	Place of collection	Gram stating	Motility	Starch hydrolysis	Gelatin liquefaction	Fluorescent pigment	Estimation of IAA	Siderophore production	HCN production
1	Pf1	Palwadi	-	+	-	-	+	3.2	0.84	8.11
2	Pf2	Varagur	-	+	-	-	+	3.0	0.82	7.98
3	Pf3	Baisuhalli	-	+	-	-	+	3.3	0.84	7.98
4	Pf4	Pulikurai	-	+	-	-	+	2.9	0.86	8.07
5	Pf5	Karagathahalli	-	+	-	-	+	3.0	0.80	8.19
6	Pf6	Kadampatti	-	+	-	-	+	2.9	0.82	8.16
7	Pf7	Perungulathur	-	+	-	-	+	3.5	0.87	8.07
8	Pf8	Vanapurm	-	+	-	-	+	3.0	0.85	8.05
9	Pf9	Royandpuram	-	+	-	-	+	3.4	0.82	7.98
10	Pf10	Thandarampatti	-	+	-	-	+	2.9	0.80	7.98

Table 2. *In vitro* efficiency of *Pseudomonas fluorescens* against *Sclerotium rolfsii* by dual culture method

Isolates	Linear growth (mm)	Inhibition zone (mm)	Per cent growth inhibition over control
Pf1	26.63	10.11	70.41
Pf2	27.31	9.84	69.95
Pf3	31.65	8.85	64.83
Pf4	29.44	9.31	67.28
Pf5	24.30	11.08	73.00
Pf6	39.97	7.31	55.58
Pf7	21.65	12.58	75.94
Pf8	33.58	8.16	62.68
Pf9	23.03	11.84	74.41
Pf10	35.41	7.95	60.65
Control	90.00	0.00	100
	S.Ed CD (0.05)	0.02 0.04	

Data in parantheses indicate angular transformed values

Table 3. Effect of culture filtrate of *Pseudomonas fluorescens* on the mycelia growth of *Sclerotium rolfsii* by Poison food technique

T.no	Concentration of cultural filtrate (%)	Solid medium		Liquid medium	
		Mycelial growth (mm)	Per cent inhibition over control (%)	Mycelial dry weight (mg)	Per cent inhibition over control (%)
T1	10	39.63	44.03	305.11	29.06
T2	20	29.31	67.43	123.73	71.23
T3	30	11.32	87.42	63.34	85.27
T4	40	0.00	100.00	3.89	99.09
T5	Carbendazim (0.1%)	0.11	99.87	1.65	99.61
T6	Control	90		430.15	
	S.Ed CD (0.05)	0.51 1.08		1.12 2.52	

Data in parantheses indicate angular transformed values

this pathogen is sensitive to *P. fluorescen* (Sakthivel and Gnanamanickam, 1987). The biocontrol mechanism to suppress fungal pathogens by *Pseudomonas* spp. normally involves the production of antibiotics and *P. fluorescens* has a gene cluster that produces a suite of antibiotics, including compounds such as 2,4-diacetylphloroglucinol (DAPG), phenazine, pyrrolnitrin, pyoluteorin and biosurfactant antibiotics (Angayarkanni *et al.,* 2005). Fluorescent *Pseudomonas* is uniquely capable of synthesizing many of these antibiotics, not only to enhance its own fitness but also to help in the maintenance of soil health and bio-protection of crops from pathogens.

MATERIALS AND METHODS

Isolation and purification of *P. fluorescens*

Pseudomonas fluorescens isolates were collected from tuberose rhizosphere soil samples collected from ten different locations were used. The collected soil samples were taken and suspended in 10 ml sterile distilled water to

get 10^{-1} dilution. Serial dilutions were made to get dilutions up to 10^{-6}. One ml of 10^{-5} and 10^{-6} dilution were pipetted out into sterile Petri plate and 15 ml of King's B medium (King *et al.,* 1954) was added and rotated clockwise and anticlockwise. The plates were incubated at room temperature (28 ± 2°C) for 48 hours for development of bacterial colonies. The Identified isolates were designated as *P. fluorescens* (Pf_1 to Pf_{10}).

Biochemical test for *Pseudomonas* spp.

For the identification of *Pseudomonas* sp. isolates, certain biochemical test were conducted according to Bergey's manual for Determinative bacteriology (Breed *et al.,* 1989). The identified *P. fluorescens* isolates were designed as Pf1 – Pf10 and used for subsequent experiments.

Gram staining: A loopful bacterial culture was transferred on a clean slide and a smear was made air dried and heat fixed. The smear was flooded for 1 min. with ammonium oxalate crystal violet. Excess strain was poured off and the slide was washed in a gentle stream of water. Lugol's iodine solution was applied and allowed to remain for 1 minute decolorized with 95 per cent ethyl alcohol. The smear was washed in gentle stream of water and counter stained with safranin for 30 seconds. The Gram-negative cells appeared red in colour and gram-positive cells appeared violet in colour (Cyrobree and Hindshill, 1975).

Motility: Semisolid agar medium was prepared and the bacterial isolates were incubated at 30°C for 72 h. and observed for motility. The distance of growth from the point of stab showed motility.

Starch hydrolysis: Filter paper was dipped in a dry old culture suspension and were placed on petri dishes containing starch agar medium and incubated for two days. The plates were then flooded with one per cent iodine solution. A colourless halo around the growth and blue colour in the rest of the plates showed utilization of starch by the microorganism (Stolpe and Godkeri, 1981).

Gelatine liquefaction: Filter paper discs were dipped in a day-old culture suspension and were placed on Petri-dishes containing gelatin nutrient agar medium. The petri dishes were incubated at 30^0C for two days and then flooded with 12.5 per cent HgCl2 solution. The development of yellow halo around the utilization of gelatin indicates positive results (Stlope and Godkeri, 1981).

Fluorescent pigment: The test tubes containing sterilized King's B medium were inoculated with the isolates of *Pseudomonas* spp. and incubated for five days and observed. Development of yellowish green fluorescent pigment was observed under the UV light (366 nm) which indicated positive results.

Estimation of IAA: Indole acetic acid (IAA) in the methanol fraction was determined by employing Salper reagent (Golden and Paleg, 1957). To 1.5 ml distilled water in a test tube 0.5 ml of methanol was mixed. Four ml fresh Salper reagent was rapidly added, kept in complete darkness for one hour and read in spectrophotometer at 535bnm. From a standard curve prepared with known concentration of IAA, the quality of IAA was in the filtrate was calculated (1 division = 0.307 µg of IAA).

Extraction of siderophore from the medium: The spent culture fluid was separated from the cells by centrifugation a 7000 rpm for 15 min. The supernatant was concentrate to one fifth of the original volume by the flash evaporation of 450C. Catechol type phenolates were extracted with ethyl acetate from the culture supernatant twice with an equal volume solvent at pH 2.0. The ethyl acetate layer was removed and evaporated to dryness and the residues were dissolved in a minimum quantity of distilled water, while hydroxamate types were measured from the untreated culture supernatant (Payne, 1994).

HCN Production: Effective endophytic *P. fluorescens* isolates were grown at 28 ± 2°C on rotary shaker in Tryptic soya broth (TSB). Filter paper (what man No. 1) was cut into uniform strips of 10 cm long and 0.5 cm wide saturated with alkaline picrate solution placed inside the conical flasks in hanging position. After incubation at 28 ± 2°C for 48 hr. the sodium picrate present in the filter paper was reduced to reddish compound in proportion to the amount of hydrocyanic acid evolved the colour eluted by placing the filter paper in a clean test tube containing 10 ml of dist. water and absorbance was measured at 625 nm (Sadasivam and Manickam, 1992). Three replications were maintained for each treatment.

Dual culture technique of *P. fluorescens*

The antagonistic activity of bio control agents (Pf_1-Pf_{10}) against *S. rolfsii* was tested by dual culture technique (Dennis and Webster, 1971). At one end of the sterile Petri dish containing 15 ml of sterilized and solidified PDA medium. *P. fluorescens* one cm long streak was gently made onto the medium using two days old culture. Similarly, at the opposite end seven mm mycelial disc obtained from seven days old culture of *S. rolfsii* was placed and incubated. A control was maintained by inoculating *S .rolfsii* alone at one end of the Petri dish. The plates were incubated at room temperature (28 ± 2°C) for seven days. The radial growth (in mm) of the pathogen and the test antagonists and the extent of the inhibition zones (in mm) developed between the two colonies were measured. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The percentage growth inhibition (I) was calculated using the formula given below (Datta *et al.,* 2004)

$$\text{Per cent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where, C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual plate

I - inhibition Per cent

Based on the dual culture technique the effective *T. viride*

were identified and used for further studies.

Poison food technique of *P. fluorescens*

PDA medium was prepared in 100 ml conical flask and autoclaved. Filtered antagonistic poison of *Pseudomonas* and of 5, 10, 15 and 20 ml were added to 45-, 40-, 35- and 30-ml aliquots respectively in flasks so as to get the final concentration of 10, 20, 30 and 40 per cent. The incorporation of fungicide carbendazim @ 0.1% in the medium was used for comparison. PDA medium without culture filtrate served as control. Each plate was inoculated at the centre with a seven old culture disc (6 mm) of pathogen and incubated at room temperature (28±2^{0}C). In case of *Pseudomonas fluorescens* the pathogen was streaked at the centre and incubated at room temperature (28±2^{0}C). Three replications were maintained for each treatment. The diameter of the mycelial growth of pathogen was measured after seven days.

RESULT AND DISCUSSION

Isolation of *P. fluorescens*

Ten isolates of *P. fluorescens* were isolated from rhizosphere soil of various locations in Tamil Nadu, which was named as Pf_1-Pf_{10}. Among the 10 isolates *P. fluorescens*, Pf_7 has recorded effective against *S. rolfsii*. These isolates are further subjected to morphological and biochemical characterization of plant growth promoting activity.

Cultural and biochemical characteristics of *P. fluorescens* isolates

The results of the Gram reaction and biochemical tests performed for the identification of the effective native isolates of *P. fluorescens* showed that all the isolates produced similar results with regard to gram staining (negative), motility (positive), Starch hydrolysis (negative), gelatin liquefaction (positive) and fluorescent pigmentation (positive). All the isolates showed positive results in IAA production. Among the isolates Pf_7 produced more quantity (3.5) of IAA followed by Pf_9, Pf_3 and Pf_1 (3.4, 3.3 and 3.2 respectively) in the decreasing order of merit. Similarly, the isolates Pf_7 produced recorded maximum siderophore (0.87) production followed by Pf_4, Pf_8, Pf_3 and Pf_1 (0.86, 0.85, 0.84 and 0.84) in the decreasing order of merit. All the isolates positive results with regard to hydrogen cyanide production (Table 1). The identified isolates were designated as Pf_1 to Pf_{10} (Table 1).

***In vitro* efficacy of *P. fluorescens* against *S. rolfsii* by Dual culture method**

In general all the native *P. fluorescens* isolates tested significantly inhibited the mycelial growth of *S. rolfsii* (Table 2). However, among the isolate Pf_7 showed the maximum growth inhibition of *S. rolfsii* up to 75.94 per cent. This was followed by the isolates Pf_9 (74.41) and Pf_5 (73.00) in the decreasing order and the least growth inhibition of pathogen (58.52 %) was exhibited by the isolates Pf_6.

Effect of culture filtrate of *P. fluorescens* on the mycelia growth and mycelial dry weight of *S. rolfsii* by Poison food technique

The results of the *in vitro* studies conducted to find out the effect of culture filtrate of *P. fluorescens* on the mycelial growth and mycelial dry weight of *S. rolfsii* are summarized in table 3. The mycelial growth of *S. rolfsii* was found to be reduced with an increase in the conc. of culture filtrates of *P. fluorescens* and the reduction was significantly the maximum in the case of with *P. fluorescens* 39.63, 29.31, 11.32 and 0.00 mm at 10, 20, 30 and 40 per cent conc. of the culture filtrate respectively as against the maximum growth of 90 mm in the control. The same trend was maintained in the case of liquid medium assay. The flasks inoculated with pathogen and amended with culture filtrate of *P. fluorescens* recorded significant reduction in the mycelial dry weight whereas, the flasks inoculated with *S. rolfsii* alone (control) recorded the maximum mycelial dry weight (430.15mg). The minimum mycelial dry weight (1.65 mg) of *S. rolfsii* was recorded in 40 per cent conc. of the culture filtrate of *P. fluorescens* which was at par with *P. fluorescens* @ 40% and carbendazim @ 0.1 per cent (3.89 and 1.65 mg respectively).

Morphological and biochemical characterization

Based on the antagonistic potential and other characteristics, seven isolates of *P. fluorescens* were studied in detail for colony, colour, growth type, fluorescence, and cell shape. The results of the biochemical tests performed for the identification of the effective native isolates of *P. fluorscens* showed that all the isolates produced similar results with regard to gram staining (negative), starch hydrolysis (negative), gelatin liquefaction (positive), catalase test (positive), oxidase test (positive) and fluorescent pigmentation (positive). All the isolates showed positive results in IAA production. Among the isolates Pf_7 produced more quantity (3.5) of IAA followed by Pf_9, Pf_3, Pf_1, Pf_8 and Pf_2.(3.4, 3.3, 3.2, 3.0 and 3.0 respectively) in the decreasing order of merit. All the isolates recorded positive results with regard to hydrogen cyanide production. The isolate Pf_7 recorded maximum production of siderophore.

***In vitro* efficiency of *P. fluorescens* against *S. rolfsii* (Dual culture)**

In the present investigation, out of ten isolates of *P. fluorescens*, isolate Pf_7 had the highest distance of inhibition (75.94 %) of *S. rolfsii* growth in dual culture assay. Similar inhibitory effect of *P. fluorescens* on wide range of fungi, including *S. rolfsii,* has been documented by several researchers. Kishore *et al.,* (2005) found 94% inhibition of *S. rolfsii* in dual culture assay with *Pseudomonas*. Kuarabachew *et al.,* (2007) and, Chakravarty and Kalita (2012) obtained 1.2 to 2.4 cm and 10.2 mm inhibition zone on King's B agar medium, respectively, by *P. fluorescens* against *Ralstonia solanacearum,* in dual culture assay.

Manjunatha *et al.,* (2012) reported that *P. fluorescens* showed the maximum inhibition of mycelial growth of *S. rolfsii* through dual plate technique.

O'Sullivan and O'Gara (1992) reported that *Pseudomonas* spp. well known for production of broad-spectrum antibiotics such as 2,4-diacetylphloroglucinol and, antibiosis has been proved to be a major mechanism involved in their biological activity. HCN and siderophores produced by *Pseudomonas* spp. also involved in their antifungal activity.

Voisard *et al.,* (1989) observed that the suppression of black rot of tobacco was due to the production of HCN by *P. fluorescens* and also HCN induced resistance in the host plant. Volatile cyanide production by rhizosphere *Pseudomonas* has been reported by several workers (Bakker *et al.,* 1991; Haas *et al.,* 1991; Rangeshwaran and Prasad, 2000; Ganesan, 2004). Singh *et al* (2003) found that *Pseudomonas* spp. was effective in the control of *S. rolfsii* infection in chickpea by induction of phenolic acid. Fakhouri and Buchenauer (2003) reported that on King's B agar medium, the isolate CW2 effectively inhibited mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici.* This isolate also produced cyanide and different antimicrobial compounds such as phenazine-1-carboxylic acid (PCA) and 2-OH-phenazine (2- OH-Phz)

Effect of culture filtrate of *P. fluorescens*on the mycelial growth and mycelial dry weight of *S. rolfsii*

The mycelial growth of *S. rolfsii* was found to the reduced with an increase in the concentration of culture filtrates of all the isolates tested and the reduction was significantly the maximum in the case of *P. fluorescens* (Pf_7) at all the concentrations tested (Table 2). Similarly Chanutsa *et al.,* (2014) reported that the isolates culture filtrate of three bacteria completely inhibited the growth of *S. rolfsii.* The antifungal metabolites produced by *P. fluorescens* might be attributed as the reason for the reduction in the growth of pathogen. *P. fluorescens* was known to produce an array of low-molecular weight metabolites some of which were potential antifungal agents (O'Dowling and O'Gara, 1994). Several studies indicated the production of lytic enzymes which was correlated with antagonistic potential of *P. fluorescens* against various soil borne plant pathogen (Velazhahan *et al.,* 1999; Meena *et al.,* 2001). The cell free extracts of Pseudomonads effectively inhibited the growth of *R. solani* (Saxena *et al.,* 1995). Culture filtrate of *P. fluorescens* was the most effective in inhibiting the mycelial growth of *S. rolfsii* (Revathy and Muthusamy, 2003). Culture filtrate of *P. fluorescens* isolates I_7 inhibited mycelial growth of *S. rolfsii in vitro* (Venkatesh, 2013).

REFERENCE

Angayarkanni T, Kamalakannan A, Santhini E, Predeepa D (2005). Identification of biochemical markers for the selection of *Pseudomonas fluorescens* against *Pythium* spp. In: Asian conference on Emerging *Trends in Plant-Miceobial Interactions*. University of Madras, Chennai. Pp. 295-303.

Asish Mahato, Bholanath Mondala (2014) *Sclerotium rolfsii* its isolates variability, pathogenicity and an eco-friendly management option. *Journal of Chemical, Biological and Physical Sciences* 4(4): 3334-3344

Bakker PAHM, Vanpeer R, Schippers B (1991) Suppression of soil borne plant pathogens by Fluorescent *Pseudomonas*, mechanism and prospects, biotic interactions and soil borne diseases (Beemster. A.B.R, Boller,G.Y., Gerlagh. M, Russen, M.A., Schippers, *B. and Tempel, A. eds*.) Elsevier, Amsterdam 217- 230

Biswas B, Bhattacharjee SK, Naveen KP (2002) Tuberose: AICRP on floriculture technical bulletin, issue 21. All India Coordinated Research Project on Spices (AICRP), New Delhi, India 1-25

Chakravarty G, Kalita MC (2012) Biocontrol potential of *Pseudomonas fluorescens* against bacterial wilt of brinjal and its possible plant growth promoting effects. *Ann Biol Res* 5083–5094

Chanutsa N, Phonkerd N, Bunyatratchata W (2014). Potential of *Pseudomonas aeruginosa* to control *Sclerotium rolfsii* causing stem rot and collar rot disease of tomato. *Journal of Advanced Agricultural Technologies* 1(2):132- 135

Cook RJ (1977) Management of the associated microbiota. In Plant diseases: An Advanced *Treatise Academic Press*, New York pp. 245-266

Datta BS, Das AK, Ghosh SN (2004) Fungal antagonists of some plant pathogens Mycopathology 1: 15-17

Dubey SC, Patel B (2001) Evalution of fungal antagonists against *Thanetephorus cucumeris* causing web blight of urd and mung bean. *Indian Phytopath* 54: 206-209

Dwivedi SK, Ganesh Prasad (2016) Integrated Management of *Sclerotium rolfsii*: An Overview.European *Journal of Biomedical and Pharmaceutical sciences* 3(11): 137-146

Ehteshamul-Haque S, Zaki MJ, Validy AA, Ghaffar A (1998). Effects of organic amendments in the efficacy of *Pseudomonas aeruginosa* in the control of root rot disease of sunflower. *Pak. J. Bot* 30: 45-50

Fakhouri W, Buchenauer H (2003) Characteristics of fluorescent pseudomonad isolates towards controlling of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. *Journal of Plant Diseases and Protection* 110 (2): 143–156

Ganesan S (2004) Studies on the biocontrol of soilborne plant pathogens. Ph.D. Thesis submitted to Madurai Kamaraj University, Madurai, Tamil Nadu

Gautam M, Kolto SJ (1979) Control of *Sclerotium* wilt of sunflower through organic amendments in soil. *Plant Soil* 53: 233-238

Haas K, Keel C, Laville Y, Maurhofer M, Oberhansli TF, Scheider U, Viosard C, Wuthrich B, Defago G, Hannacks H, Verma DPS (1991) Secondary metabolities of *Pseudomonas fluorescence* strain CHAO involved in the suppression of root diseases. In: Advances in molecular genetics of plant microbe interactions. Inter taken, Switzerland. Pp 450- 456

Johnson M, Subramanyam K, Balaguravaiah D, Sudheer MJ (2003) Management of stem rot in groundnut through soil amendments. *Ann Plant Prot Sci* 11(1):83-85

Karthikeyan A, Karunanidhi K (1996) Influence of organic amendments on the intensity of fusarium wilt of Banana. *Plant Dis Res* 11(2):180-181

King EO, Ward MK, Raney DE (1954) Two simple media for the demonstration of pyocyanine and fluorescein. *Journal of Laboratory and Clinical Medicine* 44: 301-307

Kishore (2005) Management of late leaf spot of groundnut (Arachis hypogaea) with chlorothalonil-tolerant isolates of Pseudomonas aeruginosa. *Plant Pathology 54: 401–408*

Kuarabachew H, Assefa F, Hiskias Y (2007) Evaluation of ethiopian isolates of *Pseudomonas fluorescens* as biocontrol agent against potato bacterial wilt caused by *Ralstonia solanacearum*. *Acta Agric* 90 (2): 125-135

Kulkarni SA, Kulkarni S (1998) Effect of organic amendments and green manuring on the survival of the collar rot fungus of groundnut. *Curr Res* 27:125-136

Kulkarni SA, Kulkarni S, Kulkarni S (1995) Effect of organic amendments and green manuring on the survival of collar rot fungus of groundnut. Current Res Univ Agric Sci Bangalore 24(8):135- 136

Manjunatha H, Naik MK, Patil MB, Lokesha R and Vasudevan SN (2012) Isolation and characterization of native fluorescent pseudomonads and antagonistic activity against major plant pathogens. *J Agric Sci* 25(3): 346-349

Mathur K, Bansal RK, Gurjar RBS (2006) Organic management of fusarium wilt of fenugreek (*Trigonella foenumgraecum*), a seed spice. *J Mycol Plant Pathol* 36(1):94-95

Mallavarapu GR (1995) Essential oils from some aromatic ornamental plants. Ornamental Plants Eds: *Advance in Horticulture* 12: 1046

Meena B, Marimuthu T, Vidhyasekaran P, Velazhahan R (2001) Biological control of root rot of groundnut with antagonistic *Pseudomonas fluorescents* strains. *Journal of plant Disease* Protection 108:1-13

Nagarajkumar M, Bhaskaran R, Velazhahan R (2004). Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseusomonas fluoresces* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. *Microbiology Research*. 159(1): 73-81.

Narsimhalu G, Bhaskaran R (1987) Effect of organic amendments on root rot of soybean caused by *Macrophomina phaseolina* In workshop on biological control of plant diseases (Abst.) held on 10-12 March, TNAU, Coimbatore 3

O' Dowling DN, O'Gara F (1994) Metabolites of *Pseudomonas* species involved in the biocontrol of plant disease. TIBTECH 12:133-141

O'Sullivan DJ, O'Gara F (1992) Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiol Rev* 56: 662-676

Parmar HJ, Mohamed MH, Bodar NP, Umrania VV, Patel SV, Lakhani HN (2015) *In vitro* antagonism between phytopathogenic fungi *Sclerotium rolfsii* and Trichoderma Strains. *International Journal Applied Sciences Biotechnology* 3(1): 16-19

Purkayastha RP, Menan U (1985) Exteneous supply of organic additives affecting, nodulation, phytoalexin synthesis and disease susceptibility of soybean. *J Trop Plant* Dis 2(1):9-18

Ramkrishnan G (1981) Studies on seedling diseases of cotton caused by *Rhizoctonia solani*. Ph.D. Thesis, TNAU, Coimbatore, P 128

Ramzan N, Noreen N, Perveen Z, Shahzad S (2016) Effect of seed pelleting with bio-control agents on growth and colonization of roots of mungbean by root-infecting fungi. *J Sci Food Agric* 7553

Randhawa GS, Mukhopadhyay A (1986) Tuberose. In: Floriculture in India. Allied publishers Private Limited, New Delhi 425-426

Rangeshwaran R, Prasad RD (2000) Biological control of *Sclerotium* rot of sunflower. *Indian Phytopath* 53: 444- 449

Revathy N, Muthusamy M (2003) *In vitro* inhibition of jasmine wilt pathogen *Sclerotium rolfsii* by antagonists. *Journal of Ecology* 15:319-320

Sakthivel N and Gnanamanickam SS. 1987. Evaluation of *Pseudomonas fluorescens* for suppression of sheath rot disease and for enhancement of grain yields in rice, *Oryza sativa*, L. *Applied Environmental Microbiology* 53(1):2056-2059.

Saxena KR, Patel KA, Rao KK, Mehta MH (1995) Role

of *Pseudomonas fluroscens* Pseudomonads in controlling plant disease. *Indian Journal of Mycology and plant pathology* 25: 131-132

Singh PV, Panwar S, Kumar J (2003) Response of tuberose to plant growth regulators. *J Ornamental Hort* 6(1):80-81

Singh RK, Dwivedi RS (1989) Effect of plant parts and product on morphology and growth of *S. rolfsii* a foot root rot pathogen of barley. *Acta Bot Indica* 17:125-127

Velazhahan R, Samiyappan R, Vidhyasekarn P (1999) Relationship between antagonistic activites of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* and their production of lytic enzymes. *Journal of plant Disease and Protection* 106: 244-250

Venkatesh A (2013) Studies on Biological Management of Collar rot of Pepper mint Caused by *Sclerotium rolfsii* Sacc. M.sc.Thesis, Annamalai University, Tamil Nadu, India

Voisard C, Keel C, Haas D, Defago G (1989) Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J 8:351-358

Waghe SV, Lanjewari RD (1981) Effect of different oil cake amendments on seedling survival against wilt, root and stem rot diseases of sesamum. *Oilseeds J* Pp 69-73

Yadav RP (1986) Studies on antifungal properties of certain plant extracts. (Abst.) MPAU, Rahuri, Ahamad Nager, Maharashtra P 181

Zape AS, Gade RM, Singh R, Deshmukh VA (2014) Efficacy of different antagonist against the *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium solani. The Bioscan*, 9(4): 1431-1434.