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Efficiency of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Phytophthora* spp. in citrus

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ABSTRACT

Phytophthora was recorded in all the soil samples collected from citrus orchards and nurseries. Out of 25 soil samples Isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* recorded from 11 and 5 soil samples, respectively. *In vitro*, four isolates of *Pseudomonas fluorescens* and two isolates of *Bacillus subtilis* were found effective against *Phytophthora parasitica* and other fungal pathogens of citrus *viz.*, *Pythium* sp., *Fusarium* sp. and *Colletotrichum gloeosporioides*. Efficacy tested under sick soil method, only Bs-K_1 (a) isolate of *Bacillus subtilis* was found most effective giving maximum disease control (81.34%), while other isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* were not found much efficient.

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INTRODUCTION

Citrus industry in India rank 6th in production in the world, occupying 10 per cent of area under fruit crop in country. It is third largest fruit industry after Banana and Mango. More than 150 diseases caused by fungi, bacteria, viruses and nematodes have been reported on different *Citrus* spp. cultivated in the country. The incidence and severity varies with the *Citrus* spp. / variety, age of plant, management practices, climatic conditions of the region, these all are important factors associated with citrus diseases.

Among soil borne diseases *Phytophthora* spp. are mainly responsible for wide destruction, causing varied symptoms *viz.*, damping off, seedling rot, gummosis, fruit and root rot, leaf fall and fruit drop. More than 20 per cent seedling mortality has been reported in Central India due to *Phytophthora* spp. (Naqvi, 2003). Distribution of different species *viz.*, *Phytophthora capsici*, *Phytophthora palmivora* and *Phytophthora parasitica* have been reported to cause 10-30 per cent root rot incidence from different states in North Eastern region on Khasi Mandarin (Naqvi, 2006). The management practices of *Phytophthora* spp. on citrus includes use of numerous pesticides, some which known to cause various health hazard. The regular use of metalaxyl in management of *Phytophthora* diseases in

citrus possesses the threat of development of resistant strains of the pathogen. Metalaxyl resistance is common and widespread in *Phytophthora infestans* on potato and other crop (David and Yigal, 1988).

To avoid the use of hazardous chemicals in citrus production, an emphasis has been given to find out suitable bioagent for the managements of *Phytophthora* diseases in citrus. Therefore, utilization of microbial antagonist for citrus disease management seems to be the need of an hour. Use of microbial antagonist hold special significance being an ecofriendly, disease suppression and plant growth promotion ability, target specific, biodegradable, cost effective and compatible with other mechanisms and inclusion in efficient integrated disease management strategies for citrus.

MATERIAL AND METHODS

The present investigation was carried out during 2013-2014 at Deparrtment of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MS). The soil samples were collected from different locations July to September, 2013 from the rhizosphere of citrus orchards and nurseries.

Source of pathogen and bioagents :

The fungi and bioagents involved in the studies were obtained from soil and infected samples by serial dilution and tissue isolation method.

Methods :

Isolation of *Phytophthora* and Bacterial bioagents from soil samples.

Isolation of fungus and bioagents was done from soil samples collected from different locations of citrus rhizosphere by serial dilution method. Dilution of 10^4 and 10^8 was used for the isolation of fungus and bacteria, respectively. The isolation of *Phytophthora* was done on selective medium. King's B and nutrient agar medium were used for isolation of *Pseudomonas fluorescens* and *Bacillus subtilis*, respectively.

Raising of seedlings :

Seeds of Rough lemon were collected from All India Co-ordinated Research Project on Tropical Fruits, Dr. P.D.K.V., Akola. The seeds were washed and surface disinfected with sodium hypochlorite solution. Then seeds were sown in sterilized potting mixture. Two months old seedlings were used for further study.

***In vitro* efficacy of bioagents against *Phytophthora parasitica* by dual culture method :**

Lawn culture of test fungi on PDA and broth culture of *Bacillus subtilis* and *Pseudomonas fluorescens* were prepared. In sterilized petriplates autoclaved melted Potato dextrose agar was poured and allowed to solidify for obtaining leveled surface. These plates were inoculated with the culture of test fungi. The culture of *Pseudomonas fluorescens* and *Bacillus subtilis*, were inoculated in the conical flask containing 50 ml broth of King's B and NA, respectively, three days old broth culture was used and seven days old culture of test fungi. Petriplates containing sterilized solidified PDA were inoculated by test fungi. Three plates per bacterial isolates were used. Disc of 6 mm fungus lawn culture was cut with sterilized cork borer, lifted and transplanted aseptically in the culture of each petriplates. The loopful of broth culture of the bacterial isolates were streaked on both sides of fungal disc. The control plate were kept where the culture disc grown under same condition on plain PDA without streaking of bacterial isolates. The inoculated plates were incubated at 22 ± 1^0 C for seven days.

The mycelial inhibition was calculated by the formula given below based on the average of colony diameter. The data of mycelial growth was also subjected to stastistical analysis and conclusions were drawn, Gomez and Gomez (1984).

$$PI = \frac{C - T}{C} \times 100$$

where,

PI = Per cent inhibition,

C = Growth in control plate

T = Growth in treatment plates

Preperation of carrier base cultures and efficacy of bioagents by sick soil method :

Carrier based culture of *Bacillus subtilis* and *Psuedomonas fluorescens* were prepared. *Bacillus subtilis* was inoculated in nutrient broth and *Pseudomonas fluorescens* was inoculated in King's B broth. Eight days old broth cultures were used for preparing carrier base cultures of bioagents in 1:2 proportion *i.e.* 100 ml broth culture of bacterial bioagents

was mixed in 200 g of sterilized talcum powder and air dried for a day.

Efficacy of bioagents under pot condition was carried out by sick soil method. Purified isolate of *Phytophthora parasitica* was multiplied on large scale by using sand bajara (Pearl millet) grain medium. The plastic trays having capacity of 5 kg were surface disinfected with sodium hypochlorite. The autoclaved potting mixture (Soil 5 part + Sand 3 part + FYM 1 part) were inoculated with mass multiplied culture of *Phytophthora* at 9:1 proportion of potting mixture. To the pre inoculated potting mixture 10 g carrier based cultures of bacterial bioagents as mentioned below were mixed and filled in plastic trays (Table A).

Table A : Treatment details

T_1	Soil inoculated with carrier based culture of Bs-K_1 (a) @ 10 g / kg of soil + *Phytophthora* inoculum
T_2	Soil inoculated with carrier based culture of Bs-K_6 (f) @ 10 g / kg of soil + *Phytophthora* inoculum
T_3	Soil inoculated with carrier based culture of Pf-K_4 (d) @ 10 g / kg of soil + *Phytophthora* inoculum
T_4	Soil inoculated with carrier based culture of Pf-K_5 (e) @ 10 g / kg of soil + *Phytophthora* inoculum
T_5	Soil inoculated with carrier based culture of Pf-N_1 (a) @ 10 g / kg of soil + *Phytophthora* inoculum
T_6	Soil inoculated with carrier based culture of Pf-N_2 (b) @ 10 g / kg of soil + *Phytophthora* inoculum
T_7	Inoculated with *Phytophthora* only (Control)

Pf - *Pseudomonas fluorescens* Bs - *Bacillus subtilis*

Two months old seedlings of Rough lemon were uprooted and washed in water and disinfected with the help of sodium hypochlorite solution. Twenty five seedlings per tray were maintained with three replicates and observations were recorded for mortality of the seedlings. Soil inoculated with the culture of *Phytophthora parasitica* without bioagent was served as control.

The roots of infected seedlings along with soil were chaffed in sterilized distilled water and re-isolation was done by following serial dilution method on selective medium of *Phytophthora.*

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Efficacy of *Bacillus subtilis* isolates against *Phytophthora parasitica* by dual culture technique:

Data presented in Table 1 indicated that maximum growth inhibition of *Phytophthora parasitica* recorded in *Bacillus subtilis* isolate Bs- K_1 (a), (32.57%) followed by Bs- K_6 (f), (28.33%). The other isolates were found least effective against *Phytophthora parasitica*,

The suppression of *Phytophthora parasitica* with antagonist, *B. subtilis* confirms the findings of present investigation. Amorim *et al.* (1999), who tested the *B. subtilis* isolates against *P. parasitica* and *P. citrophthora* causing diseases in citrus.

The results of present investigations was also supports the findings of Ruseela and Kumar (2008), who screened *B. subtilis* isolates against *Phytophthora meadii*, causing root rot of Vanilla.

Efficacy of *Pseudomonas fluorescens* isolates against *Phytophthora parasitica* by dual culture technique:

Data presented in Table 2 indicates the antagonistic ability of Fluorescent Pseudomonads, The maximum mycelial growth inhibition of *Phytophthora parasitica* was observed with the isolates Pf- K_5 (e) (24.28%), Pf- K_4 (d) (27.44%), Pf- N_2 (b) (24.97%) and Pf- N_1 (a) (34.35%). Other isolates were found less effective against *Phytophthora parasitica.*

Present results with Fluorescent Pseudomonads are against *Phytophthora parasitica* are in agreement to Sarathchandra *et al.* (1993); Yang *et al.* (1994); Steddom *et al.* (2002) and Amorim *et al.* (1999), who have reported the ability of *Pseudomonas fluorescens* to inhibit the growth of *Phytophthora* spp., *in vitro.*

Observations on same line in respect of *Pseudomonas fluorescens* was also reported by Anith *et al.* (1999), who observed the antifungal activity of strain E085 against number of soil borne plant pathogens, *in vitro.*

Efficacy of *Bacillus subtilis* and *Pseudomonas fluorescens* isolates against other fungal pathogens of citrus :

Data presented in Table 3 and indicate that the selected bacterial isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* showed maximum mycelial growth inhibition of *Pythium* sp. (54.34%), *Fusarium* sp. (42.22%) and *Colletotrichum gloesporioides* (38.51%) with Bs- K_1 (a) followed by Pf- N_2 (b).

Table 1: Efficacy of *Bacillus subtilis* isolates against *Phytophthora parasitica* by dual culture technique

Sr. No.	Isolates	Average radial mycelial growth in mm	Per cent growth inhibition
1.	Bs- K_1 (a)	56.91	32.57
2.	Bs- K_6 (f)	60.50	28.33
3.	Bs- M_4 (d)	67.41	20.13
4.	Bs- W_1 (a)	72.50	14.11
5.	Bs- W_2 (b)	71.50	15.30
6.	Control	84.41	-
	'F' Test	Sig.	-
	S.E. (m) $\pm$	0.94	-
	C.D. (P = 0.01)	4.06	-

Bs - *Bacillus subtilis*

Table 2 : Efficacy of *Pseudomonas fluorescens* isolates against *Phytophthora parasitica* by dual culture technique

Sr. No.	Isolates	Average radial mycelial growth in mm	Per cent Growth inhibition
1.	Pf- K_1 (a)	70.41	16.58
2.	Pf- K_4 (d)	61.25	27.44
3.	Pf- K_5 (e)	63.91	24.28
4.	Pf- K_6 (f)	66.67	21.02
5.	Pf- M_2 (b)	69.33	17.86
6.	Pf- M_5 (e)	69.58	17.5
7.	Pf- N_1 (a)	55.42	34.35
8.	Pf- N_2 (b)	63.33	24.97
9.	Pf- W_1 (a)	70.83	16.09
10.	Pf- W_2 (b)	71.00	15.89
11.	Pf- W_3 (c)	71.66	15.10
12.	Control	84.41	-
	'F' Test	Sig.	-
	S.E. (m) $\pm$	0.29	-
	C.D. (P = 0.01)	1.14	-

Pf - *Pseudomonas fluorescens*

Table 3 : Efficacy of *Bacillus subtilis* and *Pseudomonas fluorescens* isolates against other fungal pathogens of citrus

Sr. No.	Isolates	Average radial mycelial growth in mm			Per cent growth inhibition		
		Pythium sp.	*Fusarium* sp.	*Colletotrichum gloesporioides*	*Pythium* sp.	*Fusarium* sp.	*Colletotrichum gloesporioides*
Bacillus subtilis							
1.	Bs- K_1 (a)	41.16	52	37.25	54.34	42.22	38.51
2.	Bs- K_6 (f)	72.50	67.33	46.58	19.59	25.18	23.10
Pseudomonas fluorescens							
1.	Pf- K_4 (d)	67.25	69.58	46.16	25.41	22.68	23.80
2.	Pf- K_5 (e)	60.75	68.08	47.25	32.62	24.35	22.00
3.	Pf- N_1 (a)	63.50	69	45.33	29.57	23.33	25.17
4.	Pf- N_2 (b)	48.83	67.16	44.83	45.84	25.37	25.94
	C) Control	90	90	60.58	-	-	-
	'F' Test	Sig.	Sig.	Sig.	-	-	-
	S.E. (m) $\pm$	3.01	0.40	0.35	-	-	-
	C.D. (P = 0.01)	12.65	1.69	1.45	-	-	-

Table 4 : Efficacy of selected *Bacillus subtilis* and *Pseudomonas fluorescens* isolates against *Phytophthora* root rot in citrus (Rough lemon)

Sr. No.	Isolates	Per cent disease incidence	Per cent disease control
Bacillus subtilis			
1.	Bs- K_1 (a)	18.66	81.34
2.	Bs-K_6 (f)	89.33	10.67
Pseudomonas fluorescens			
1.	Pf- K_4 (d)	88.00	12.00
2.	Pf- K_5 (e)	93.33	6.67
3.	Pf- N_1 (a)	77.33	22.67
4.	Pf- N_2 (b)	85.33	14.67
	Inoculated with *Phytophthora* only	100	0
	'F' Test	Sig.	
	S.E. (m) $\pm$	1.42	
	C.D. (P = 0.01)	5.97	

Minimum inhibitory effect was achieved by other isolates of *Bacillus subtilis* and *Pseudomonas fluorescens* against fungal pathogens of citrus.

Ruseela and Kumar (2008) reported the suppression of *Phytophthora medii*, *Fusarium oxysporum* f. sp. *vanillae* and *Colletotrichum vanillae* in Vanilla. These observations collaborates the present findings

The similar results also reported by Kupper *et al.* (2003) in case of *Bacillus subtilis* against *Colletotrichum acutatum* causing post bloom fruit drop in citrus.

The results in case of *Pythium* sp. causing disease in Mandarin confirm the finding of Nemec *et al.* (1996). Results on same line were also reported by Berger *et al.* (1996); Kumar and Bezbaruah (1996); Havega *et al.* (1999); Jager and Korsten (1998) and Thrane *et al.* (2000).

Efficacy of selected *Pseudomonas fluorescens* and *Bacillus subtilis* isolates against *Phytophthora* root rot in citrus (Rough lemon) :

Data presented in Table 4 indicate that two selected isolates of *Bacillus subtilis* and four isolates of *Pseudomonas fluorescens* were screened against root rot caused by *Phytophthora parasirica* by sick soil method.

Mortality due to root rot was assessed by counting healthy and diseased plants and per cent mortality was recorded.

Data presented indicated that Bs- K_1 (a) recorded minimum disease incidence (18.66%) with maximum disease control (81.34%).

The other isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* recorded 6.67 per cent – 22.67 per cent disease control.

Thus the Bs- K_1 (a) was found highly efficient for controlling the root rot caused by *Phytophthora parasitica* and it may be perform well under field condition as it gave maximum disease reduction under artificial epiphytotic condition.

The results obtained under sick soil condition correlates the finding of Amorim *et al.* (1999) who tested the *Bacillus subtilis* for control of *Phytophthora citrophthora* in citrus.

A similar finding was also reported by Berger *et al.* (1996) while testing the biocontrol activity of *Bacillus subtilis* against *Phytophthora* spp. in *Aster*, *Daphae*, *Photinia*, *Hemerocallis* and *Brassica.*

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