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Mitigating *Phytophthora* foot rot and slow decline diseases of black pepper through the deployment of bacterial antagonists

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Abstract

Field trial was conducted at ICAR-IISR Experimental Farm, Peruvannamuzhi, Kozhikode district, Kerala, during 2008-2013 for managing foot rot and slow decline diseases of black pepper using IPM modules. Planting material of black pepper was produced by raising two node runner cuttings treated with chemicals/bioagents. A plot having non-living standards in 3 x 2 m runner was selected. Pits of 50 cm^3 were made and buffered with lime and cow dung. Planting was done with three month old rooted plants where roots were primed with bacterium/chemical. The experiment was in RCBD with eight treatments *viz*., *Pseudomonas aeruginosa* (Bp 35) + Phorate, *P. putida* (Bp 25) + Phorate, *P. fluorescence* (IISR 6) (later identified as *P.aeruginosa*) + Phorate, *Bacillus megaterium* (Bp 17) + Metalaxyl Mz, *Curtobacterium luteum* (TC10) + Metalaxyl Mz, *P. aeruginosa* (IISR 853) + Metalaxyl Mz and Phorate + Metalaxyl Mz (chemical check) and an absolute control with no treatments. The treatments were imposed at planting in May and subsequent applications in September and thereafter every year. During the initial two years, plants were maintained weed free by plastic mulching. Shades and irrigation were provided during summer in the first two years and thereafter only mulching and irrigation were continued. All the plants were sprayed with $ZnSO_4$ (0.25%), DAP (0.5%) and $MgSO_4$ (0.25%) during May and September from second year onwards. For controlling 'Pollu' beetle (*Longitarsus nigripennis*) and anthracnose (*Colletotrichum gloeosporioides*), quinalphos (0.075%) + Bordeaux mixture (1%) was sprayed twice in September and November. Soil biological properties and pathogen population were monitored regularly. No foot rot incidence due to *Phytophthora* spp. could be noticed in any of the treatments till the end of the experimental period. However, nematode infection, manifested as yellowing of the vines, as well as nematode population showed a gradual increase. Among the treatments, *C. luteum* (TC 10) with Metalaxyl Mz showed significant reduction in nematode population with better growth and yield.

Keywords: black pepper, *Curtobacterium luteum,* endophytic bacteria, foot rot, IPM, *Phytophthora capsici*, *Piper nigrum*, *Radopholus similis,* slow decline

Introduction

Piper nigrum commonly called as black pepper, is a perennial trailing vine grown for its black berries. It is a highly export oriented spice grown in the humid tropical evergreen forests of the Malabar Coast in India. It is now being

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cultivated in countries like Indonesia, Brazil, Malaysia, Sri Lanka, Vietnam, China and several other countries. It grows successfully in humid tropics with adequate rainfall and humidity between 20°N-S latitude, and grows from sea level up to1500 meters. *Phytophthora* foot rot caused by *Phytophthora capsici* is the most destructive disease (Alizadeh & Tsao 1985), followed by slow decline caused by the association of burrowing nematode *Radopholus similis,* and the root knot nematode *Meloidogyne incognita* with *P. capsici* (Anandaraj *et al.* 1996; Ramana & Eapen 2000). All the plant parts like, leaf, stem, spike and roots are vulnerable to infection by *Phytophthora* sp. Slow decline is another important debilitating disease where the affected vines show various levels of feeder root infection followed by foliar yellowing. The expression of symptoms on leaf and stem occur after substantial damage of the feeder roots.

Mainly copper based fungicides and organophosphate insecticides are the current recommendations, being widely used to combat *Phytophthora* and nematodes in black pepper. Even though most effective, they are polluting the environment and residue remains with the product that are hazardous. Besides, these insecticides having red label claim, are banned in India. So during the past two decades researchers pursued options like host resistance, organic amendments and biocontrol agents to chalk out an integrated pest management (IPM) strategy with varied success rates. Some of the prominent recommendations emanated in India are combined application of potassium phosphonate, neem oil cake and biocontrol agents like *Trichoderma harzianum, T. viride, Pseudomonas fluorescens* and *Pochonia chlamydosporia* (Sarma *et al.* 1989; Ramachandran *et al.* 1991; Ramachandran & Sarma 1995; Rajan *et al*. 2002; Saju *et al*. 2002); spraying and drenching Bordeaux mixture (1%) at fortnightly intervals (Thankamani *et al.* 2008; Shasidhara *et al.* 2009; POP IISR 2015), soil drenching of Copper oxychloride (0.2%) and foliar application of Bordeaux mixture (1%), soil drenching and foliar spraying of Metalaxyl Mz (0.125%) coupled with soil application of nematicides like Phorate or carbofuran (Mohandas & Ramana 1987) and deployment of resistant varieties like IISR Shakti, Pournami etc. (Bhai *et al.* 2007; 2010). However, farmers are more dependent on chemicals, may be due to their ignorance on biological control or due to the non-availability of quality biocontrol products. So soil application of fungicides like Metalaxyl Mz or copper oxychloride along with nematicides Phorate or carbofuran were more prevalent (POP, IISR 2015, Shashidhara *et al.* 2009). But off late, farmers prefer organic cultivation and more over in states like Kerala (India), Government has banned the use of red and yellow labeled pesticides. In this context it is highly imperative to search for an alternative to the highly toxic pesticides like Phorate or Carbofuran.

In our pursuit for effective biocontrol agents against *Phytophthora* and nematodes, number of endophytic bacteria were isolated from black pepper tissues, characterized and screened against *P.capsici* and nematodes both *in vitro* and *in planta*. *P. putida*, *P. aeruginosa*, *Bacillus megaterium* and *Curtobacterium luteum* were some of the promising candidates shortlisted in the above screening (Aravind *et al.* 2009; 2010; 2012). In this study, these potential bacteria and two other rhizosphere bacteria were evaluated with agrochemicals under field conditions for the management of *Phytophthora* foot rot and slow decline diseases. Role of these bacteria in combating the diseases and also increasing the growth and yield of this perennial vine is reported in this paper.

Materials and methods

The experiment was conducted during 2008-2013 in two phases. In the first phase nucleus planting material was produced from runner shoots primed with respective bioagents as per treatment design and in the second phase the plants thus produced were planted in the experimental field for the evaluation of integrated pest management strategies(IPM).

First phase

Planting material

As nucleus planting material, runners of black pepper (variety Panniyur I) were collected from

a farmer's plot in Kodagu, Karnataka. The particular variety was selected because of two reasons. Firstly the variety is susceptible to both foot rot and slow decline diseases. Secondly, the variety grows well under open conditions. The runner shoots thus collected were washed thoroughly in running tap water and made into two-node stem cuttings.

Bioagents

Four endophytic bacteria *viz*., *Pseudomonas aeruginosa* BP35 (MTCC 5410), *P. putida* BP25, *Bacillus megaterium* BP17 (MTCC 5409), *Curtobacterium luteum* TC10 and two rhizobacteria *viz*., *P.fluorescence* strain IISR 6 (MTCC 5178) later identified as *P. aeruginosa* (Kumar *et al.* 2012) and strain IISR 853 (MTCC 5411) were used as bioagents. All the endophytic bacteria were originally isolated from black pepper, characterized and their bioefficacy proved through extensive screening studies (Aravind *et al.* 2010). The strain IISR 853 was isolated from *Strychnos nuxvomica*, was proved inhibitory to *R. similis* (Beena 2008). The strain IISR 6 has already been recommended against *P. capsici* in black pepper gardens (Thankamani *et al.* 2008). Bacterial cultures were raised in Nutrient broth and 48h old culture of OD 1 having a cfu of $10^{10\text{-}12}$ mL^{-1} was used for treating the cuttings initially.

The two-node cuttings were dipped in the respective bacterial suspension (~cfu 10^{10} mL^{-1}) for 30 min and planted in sterile nursery mixture containing soil, sand and cow dung (1:2:1) in polythene bags of size 10 x 20 cm and kept for rooting under humid conditions by keeping under a chamber made of polythene sheet. The cuttings were watered once in three days and grown up to three leaf stage (Fig. 3).

Second phase

Design of experiment

The experiment was in a randomized complete block design. There were eight treatments and three replications/treatment and eight plants/ replication. The treatments were T_1- Bp35 + Phorate, T_2- Bp25 + Phorate, T_3- IISR6 + Phorate, T_4- Bp17 + Phorate, T_5- TC10 + Metalaxyl Mz, T_6- IISR853 + Metalaxyl Mz, T_7- Metalaxyl Mz + Phorate and T_8 Absolute control with no treatments. The treatments were designed for integrated management where both foot rot and slow decline diseases have to be controlled. Here the bacterial antagonists of *P. capsici viz*., BP35, BP25 and IISR6 were integrated with nematicide Phorate (30g/vine) while bacterial antagonists of nematodes *viz*., BP17, TC10 and IISR853 were integrated with fungicide Metalaxyl Mz.(0.125%). The compatibility of all these bacterial antagonists with target chemicals has already been studied (Sasmita 2009; Sasmita *et al.* 2014).

Bioagents and chemicals

The four endophytic bacteria (BP35, BP25, BP17 and TC10) and two rhizospheric bacteria (IISR6 and IISR 853) were used for field evaluation The chemicals *viz*., Metalaxyl Mz (0.125%) and Phorate (30g/vine) have proven efficacy against *P. capsici* and nematodes, respectively, and are being recommended for use in black pepper gardens (POP 2010). Bacterial cultures were made individually in Nutrient broth and grown for 48h and 2.4 liter of OD 1 culture having a cfu of 10^{10} was inoculated into 120 kg of dried and powered cow dung and applied @ of 5 kg $vine^{-1}$.

Field evaluation

The field evaluation was conducted during 2008-2013 at IISR experimental farm Peruvannamuzhi, Kozhikode, Kerala, India. The experimental farm selected is located in the foot hills of Western Ghats of Kerala at an altitude of 60m above MSL with an average rainfall of 4300 to 5300mm annually. The temperature of the area is ranging from 23-40^0C and the sunshine hours varying from 45 to 260 hours per month. About 0.17ha area, previously planted with black pepper and have the history of foot rot and slow decline infections, was cleared and used for the study. In order to minimize the interaction with live standards, concrete/granite poles were used as standards for trailing the vines. Soil is lateritic with clay-loam texture (27% sand and 38% clay) with an acidic pH of 4.0-4.7. The nutrient status of the top 15 cm soil layer where: available nitrogen 292 kg ha^{-1}, available phosphorus 26

kg ha^{-1}, available potassium 527 kg ha^{-1} and organic carbon 0.76-1.27%.

Planting and cultural interventions

With the onset of the first monsoon showers in May, pits of 50 x 50 x 50 cm were opened at a spacing of 3 x 2m. Lime was applied in each pit @ 1 kg for improving the soil pH. At the time of planting the pits were filled with well decomposed cow dung @1 kg pit^{-1} along with top soil. The two-node rooted plants raised as above were planted in the pits as per the treatments design. Since there is no overhead canopy due to non-living standards, individual plants were shaded with coconut fronds to protect from the summer scorching during the initial establishment period (Fig. 4). The interspace area was spread with black polythene sheet in order to reduce the weed population (Fig. 5). Drainage channels were provided to avoid water stagnation (Fig. 6).

The treatments were imposed at the time of planting in May and subsequent applications in September. The same schedule was followed every year till the end of the experiment. Inorganic fertilizers were not applied at any time throughout the experimental period. FYM was applied to all the vines @10 kg $vine^{-1}$ twice during the monsoon and post monsoon seasons. Foliar sprays with micronutrients like $ZnSO_4$ (0.25%), DAP (0.5%) and $MgSO_4$ (0.25%) were given during May and September from second year onwards @1 liter/vine. For controlling Pollu' beetle (*Longitarsus nigripennis*) and anthracnose (*Colletotrichum gloeosporioides*), quinalphos 0.075% + Bordeaux mixture 1% was given as foliar sprays twice during September and November. Opened drainage channels before the onset of monsoon to avoid water stagnation which is hostile for the buildup of *Phytophthora* population. Similarly life saving irrigation was provided during summer months from January to May once/twice in a week with 25-30 liters of water/vine. Along with weeding, mulching and earthing up was adopted during post monsoon season to maintain soil moisture in the rhizosphere.

Soil sampling and recording of observations

Observations on population dynamics of total culturable fungi, bacteria, introduced antagonists, *P. capsici* and plant parasitic nematodes as well as pH and moisture content of the soil were recorded at periodic intervals. Soil samples for analysis was collected at 10 cm away from the plant base at a depth of 10-15 cm. Soils were drawn randomly, pooled together and a composite sample of 100g was collected from each replication and used for biological and chemical analyses. Observations on growth parameters like height of the vine, number of leaves and branches and branch length were recorded during the first year of planting. Yellowing of vines due to nematodes, foot rot incidence due to *Phytophthora* sp. and yield (third year) were recorded (In case of foot rot, if at all plants show yellowing, it is followed by sudden wilting and blackening of leaves. while in case of slow wilt due to nematodes, yellowing is gradual and there is no foliar wilting). Yellowing was rated visually on a 0-4 scale (score) where 0= No yellowing (Absolute healthy), 1- 25% (slight yellowing), 2=26-50% (moderate yellowing), 3= 51-75% (severe yellowing) and 4= >75% (dead vines due to yellowing) and percent intensity (PI) of yellowing was calculated based on the formula,

(Sum of ratings) / (Maximum score × No. of plants scored) × 100

In this experiment the initial status of microflora including plant pathogenic fungi and nematode as well as total culturable fungi, bacteria and non-parasitic nematode population were assessed to study the population build up. Rhizosphere soil samples were collected from each treatment twice (2 months and 3 months after the second application of treatments). *P. capsici* population was estimated by soil baiting technique (Tsao 1977) whereas nematode population was estimated by sieving and centrifugal floatation method (Jenkins 1964; Barker 1985). Total culturable bacteria and fungi were assessed using dilution plating of soil. For this, 1 g subsample was processed independently and plated as follows. Each 1g sample was added to 0.2% sterile water agar (90 mL), stirred using magnetic stirrer for 5 min, and serially diluted and the dilutions of 10^{-6} and 10^{-8} were plated

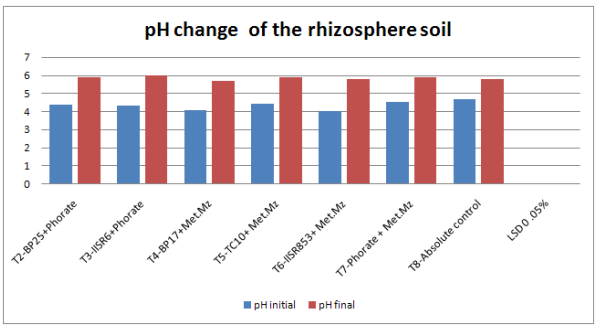

Fig. 1. pH change of the experimental field after IPM practices

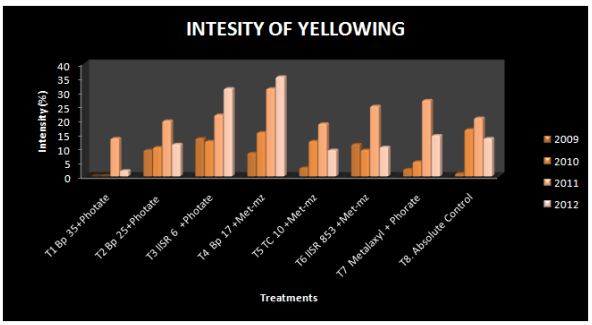

Fig. 2. Intensity of yellowing

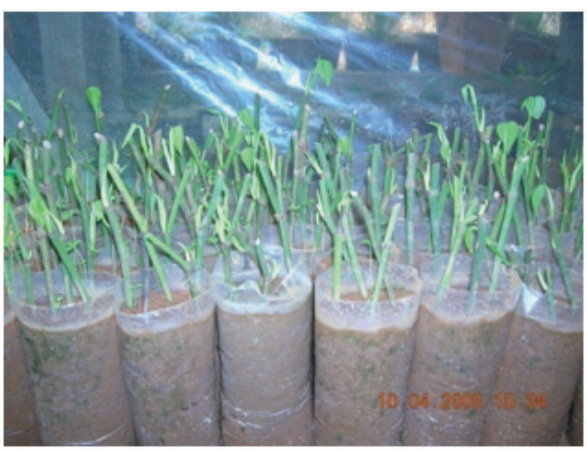

Fig. 3. Establishment of two node cuttings in humid polythene chamber

Fig. 4. Providing shade during initial establish-ment of the plants using coconut fronts

Fig. 5. Interspace mulching with black polythene sheet (300mm gauge) in the first two years of plant establishment

Fig. 6. Basal mulching and providing drainage channels

in nutrient agar (NA) for bacterial counts and dilutions of 10^{-3} and 10^{-5} in Rose Bengal agar (RBA) for fungal counts. For enumerating the introduced bacteria, NA amended with selective antibiotics and TTC were used (Aravind *et al.* 2008). The antibiotics used were Thrimethomorpherin and colistin-100 µL/100 mL, Chloramphenicol-125 µL/100 mL, Kanamycin 50 µL/100 mL, Polymixin B 20 µL/ 100 mL respectively for Bp 17, Bp 25, Bp35 and Tc10. There were three replications for each dilution for each sample. Incubation was done at 28°C for 72-96 h. The microbial counts were recorded as the number of colony forming units (CFUs) $gram^{-1}$ soil.

For the isolation of endophytic bacteria, the roots were washed thoroughly in tap water and cut sections with a sterilized scalpel. Cut root sections were sterilized using sodium hypochlorite (0.5%) for 2 min, rinsed in sterile water thrice and dried on sterile filter paper. The samples were ground using motor and pestle in 2 ml of PBS and then diluted up to 10^3 with PBS and plated in NA incorporated with corresponding antibiotics (*P. aeruginosa*-Kanamycin 50 µL/100 mL, *P. putida*-Chloramphenicol 125 µL/100 mL), *C. luteum*-20 µL/100 mL and for *Bacillus megaterium* –Colistin and Trimethopherin- 100 µL/100 mL) (Aravind *et al.* 2008) and incubated at 28-30°C for 48h.

Aanalysis for organic carbon, available phosphorous and total nitrogen was carried out using the protocols of Nelson & Sommers (1982); Olsen & Sommers (1982) and Novozamsky *et al.* (1983), respectively.

Statistical analysis

The data was analyzed statistically using ANOVA with the package SAS software (Version 9.3) and then subjected to mean separation by Least Significant Difference test, $P<0.05$.

Results and discussion

The presence of the pathogens and other culturable microflora in soil samples were estimated at the time of planting (Table 1) and after two and three months. Except treatment T_1 (Bp35 and Phorate), no other treatments showed the presence of *P. capsici* population at the time of planting. This may be due to the exposed condition where the soil experienced a sort of solarization effect due to the total absence of overhead canopy as well as the absence of weed flora (since the plots were maintained weed free in the initial years of cultivation). But significant difference was observed in the population of *R. similis* and *M. incognita* in the initial years (Table 1). The number of *R. similis* ranged from 1- 6 /100 g soil. The population of *R.similis* after three months was highest in absolute control (5.7/ 100 g). The least population (0.33/100 g) with a reduction of 94.74% was observed in T_2 (Bp25 + Phorate) followed by T_5 (TC10 + Met. Mz) (1/ 100 g) with a reduction of 82.45% and T_6 (IISR853 + Met. Mz) (1.33/100g) with a reduction of 76.67% (Table 2). The population reduction was due to the presence nematode antagonists and nematicides in the treatments. The least total culturable bacterial population was observed in Treatment T_8 and least fungal population in T_5. However the variation was very minimum (Table 2). Significant difference was observed in the colonization of different biocontrol agents in Treatments T_1-T_6 when compared to absolute control (T_8) and T_7 (Met. Mz + Phorate). The higher colonization observed in T_1-T_6 indicated the compatibility of these bioagents with the chemicals (Sasmita *et al.* 2014).

The soil samples from the interspaces of the plants in different treatments were also collected after 5 months of planting and were analyzed as above for total culturable fungi, bacteria, plant parasitic nematodes, saprophytic nematodes besides *P. capsici* and *R. similis* (Tables 1 & 3). Besides interspace soil samples, soil was also collected and analyzed from the plot corners and outside the plot where natural shade condition prevails. No *P. capsici* could be isolated from any of the samples. However, both parasitic and non-parasitic nematode population was detected in the interspaces. Treatments T_4 and T_2 showed the maximum population of nematodes. The total culturable fungal and bacterial population was comparatively higher in the corners of the field

Table 1. *Phytophthora* and *R. similis* population in the interspace and rhizosphere of plants

Treatment	Interspace population at the time of planting		Rhizosphere population after different intervals			
	**R. similis*	*P. cap*	*R. similis* (no./100 g soil) (2 maa)	*R. similis* (no./100 g soil) (3 maa)	*P. cap* (2 maa)	*P. cap* (3 maa)
T_1-BP35 + Phorate	0.00	0.33	1.00	1.00	0.00	0.00
T_2-BP25 + Phorate	0.00	0.00	1.00	0.33	0.00	0.00
T_3-IISR6 + Phorate	0.00	0.00	1.00	3.67	0.00	0.00
T_4-BP17 + Met. Mz	0.00	0.00	2.00	2.33	0.00	0.00
T_5-TC10 + Met. Mz	0.00	0.00	1.67	1.00	0.00	0.00
T_6-IISR853 + Met. Mz	0.00	0.00	1.33	1.33	0.00	0.00
T_7-Phorate + Met. Mz	0.00	0.00	0.33	3.33	0.00	0.00
T_8-Absolute control	0.00	0.00	5.67	5.70	0.00	0.00
LSD (P<0.05)	0.00	0.00	3.203	1.554	0.00	0.00

*maa= months after application

where no chemicals or biocontrol agents reached. Among the treated plants, T_7 holds the least no. of total culturable fungi and bacteria (Table 3). Colonization of the introduced antagonists in the root system was also analyzed. Soil colonization of TC10 was comparatively lesser compared to BP 17 in the presence of Metalaxyl Mz and the colonization of BP35 was higher compared to that of BP25 in presence of Phorate (Table 4) Since black pepper being a perennial climbing vine, the growth can be measured only in the early stages. The growth parameters at six months of planting showed significant difference between control and treatments and among the treatments T_5 followed by T_6 showed increased plant height, no. of leaves and no. of branches (Table 5). However, these isolates were on par based on statistical analysis.

During the experimental period, the rain fall ranged from 3600 mm to 5400 mm and the maximum rainfall was obtained during 2009 and 2011 (number of rainy days were 154 and 158, respectively). Even with the highest rainfall and more number of rainy days, *P. capsici* infection, either soil phase or aerial phase was not observed in the experimental plot which indicated the effect of cultural management practices (mulching the interspace with polythene sheet and nonliving standards which allowed direct sunlight) rather than the effect of individual treatments. The pH was also raised to almost neutral level as shown in Fig. 1. This is mostly because of the application of lime before planting to improve soil conditions. The pH of the particular area before planting and application of lime was found between 4.11-4.51 as evinced from the pH of the interspaced soil. The treatments have effect on soil microbial population especially burrowing nematode *R. similis.* Plant parasitic nematodes gradually increased in the plot, so also the yellowing of the vines (Fig. 2). The yellowing was aggravated during the peak summer season. So a direct correlation could be noticed between yellowing and nematode population. However, the intensity was more in exposed areas when compared to border areas where there is shade from the adjacent plot.

The yield data showed significant difference between control and treatments. The treatments T_5 showed the maximum yield (303.04% increase) followed by T_6 (208.06% increase) and T_3 (Table 6). The same treatments also showed increased canopy size that gives an indirect indication that the introduced

Table 2. Rhizosphere microbial build up three months after imposition of treatments

Treatments	Total fungi (log cfu)	Total bacteria (log cfu)/g	Introduced antagonists (log cfu)/g	*P. capsici*	*R. similis* (no./100 g soil)	Total PPN (no./100 g soil)	Total NPN (no./100 g soil)	Moisture
T_1-BP35 + Phorate	4.94(87x10^3)	10.36 (23x10^9)	10.29(19x10^9)	0.00	1.00	22.00	22.67	11.25
T_2-BP25 + Phorate	4.88(75x10^3)	10.63(43x10^9)	9.96 (91x10^8)	0.00	0.33	16.00	10.67	8.75
T_3-IISR6 + Phorate	4.78(61x10^3)	10.43(27x10^9)	9.98 (97x10^8)	0.00	3.67	25.00	34.67	9.78
T_4-BP17 + Met. Mz	4.85(71x10^3)	10.41(26x10^9)	9.64 (44x10^8)	0.00	2.33	22.67	22.00	10.33
T_5-TC10 + Met. Mz	4.72(52x10^3)	10.51(32x10^9)	9.91 (81x10^8)	0.00	1.00	4.00	14.33	13.17
T_6-IISR853 + Met. Mz	4.77(58x10^3)	10.50(32x10^9)	9.94 (87x10^8)	0.00	1.33	7.00	21.33	13.77
T_7-Phorate + Met. Mz	4.60(40x10^3)	10.51(32x10^9)	0.00 -	0.00	3.33	11.33	16.33	15.00
T_8-Absolute control	4.93(35x10^3)	10.32(21x10^9)	0.00 -	0.00	5.70	11.00	21.33	16.02
LSD (P<0.05)	0.044	0.056	0.320	0.00	1.554	10.101	14.797	1.949

antagonists (*C. luteum*) have growth promotive effect too.

In our study, six antagonists were evaluated for their field efficacy on suppression of disease and growth promotion. Among them, *C luteum* and *P. putida* were found promising and is supported by the work of Denise *et al.* (2002) and Pinho *et al.* (2009). Denise *et al.* (2002) identified six most promising colonizing strains *viz*., *Cellulomonas*, *Clavibacter*, *Curtobacterium* and *Microbacterium* from aerial tissues of soybean, wheat and prairie plants. These endophytic species exhibited high colonization ability to survive inside the host and were found as suitable biocontrol agents. Similarly the effect of endophytic bacteria such as *Acinetobacter johnsonii*, *C. luteum*, *B. pumilus*, *B. amyloliquefaciens* as most effective strains on the control of *M. incognita* of tomato was reported by Pinho *et al.* (2009).

We also studied the colonization ability of the bacterial endophytes inside roots along with agrochemicals that can be used in IPM (Table 4). The result showed that BP35 (*P. aeruginosa*) was inhibited lesser (12.57%) when compared to BP25 (*P. putida*) (40.90%) in the presence of nematicide Phorate. The endophyte BP17 (*B. megaterium*) was inhibited lesser (33.33%) when compared to TC10 (*C. luteum*) (35.64%) in the presence of the fungicide Metalaxyl Mz. The treatments were designed for integrated management where both foot rot and slow decline diseases has to be controlled. Moreover, it is aimed to replace Phorate which is a banned pesticide. The recommended practice was using Metalaxyl mancozeb along with phorate in managing the diseases. Here the bacterial antagonists of *P. capsici viz*., BP35, BP25 and IISR6 were integrated with nematicide Phorate (30g/vine) while bacterial antagonists of nematodes *viz*., BP17, TC10 and IISR853 were integrated with fungicide Metalaxyl Mz.(0.125%). Compatibility of the endophytes *in vivo* in presence of chemicals showed varying levels of colonization. Among the four endophytes, BP35 showed higher colonization than BP25 (36.11-47.1% reduction) followed by BP 17 and TC 10 (22-36%). Even though rhizosphere colonization of TC10 was less

when compared to other antagonists, effect wise TC10 showed comparatively better growth promotion in terms of height of the plant (69.76 cm) (Table 5) and is at par with IISR 853 with Metalaxyl mz. (67.31cm). This may be due to endophytic colonization.

The analysis of the interspace soil samples from the field showed no *P. capsici* population. This may be due to the prevailed atmospheric conditions. The atmospheric temperature during the period (December to February) was very high ie.31.84°C, with no rainfall and very low relative humidity (min 55.66% and max. 86.10%). These conditions are very unfavorable for the survival of *P. capsici* in the soil. More over the pH of the soil was 4.11-4.51 (Table 3), which is very acidic and not congenial for the growth. The population of *R. similis* was also negligible in the interspaces. This may be due to the absence of any living plant hosts in the interspaces (Table 3).

In the rhizosphere soil analysis, only the treatment T_1 showed the presence of *P. capsici*. The *R. similis* population was considerably reduced in the treatment T_2 and T_5. This is because T_2 contains the nematicide Phorate and T_5 contains the nematode antagonist *C. luteum*, isolated from tissue cultured Black pepper plants, which is effective against *R. similis*. There is considerable build up of antagonistic population in the rhizosphere. Among the treatments, T_5 and T_6 showed growth promotive effects with increased height of the plant, number of leaves and number of branches (Table 5). According to Aravind *et al.* (2008), diversity of the bacterial endophytes

Table 3. Interspace microflora five months after planting and treatment imposition

Treatments	Total fungi (log cfu g^{-1})	Total bacteria (log cfu g^{-1})	*P. capsici*	*R. similis* (no./100 soil)	Total PPN* (no./100 g soil)	Total SN* (no./100 g soil)	Moisture	
T_1-BP35 + Phorate	3.85	6.17	0.00	0.00	0.00	2.67	15.00	4.51
T_2-BP25 + Phorate	4.37	6.28	0.00	0.00	2.00	18.67	13.53	4.21
T_3-IISR6 + Phorate	3.85	6.09	0.00	0.00	0.33	11.33	14.57	4.22
T_4-BP17 + Met. Mz	3.82	5.97	0.00	0.00	0.67	16.67	17.71	4.45
T_5-TC10 + Met. Mz	4.01	6.14	0.00	0.00	0.00	4.33	17.09	4.15
T_6-IISR853 + Met. Mz	3.95	6.11	0.00	0.00	1.67	4.33	18.53	4.49
T_7-Phorate + Met. Mz	3.73	5.93	0.00	0.00	2.00	16.0	18.76	4.16
T_8-Absolute control	3.84	6.33	0.00	0.00	0.00	6.0	30.81	4.11
Outside (sides)	3.91	6.10	0.00	0.00	0.67	9.67	21.02	4.28
Outside (corners)	4.85	6.09	0.00	0.00	0.00	12.67	23.16	4.12
LSD (P<0.05)	0.22	0.18	0.00	0.00	10.11	1.45	4.56	0.163

*PPN=Plant parasitic nematodes; **SN=Saprophytic nematodes

Table 4. Root colonization by endophytes in different treatments

Chemicals	Bp 17	Bp 25	Bp 35	TC 10
Metalaxyl Mz 0.125%	3.83 (33.33%)			3.54 (35.64%)
Phorate		3.96 (40.90%)	4.38 (12.57%)	
Control	5.75	6.70	5.01	5.50

from black pepper have biocontrol efficacy against *P. capsici* and among them *P. aeruginosa, P. putida* and *B. megaterium* were selected for biological control of *Phytophthora* foot rot. In the present study three strains of *P. aeruginosa viz*., Bp 35 (endophytic isolate), IISR6 and IISR 853 (rhizosphere isolates) were used. However, only the rhizosphere isolates were found to be more effective. This may be due to its rhizosphere colonization abilities (Edna 2009). Fluorescent pseudomonads' have frequently been suggested to be important natural antagonists to plant pathogens (Elengovan & Gnanamanikkam 1992). Diby *et al.* (2005) suggested that rhizosphere bacteria involving *P. fluorescens* were antagonistic to *P. capsici* and prevents its growth. The nutrient status of the soil also showed considerable variation from the initial status (Table 1).

Foot rot and slow decline disease management has become a major anxiety to black pepper farmers. Integrated Pest Management (IPM) would be the ideal strategy to tackle this problem. Out of the various components of IPM, biocontrol has got higher preference. So the overall result of the present study is summarized as follows.

The experimental plot has a previous history of foot rot infection. The particular plot was

Table 5. Plant growth effect after six months of planting

Treatments	Ht. of the plant (cm)	No. of leaves	No. of branches	Branch length (cm)
T_1-BP35 + Phorate	32.79	6.17	0.0	0.00
T_2-BP25 + Phorate	45.47	7.42	0.29	6.05
T_3-IISR6 + Phorate	36.65	6.88	0.13	1.90
T_4-BP17 + Met. Mz	47.36	8.34	0.79	19.08
T_5-TC10 + Met. Mz	69.76	11.29	1.00	20.57
T_6-IISR853 + Met. Mz	67.31	11.75	0.88	15.90
T_7-Phorate + Met. Mz	27.04	5.67	0.13	2.29
T_8-Absolute control	17.00	3.92	0.21	1.42
LSD (P<0.05)	7.09	1.166	0.26	4.81

Table 6. Yield data from 2010-2013

Integrated Treatments	Average fresh yield plot^{-1} (kg)				T x Y Mean	Yield (kg ha^{-1}) 3 x 2m spacing (1600 plants)
	2010	2011	2012	2013		
T_1-BP35 + Phorate	0.394 h	0.685 h	4.383 gh	0.405 h	1.467 c	478.80
T_2-BP25 + Phorate	1.212 h	1.747 gh	12.367 d	10.518 de	6.431 b	2288.50
T_3-IISR6 + Phorate	2.522 gh	2.49 3 gh	14.352 bcd	14.276 bcd	8.416 b	2862.80
T_4-BP17 + Met. Mz	5.340 fgh	2.147 gh	14.253 bcd	9.350 def	7.773 b	2360.0
T_5-TC10 + Met. Mz	6.918 h	2.527 gh	20.989 a	18.379 ab	12.20 a	3936.80
T_6-IISR853 + Met. Mz	3.277 efg	2.737gh	18.020 ab	13.267 cd	9.325 ab	3128.70
T_7-Phorate + Met. Mz	2.671 gh	2.657 gh	12.137 d	12.713 d	7.544 b	2485.00
T_8-Absolute control	0.173 h	0.850 h	4.65 3 fgh	6.430 efg	3.027 c	1108.30
Mean	2.813c	1.980c	12.64 a	10.67b		

values with the same letters within each column are not significantly different (P<0.05).

abandoned due to severe foot rot incidence during 90's and the same plot was chosen for the present studies. However, the *P. capsici* population was not found in any of the treatments except T_1. This is due to the IPM practices adopted as mentioned elsewhere. For nematode suppression, the treatments (*P. putida strain* BP25 + Phorate and *C. luteum* strain TC10 + Met-Mz were effective. The introduced bacterial antagonistic populations in the rhizosphere of the plants were increased to an extent. The treatments TC10 + Met-Mz and IISR 853 + Met-Mz showed promising in plant growth attributes. The treatments with *C. luteum* (TC10) + Met-Mz showed the maximum yield. The same treatments also showed increased canopy size which gives an indirect indication that the introduced antagonists (*C. luteum*) have growth promotive effect too. This is supported by the work of Sturz *et al.* (1997) where he reported the consistent efficacy of *C. luteum* in promoting plant growth independently or along with *Rhizobium* spp.

There are reports that endophytic organisms contribute essential vitamins to plants (Pirttila *et al.* 2004). Besides, Compant *et al.* (2005) attributed osmotic adjustment, stomatal regulation, modification of root morphology, enhanced uptake of minerals and alteration of nitrogen accumulation and metabolism to endophytes. Research has also been directed to search endophytes that can significantly increase yields after inoculation. Aravind *et al.* (2012) reported pre-plant bacterisation as a method for delivering endophytic bacteria for the production of disease free planting material of black pepper. Pre-plant root bacterisation of black pepper plantlets had positive effect on growth performances such as height of plants, number of leaves, root biomass and total biomass as is seen in the present study.

So based on the findings and observations made during the study, a holistic management strategy is charted out for mitigating the threatening diseases of black pepper *viz*., foot rot and slow decline. This includes application of lime @500 g-1 kg/plant base before planting in acidic soil and repeated applications in alternate years. To prevent the invasion of nematodes, it is suggested, root priming of planting material with endophytic bacterium *C. lutium* (for 30 min) and soil application of the same during planting and during post monsoon season. This could be repeated every year. Foliar application of Bordeaux mixture 1% and Quinalphos 0.075% during August-September (Post-monsoon) help in checking aerial infection by *Phytophthora* spp., *Colletotrichum gloeosporioides* and *Longitarsus nigripennis*. It is important to keep the rhizosphere weed free to avoid competition of developing root system. In this study it was done by interspace mulching with black polythene sheet of 300mm to avoid weed growth (Fig. 4). Since there is no basal application of inorganic fertilizer, it is essential to supply micro nutrients as foliar spray (0.5%). Raking the soil and exposing to sunlight before planting /replanting help to reduce the pathogen build up as done in the present experiment as a cultural practice. As cultural intervention shade must be regulated or provided so as to get 50% penetration of sunlight. Opening drainage channels before the onset of monsoon helps to avoid water stagnation which is hostile for the buildup of *Phytophthora* population. Similarly life saving irrigation has to be provided during summer months from January to May once/twice in a week with 25-30 liters of water/vine. Along with weeding, mulching and earthing up have to be adopted during post monsoon season to maintain soil moisture in the rhizosphere. These IPM strategies help to a great extent in managing these diseases.

On the basis of overall growth and yield of black pepper, the treatment with the endophytic bacterium, *C. luteum* TC 10 + Metalaxyl Mz was the best among all other treatments followed by *P. aeruginosa* IISR 853 + Metalaxyl Mz. However, the use of IISR 853, though found to be very effective, may be discouraged as *P. aeruginosa* has some clinical implications. Soil application of *Curtobacterium*

luteum along with Metalaxyl Mz 0.125% significantly reduced the plant parasitic nematode population and enhanced the growth and yield of black pepper. So through continuous monitoring, judicial application of pesticides along with suitable biocontrol agents and cultural interventions, the *Phytophthora* infections and *R. similis* damage could be successfully managed in the black pepper. This strategy of using the endophyte *C. luteum* along with Metalaxyl –Mz is a suitable alterative to the existing recommendation of Metalaxyl Mz + Phorate where Phorate being a red label pesticide, is permanently banned in India.

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