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Rhizospheric *Pseudomonas fluorescens* as rejuvenating and root proliferating agents in black pepper

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ABSTRACT: **Six strains of *Pseudomonas fluorescens*, which had been found efficient in root-rot suppression in black pepper (*Piper nigrum* L.) caused by *Phytophthora capsici* were tested for their ability in rejuvenating *Phytophthora* infected black pepper cuttings. Among the treatments, which had only bacterial application, IISR-6 ensured survival of 67% of the infected cuttings where as only 22% of the untreated plants survived 3 months after treatment. The combined application of IISR-51, IISR-6+IISR-13 and IISR-51+IISR-11 with Metalaxyl - Mancozeb resulted in 100% survival of the infected plants, showing an additive effect. Experiments conducted for root-proliferation activity of the *P. fluorescens* showed that the bacterial strains helped increase the root biomass of the plants (30-135%). All the strains increased the root length and root area in the treated plants (12-127%, 43-200%, respectively). Strains were also found to produce plant growth hormones, *viz.* IAA & GA.**

KEY WORDS: Black pepper, root-rot, *Phytophthora capsici, Pseudomonas fluorescens*, rejuvenation, root proliferation

INTRODUCTION

Black pepper is an important export oriented crop. Foot-rot of black pepper caused by *Phytophthora capsici* is a very serious disease, which caused severe economic loss to the farmers (Sarma, 2003). It is inevitable that fewer pesticides will be used in the future and that greater stress would be towards disease management using rhizosphere microorganisms. Fluorescent pseudomonads are some of the effective candidates for biological control of soil-borne plant pathogens owing to their rhizosphere competence, plant growth promotion and disease suppression (Kloepper and Schroth, 1981).

Lynch and Whipps (1991) proved plant growth promotion by rhizobacteria due to chemical and physical stimulation of plant roots resulting in more rapid emergence, higher chlorophyll level and increased stature. Fluorescent pseudomonads produce plant growth regulators and even suppress deleterious soil-borne pathogens (Dubeikovsky *et al.*, 1993). Liu *et al.* (2000) observed longer shoots/

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roots in plants treated with strains of Plant Growth Promoting Rhizobacteria (PGPR).

In the present study, short listed efficient strains of PGPRs were tested for their effectiveness in rejuvenating black pepper cuttings infected with *P. capsici* in order to adopt the technology for nursery management, if found feasible. Studies were also conducted with 5 strains of rhizobacteria for their efficacy in root proliferation in black pepper.

MATERIALS AND METHODS

The experiments were conducted with 40 different treatments in completely randomized design (CRD). The *P. fluorescens* strains used for the study were IISR-51, IISR-13, IISR-18, IISR-11, IISR-8 and IISR-6. The strains were multiplied in nutrient broth at 28°C for 48 h with constant agitation (150 rpm) and bacteria were harvested by pelleting at 7000 rpm for 10 min. The cells were resuspended in 10 mM $MgSO_4$. This inoculum was used to treat the plants.

Infected black pepper cuttings from a nursery were used for the study. There were 40 treatments altogether (Table 1), which also included a fungicide, Ridomil Mancozeb (2.5g/L), drenched in soil @

Table 1. Treatment details

Sl. no.	Treatment
1	*P. fluorescens* –IISR 51
2	IISR 51+ Metalaxyl - Mancozeb (2g/L)
3	IISR 51+ Phorate
4	IISR 51+ Metalaxyl - Mancozeb (2g/L)+ Phorate
5	*P. fluorescens* - IISR 11
6	IISR 51+ Metalaxyl - Mancozeb (2g/L)
7	IISR 51+ phorate
8	IISR 51+ Metalaxyl - Mancozeb (2g/L)+ Phorate
9	*P. fluorescens* - IISR 6
10	IISR 6+ Metalaxyl - Mancozeb (2g/L)
11	IISR 6+ Phorate
12	IISR 6+ Metalaxyl-Mancozeb (2g/L)+ Phorate
13	*P. fluorescens* – IISR 13
14	IISR 13+ Metalaxyl - Mancozeb (2g/L)
15	IISR 13+ Phorate
16	IISR 13+ Metalaxyl-Mancozeb (2g/L) + Phorate
17	*P. fluorescens* –IISR 18
18	IISR 18+ Metalaxyl-Mancozeb (2g/L)
19	IISR 18+ Phorate
20	IISR18+ Metalaxyl-Mancozeb (2g/L)+ Phorate
21	*P. fluorescens* –IISR 8
22	IISR 8 + Metalaxyl - Mancozeb (2g/ L)
23	IISR 8 + Phorate
24	IISR 8+ Metalaxyl - Mancozeb (2g/L)+ Phorate
25	IISR 51+IISR 11
26	IISR 51+ IISR 11 + Metalaxyl - Mancozeb (2g/L)
27	IISR 51+ IISR 11+ Phorate
28	IISR51+ IISR11+ Metalaxyl-Mancozeb (2g/L)+ Phorate
29	IISR 6+IISR 13
30	IISR 6+IISR 13 + Metalaxyl-Mancozeb (2g/L)
31	IISR 6 + IISR 13+ Phorate
32	IISR 6 + IISR 13+ Metalaxyl - Mancozeb (2g/L)+ Phorate
33	IISR 18+ IISR 8
34	IISR 18+ IISR 8 + Metalaxyl - Mancozeb (2g/ L)
35	IISR 18 + IISR 8 + Phorate
36	IISR 18+ IISR 8 + Metalaxyl-Mancozeb (2g/L)+ Phorate
37	Control
38	Metalaxyl-Mancozeb (2g/L)
39	Phorate
40	Metalaxyl-Mancozeb (2g/L)+Phorate

250ml/bag and Phorate @ 5g/bag (all standard recommended dosages). Treatments with combination of fungicide and bacterial strains also were maintained. Five replicates were maintained for each treatment with a plot size of 3 black pepper cuttings. Before planting, the roots were tested for *P. capsici* (by plating), *Radopholus similis* and *Meloidogyne incognita* (enumeration under microscope). The roots were thoroughly washed in running tap water to remove adhering soil and dipped in the bacterial suspension (10^{10} cfu / ml) for 30 min. and planted in polythene bags containing 5kg of potting mixture. Then the soil was drenched with the bacterial suspension (10^{8} cells/g of soil). The plants were watered regularly. Observations were made on the survival of the infected plants. The values obtained were mean of 5 replicates and means were separated by Duncans' Multiple Range Test.

Effect of *P. fluorescens* strains in root proliferation

In another experiment, five strains of *P. fluorescens* (IISR-51, IISR-13, IISR-11, IISR-8, and IISR-6) were evaluated in the greenhouse for root initiation and proliferation in black pepper plants. Two-node cuttings of black pepper were prepared and dipped in bacterial suspension (10^{8} cells/ml) for 30 min. These cuttings were planted in sterile coir compost and the bags were drenched with bacterial suspension (Log-8 cfu/ml). These cuttings were uprooted after 60 days and thorough examination of the root system *viz.* root length, total number of roots, the total area of roots and the root biomass were estimated after scanning and analysis using the software, GS-Root® (PP Systems, Winter Street, USA) and compared with that of the untreated plants. The values obtained were mean of 5 replicates and means were separated by Duncans' Multiple Range Test.

In order to find out the mechanism by which the *Pseudomonas* strains enhance the growth in black pepper, the strains were tested for the production of Indole Acetic Acid (IAA) and Gibberellic Acid (GA), which are growth-promoting hormones in plants. The assay was performed as per the protocol of Hasan (2002).

RESULTS AND DISCUSSION

P. capsici was detected in the roots of the infected plants before planting. Population of *R. similis* was detected (15-25/g root) in the roots and *M. incognita* was not detected. Observations were made on percentage survival of the infected cuttings, which was recorded over a period of 3 months. Strains of *P. fluorescens* could rejuvenate the infected black pepper cuttings to varying levels. After 3 months, out of the bacteria-only treatments, the plants treated with IISR-6 resulted in maximum number of surviving plants (67%), followed by IISR-13 (56%) whereas untreated set had only 22 per cent survival (Table 2). Earlier studies showed that the antagonism by these strains of *P. fluorescens* against *P. capsici* is by different modes *viz.* production of volatile and non-volatile inhibitory compounds including hydrogen cyanide, production of siderophores and mycolytic enzymes which reduced the proliferation of the pathogen (Diby and Sarma, 2005a & 2005b). This could also be explained as a means of systemic resistance offered by the bacterial strains to the host plant (Diby and Sarma, 2005). Metalaxyl – Mancozeb, showed an additive effect with the bacterial strains in protecting the plant. Survival of 100 per cent of the infected plants was obtained when bacterial treatment (IISR-11, IISR-51+IISR-11 and IISR-6+IISR-13) was combined with Metalaxyl–Mancozeb (Table 2). When applied alone, IISR-6 was found to be superior to other strains in rejuvenating the infected plants. The ability of certain fluorescent pseudomonads in protecting black pepper cuttings from infection by *P. capsici* in an artificially inoculated system has been demonstrated earlier wherein the antagonists acted as preventive agents (Jubina and Grija, 1998; Anith *et al.*, 2002), while the present study demonstrated the ability of *P. fluorescens* strains as rejuvenating agents in black pepper cuttings, which had already been infected naturally.

Where combination of bacterial strains was used, there was no additive or synergistic effect. Phorate had no effect on the survival of the plant since the population of *R. similis* was only very less and the major pathogen being *P. capsici*.

Table 2. Survival of infected cuttings after three months of treatment

Sl.no	Treatment	Survival (%)	Sl.no.	Treatment	Survival (%)
1	*P. fluorescens* –IISR 51	44.4 bcdef	21	*P. fluorescens* –IISR 8	22.2 def
2	IISR 51+ Metalaxyl - Mancozeb (2g/L)	77.8 abc	22	IISR 8+ Metalaxyl - Mancozeb (2g/L)	88.9 ab
3	IISR 51+ Phorate	22.2 cdef	23	IISR8+ Phorate	00.0 f
4	IISR 51+ Metalaxyl - Mancozeb (2g/L)+ Phorate	33.3 cdef	24	IISR 8 + Metalaxyl - Mancozeb (2g/L) + Phorate	88.9 ab
5	*P. fluorescens* - IISR 11	22.2 cdef	25	IISR 51+IISR 11	11.1 ef
6	IISR 11+ Metalaxyl - Mancozeb (2g/L)	100.0 a	26	IISR 51+ IISR 11 + Metalaxyl - Mancozeb (2g/L)	100.0 a
7	IISR 11+ Phorate	22.2 cdef	27	IISR 51+ IISR 11+ Phorate	22.2 cdef
8	IISR 11+ Metalaxyl - Mancozeb (2g/L)+ Phorate	22.2 cdef	28	IISR51+ IISR11+ Metalaxyl- Mancozeb (2g/L)+ Phorate	55.6 bcde
9	*P. fluorescens* - IISR 6	66.7 abcd	29	IISR 6+IISR 13	44.4 bcdef
10	IISR 6+ Metalaxyl - Mancozeb (2g/L)	88.9 ab	30	IISR 6+IISR 13 + Metalaxyl- Mancozeb (2g/L)	100.0 a
11	IISR 6+ Phorate	44.4 bcdef	31	IISR 6 + IISR 13+ Phorate	11.1 ef
12	IISR 6+ Metalaxyl - Mancozeb (2g/L)+ Phorate	77.8 abc	32	IISR 6 + IISR 13+ Metalaxyl - Mancozeb (2g/L)+ Phorate	44.4 bcdef
13	*P. fluorescens* – IISR 13	55.5 bcde	33	IISR 18+ IISR 8	11.1 cdef
14	IISR 13+ Metalaxyl - Mancozeb (2g/L)	66.6 abcd	34	IISR 18+ IISR 8 + Metalaxyl - Mancozeb (2g/ L)	88.9 ab
15	IISR 13+ Phorate	55.5 bcde	35	IISR 18 + IISR 8 + Phorate	66.7 abcd
16	IISR 13+ Metalaxyl - Mancozeb (2g/L)+ Phorate	66.7 abcd	36	IISR 18+ IISR 8 + Metalaxyl- Mancozeb (2g/L)+ Phorate	66.7 abc
17	*P. fluorescens* –IISR 18	22.2 cdef	37	.Control	22.2 cdef.
18	IISR 18+ Metalaxyl - Mancozeb (2g/L)	44.4 bcdef	38	Metalaxyl-Mancozeb (2g/L)	66.7 bcde
19	IISR 18+ Phorate	11.1 ef	39	Phorate	22.2 ef
20	IISR 18+ Metalaxyl - Mancozeb (2g/L)+ Phorate	44.4 bcdef	40	Metalaxyl-Mancozeb (2g/L)+ Phorate	44.4 bcdef

In the experiment for root proliferation activities, the bacterial strains significantly increased the total root biomass (30-135%) of the black pepper plants (Table 3). The number of roots have been significantly increased (82-137%) apart from increasing the root length (12-127%) and thereby root area (43-200%). These beneficiary attributes can be corroborated with the hormonal and nutritional factors by which the rhizobacteria influence the plant (Diby and Sarma, 2005c). Plant growth regulators, *viz.* IAA and GA were produced by these strains, as detected in chromatographic

Table 3. Different root-growth parameters of plants under treatment

P. fluorescens strains	Root biomass (g)	Total root length (cm)	Total root area (mm^2)	Total number of roots
IISR-6	46.95 ^{ab}	1048.046 ^{a}	2410.088 ^{a}	506.7 ^{a}
IISR-8	48.39 ^{ab}	0754.308 ^{bc}	1349.492 ^{bcd}	415.9 ^{ab}
IISR-11	47.40 ^{ab}	0962.685 ^{ab}	2004.929 ^{ab}	480.9 ^{a}
IISR-13	33.06 ^{bc}	0518.401 ^{cd}	1155.100 ^{cd}	389.8 ^{ab}
IISR-51	59.63 ^{a}	0917.521 ^{ab}	1668.761 ^{bc}	490.2 ^{a}
Control	25.35 ^{c}	0461.294 ^{d}	0803.061 ^{d}	213.9 ^{b}

studies. The TLC plate was observed in an Alpha Imager-Image Analysis system and in a UV-trans illuminator, GA showed greenish fluorescens. IAA showed violet red colour in visible light and orange in UV light. GA was detected with all the strains and IAA, with strains, IISR-6, IISR-11, IISR-13 and IISR-51. Earlier studies showed that these strains could solubilize complex forms of P in the soil thus making it available to the plant. The intake of other minerals such as N and P also was found to be more with *P. fluorescens* treated black pepper plants (Diby *et al.*, 2005c). These factors not only stimulated the root for higher absorption of nutrients and minerals but also affected better root health.

Most root-promoting bacteria synthesis IAA and it has been clearly demonstrated that they stimulate the formation of lateral and adventitious roots (Barbieri and Galli, 1993). The other evidences include that of *P. putida* GR12-2 cells that produced wild type levels of IAA stimulated the formation of many short adventitious roots on mung bean cuttings and in IAA over producing mutant stimulated the formulation of even more adventitious roots than the wild type strain (Mayak *et al.*, 1997).

The results showed the effectiveness of *P. fluorescens* strains in rejuvenation of infected black pepper cuttings, which could be explored for effective nursery management. The study also proved the rhizobacteria-mediated root proliferation in black pepper.

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