Short Communication

Inoculation with bacterial endophytes and the fungal root endophyte, *Piriformospora indica* improves plant growth and reduces foliar infection by *Phytophthora capsici* in black pepper

Teenu Paul, N.S. Nysanth, M.S. Yashaswini and K.N. Anith*

College of Agriculture, Kerala Agricultural University, Thiruvananthapuram 695 522, Kerala, India

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Abstract

Foot rot disease caused by the oomycete fungus *Phytophthora capsici* is one of the major problems faced by black pepper growers. Two bacterial endophytes, *Bacillus velezensis* PCSE10 and *Rhizobium radiobacter* PCRE 10 isolated from the wild relative of black pepper, *Piper colubrinum*, and the root endophytic fungus, *Piriformospora indica* and their combined effect were evaluated for plant growth promotion and suppression of foliar infection caused by *P. capsici* in bush pepper plants (var. Panniyur 1). *B. velezensis* PCSE10 exhibited *in vitro* antagonism against *P. capsici*. In a detached leaf assay, *R. radiobacter* PCRE10 significantly reduced the lesion size. *R. radiobacter* PCRE10 was found to be compatible with *P. indica* in dual culture plate assay. Suppression of foliar infection of *P. capsici* in plants treated with the individual endophytes and their combination was assessed by challenge inoculation with the pathogen on the foliage. Plants treated with combination of *P. indica* and *R. radiobacter* PCRE10 recorded lowest lesion size (0.43 cm), which recorded 10.41 per cent disease suppression over the pathogen control with the lowest disease index of 0.2. Disease suppression on inoculation with *P. indica* was the least compared to all other treatments. Combined application of *P. indica* and *R. radiobacter* PCRE10 as well as single inoculation of *P. indica* showed discernible improvement in growth parameters of bush pepper (var. Panniyur 1). Plants treated with single inoculation of *P. indica* showed the highest fungal root colonization of 35.50% followed by the combination of *P. indica* and *B. velezensis* (31.10%), as well as *P. indica* and *R. radiobacter* PCRE10 (19.35%).

Keywords: Bacterial endophytes, Black pepper, *Phytophthora capsici*, *Piper nigrum*, *Piriformospora indica.*

Black pepper (*Piper nigrum* L.) is one of the major spice crops having its origin in the Western Ghats region in South India. A major portion in the Indian spice export earning is contributed by black pepper, the "King of spices". Indian black pepper is having high demand in international market since ancient ages. Black pepper has wide use apart from its consumption in human dietaries. It is a major component in most of the ayurvedic medicines and used as a preservative in food industry. Essential oils from pepper are used in perfumery and also as insecticides.

Foot rot disease caused by the oomycete fungus, *Phytophthora capsici* is one among the major constraints of black pepper production especially during the southwest monsoon season (Sarma et al., 1994). Black pepper is susceptible to the fungus at all stages of its growth. The pathogen attacks the above and below ground parts of the vine such as root, collar, leaves, stem, berries and inflorescence. Management of this deadly disease is possible only through phyto-sanitary measures and repeated prophylactic spraying or drenching of vines with copper fungicides. In order to avoid the adverse

*Author for Correspondence: Phone: 9446413861, Email: anith.kn@kau.in

effect of toxic chemicals, biocontrol agents such as rhizospheric and endophytic bacteria as well as fungi have been recommended.

Endophytic bacteria can be defined as a class of endosymbiotic microorganisms that usually colonize the internal tissues of host plants without causing any injuries (Schulz and Boyle, 2006). They are involved in imparting disease resistance, nitrogen fixation, solubilization of immobilized phosphorus, nutrient cycling, stress tolerance, production of plant hormones, siderophores and volatile organic compounds that improve plant health (Sturz et al., 2000). As they are ecologically highly competitive, they can effectively colonize the rhizosphere and enhance plant growth, trigger defense mechanisms against various plant pathogens and promote plant growth (Govindasamy et al., 2017). *Bacillus velezensis* PCSE10 and *Rhizobium radiobacter* PCRE10, two bacterial endophytes isolated from the exotic wild pepper, *Piper colubrinum* were reported to have plant growth promotion and biocontrol activity against *P. capsici* infecting black pepper plantlets of the variety Karimunda (Kollakkodan et al., 2017; 2021). *Piriformospora indica*, belonging to the order Sebacinales of Basidiomycota, is a biotrophic mutualistic root endosymbiotic fungus (Verma et al., 1998; Varma et al.,1999; Weiss et al., 2004). It is axenically cultivable and imitates the abilities of arbuscular mycorrhizal fungi (AMF). *P. indica* is having wide host range including bryophytes, pteridophytes, gymnosperms and angiosperms (Shahollari et al., 2005; Deshmukh et al., 2006). Symbiotic association of *P. indica* induces abiotic stress tolerance against salinity, drought, heavy metal toxicity and low temperature (Sheramati et al., 2008; Zarea et al., 2012). Besides, it improves the defensive capacity of several crop plants against bacterial, fungal, nematode and viral pathogens (Daneshkhah et al., 2013; Deshmukh and Kogel, 2007; Lakshmipriya et al., 2017; Sherameti et al., 2008; Varkey et al., 2018; Athira and Anith, 2020). Colonization of black pepper by *P. indica* reported for the first time by Anith et al. (2011). It was also reported that inoculation with the fungus improved the growth of bush pepper plants and resulted in early flowering. It also improved secondary metabolite production in the berries (Anith et al., 2018).

Here, we report that inoculation with a combination of the endophytic fungus, *P. indica* and endophytic bacterial strains obtained from wild pepper species, *P. colubrinum* promoted plant growth in bush pepper and suppressed foliar infection by foot rot pathogen. *In vitro* screening of antagonism of bacterial bioagents *with P. indica* was carried out on potato dextrose agar (PDA) medium using dual culture plate assay as described by Anith et al. (2015). A mycelial disc of 4 mm was cut out from a fresh culture of *P. indica* grown on PDA and placed at the centre of Petri plate containing PDA medium. Inoculated plates were incubated at 28 ^{o}C for five days. Single colonies of *Bacillus velezensis* PCRE10 and *Rhizobium radiobacter* PCRE10 were obtained by streak plating them on Nutrient agar (NA) medium. After five days of incubation of *P. indica* plates, using an inoculation loop, a heavy inoculum from single colony of the bacterial strain was applied as a line of streak of 2.5 cm length at two opposite edges of the Petri plate equidistantly at 2.0 cm away from the periphery of the Petri plates. The plates were further incubated at 28 ^{o}C and observed for the development of inhibition zone. Plates with media alone and not streaked with bacteria were kept as control. *In vitro* screening of endophytic bacteria for antagonism against *Phytophthora capsici* was carried out in Petri plates containing PDA using dual culture plate assay to assess the direct antagonistic effect of the bacterial endophytes against the foot rot pathogen as described above with *P. capsici* as the test fungus.

Screening by detached leaf assay was carried out to evaluate the ability of antagonistic bacterial isolates to suppress the infection of *P. capsici* (Kollakkodan et al., 2021). Bacterial inoculum used in the study was prepared by growing them in agar plates as described by Varkey et al., (2018). Using a sterile

loop, bacterial cells from single colony were heavily cross streaked on nutrient agar plates. The plates were incubated overnight at 28 ± 2 °C. Plates were then drenched with 10 ml of sterile distilled water. Using a sterile glass spreader, the bacterial cells were scrapped out and collected aseptically. The OD values of cell suspension was adjusted to 1.0 at 660 nm using sterile distilled water resulting in uniform suspension of approximately 10^7 cfu ml^{-1}. Mature leaves from the black pepper variety Panniyur 1 were collected from Instructional farm, College of Agriculture, Vellayani and washed well in water. They were allowed to dry in a laminar air flow chamber and spray-drenched with bacterial suspension separately till run off. Leaves sprayed with sterile water served as control. A mycelial disc (8 mm) from freshly grown *P. capsici* was cut out and placed on the lower side of the leaf after providing a pinprick. Moist sterile cotton was placed over the mycelial disc to maintain humidity. The petiole end of leaves was also covered with moist cotton to avoid drying of leaves. Leaves sprayed with sterile water inoculated with pathogen were also kept as control. Lesion size on leaves and the time taken for appearance of lesion were observed to assess the effectiveness of bacterial endophytes. The experiment was designed as CRD with five replications.

Indole Acetic Acid production by endophytic bacteria was estimated as per the procedure described by Gordon and Weber (1951) employing Salkowski method. One set each of 100 ml nutrient broth was inoculated with the endophytic bacteria with or without tryptophan (0.1%) and incubated at 28 °C for 5 days with constant shaking in a rotary shaker (Scigenics Biotech, Chennai, India) at 100 rpm. Uninoculated broth was taken as control. After five days of incubation, the broth cultures were centrifuged at 4500 rpm for 20 min at 4 °C. To one ml of the supernatant, two ml Salkowski reagent was added. Salkowski reagent was prepared by mixing two ml 0.5M $FeCl_3$ and 49 ml water and 49 ml 70 % perchloric acid. The tubes containing the culture filtrate and Salkowski reagent were kept in dark for 25 min for colour development. Absorbance was measured at 530nm (Shimadzu 900i spectrophotometer, Shimadzu Corporation, Japan). The absorbance of the samples obtained was plotted against a standard to determine the concentration of IAA produced.

Endophytic fungus, *P. indica* and endophytic bacterial strains were tested for their ability to promote plant growth in bush pepper**.** Bacterial inoculum was prepared by growing them in agar plates as described above. The OD of cell suspension was adjusted to 1.0 at 660 nm using sterile distilled water resulting in uniform suspension of approximately 10^7cfu ml^{-1}. Mass multiplication of root endophytic fungus *P. indica* was carried out by the procedure by Anith et al. (2018). The fungal inoculum was produced in 500 ml capacity Erlenmeyer flasks containing 100 ml of potato dextrose both (PDB; pH 7.00). PDB was inoculated with three mycelial discs (8 mm) from freshly grown PDA plate and then incubated at 28 °C for 10 days with constant shaking in a rotary shaker (Scigenics Biotech, Chennai, India) at 100 rpm. The mycelium was harvested by filtration after 10 days using a sterile muslin cloth and then washed twice with sterile water to remove media contents. The weight of harvested mycelium was recorded. Freshly harvested mycelium was mixed with sterile vermiculite to get a final concentration of one per cent (v/w) fungal mycelial mass. Vermiculite was sterilized by autoclaving for one hour at 121° C for three consecutive days.

Polythene bags were filled with equal proportion of sand, soil and farm yard manure. Young healthy lateral branches (one year old) from high yielding vines of variety Panniyur 1 were collected and pruned to 2-3 nodes. All leaves except the flag leaf were removed. A sharp slanting cut was made at the basal portion of the stem and dipped in bacterial cell suspension for 20 min with intermittent shaking and planted in the case of treatments involving endophytic bacteria. Fungal inoculum mixed with vermiculite was applied in the planting holes at the

rate of 25 g per polybag for the treatments with *P. indica*. Cuttings were planted in the polybags and kept in a shade house for three months for establishment. Watering was done regularly. After three months the rooted cuttings were transferred to larger earthen pots (25 cm diameter, 30 cm height) filled with a mixture of soil, sand and farm yard manure in equal proportions. The polythene bags were removed carefully without disturbing the root system. Fungal mycelial mass mixed with sterile vermiculite (1% v/w; 50 g per pot) was applied to the small pit made for planting. Fifty mL bacterial cell suspension (10^7cfu/ mL) was poured into the root zone in the case of treatments requiring bacterial treatment. Pots were arranged in completely randomized design (CRD). Experiment was laid with six treatments with five replications having one plant each. 50 mL water soluble chemical fertilizer (N:P:K- 19:19:19) solution (0.5 %) was given to each pot at 15 days intervals starting from 30th day of planting. Number of leaves and longitudinal leaf length were taken at monthly intervals after transplanting. Spikes were harvested at maturity. Observations on number of spikes harvested per plant, mean spike length (cm) and dry weight of berries were also taken.

Root colonization by *Piriformospora indica in* bush pepper plants was examined after one month of transplanting. Five root samples each from the treated and untreated plants were excavated from the pots without damaging the plants. Staining was done as described by Anith et al. (2011). The collected roots were washed thoroughly in running water to remove soil and other debris. Then the roots were cut into bits of 1 cm length. The root bits were kept in freshly prepared 10 % KOH solution for 15 mins and boiled for 5 min. Roots were then washed in distilled water twice. Acidification of root bits was carried out with 1M HCl for 5 min and stained in 0.2 % lactophenol-trypan blue for 15 min. Lactophenol solution was utilized for de-staining. Roots bits after de-staining were observed under a compound bright field microscope. Percentage of root colonization was estimated using following formula.

$$\text{Root colonization (\%)} = \frac{\text{Number of root segments colonized} \times 100}{\text{Total number of root segments observed}}$$

The ability of bacterial bioagents *with P. indica* to suppress the foliar disease caused by *P. capsici* was evaluated under *in vivo* conditions. Virulent strain of *P. capsici* was used for inoculation on intact leaves of endophyte treated bush pepper plants kept in earthen pots. Mycelial plugs from fully grown PDA plates were made using 4 mm cork borer. Inoculation of *P. capsici* was done on lower leaf surface of three intact leaves of eight months old bush pepper plants. The mycelial plugs were covered with a thin layer of moist cotton and leaves were covered with polythene covers to provide humidity. Inoculation was done in all plants except in the absolute control. Development of lesion was observed and the lesion size on inoculated leaves was recorded. Copper oxychloride (0.2%) was sprayed on the foliage of control plants one week before pathogen inoculation.

Disease index was calculated six days after inoculation based on a score chart of 0-5 scale based on the lesion size on inoculated leaves. Scale for scoring *Phytophthora capsici* induced foliar infection in black pepper is shown as below.

Score	Lesion size (cm)
0	No Lesion
1	0.1 – 2 cm
2	2.1 – 3 cm
3	3.1 – 4 cm
4	4.1 – 5 cm
5	> 5 cm

Disease index (DI) was calculated using the formula:

$$D = \frac{\text{(Sum of individual ratings)} \times 100}{\text{Number of leaves assessed} \times \text{maximum grade used}}$$

Statistical analysis was done using R based analysis platform of Kerala Agricultural University, GRAPES (General Rshiny Based Analysis Platform Empowered by Statistics; https://www.

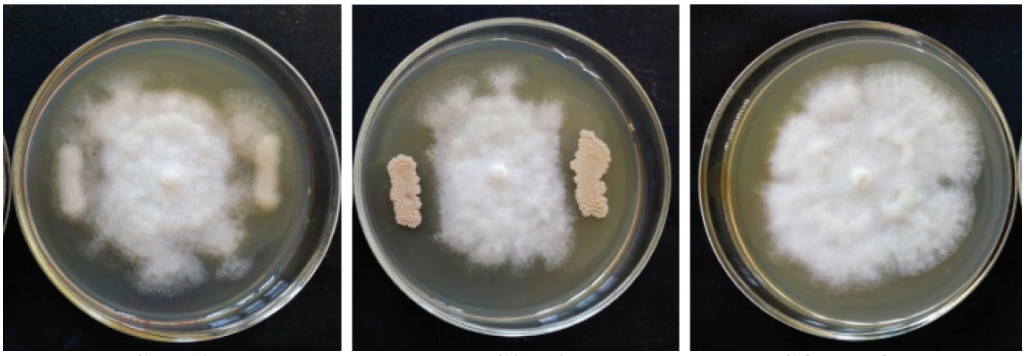
kaugrapes.com/home) by one way Analysis of Variance (ANOVA) and the treatment means were compared using Duncan's Multiple Range (DMRT) at a probability of 0.05 %.

The bacterial endophytes used in the present study were isolated from the wild relative of black pepper, *Piper colubrinum* (Kollakkodan et al., 2017). *P. colubrinum* has innate resistance against infection caused by *Phytophthora capsici*. It is used as root stock for production of disease resistant black pepper. This exotic pepper variety has immense potential as a donor plant in breeding programs for improvement of the cultivated species, *P. nigrum* (Dicto and Manjula, 2005). Kollakkodan et al. (2017; 2021) reported that many endophytic bacteria from *P. colubrinum* have antagonistic activity against *P. capsici*.

The broad host range beneficial fungal root endophyte *Piriformospora indica* was also used as an inoculant. *P. indica* is an AM like fungus, originally isolated from the roots of xerophytic woody shrubs in the Thar Desert in India (Verma et al., 1998; Varma et al., 1999). As reported by several authors, it interacts beneficially with a wide range of plants and is known to enhance plant growth, biomass production, phosphorus acquisition and acts as a bio-protector against abiotic and biotic stress including root and leaf fungal pathogens (Waller et al., 2005; Serfling et al., 2007; Shahollari et al., 2007; Fakhro et al., 2010; Yadav et al., 2010; Wang et al., 2015; Lakshmipriya et al., 2017; Varkey et al., 2018). The present study was divided into two, *in vitro* and *in vivo* experiments. Assessment of *in vitro* antagonism between the endophytic fungus and the bacterial bioagents is one of the most important pre-requisites to assess their compatibility. *In vitro* trials are preliminary screening methods, which can be used to choose the combination of biological agents for efficient consortium development (Anith et al., 2003; Lemessa and Zeller, 2007). Combination of organisms to be used in the *in vivo* experiment would be selected mostly by *in vitro* assay.

In the present study, antagonism against *P. indica* on PDA medium was done by dual culture plate to know about the *in vitro* interaction. Compatibility was assessed by lack of inhibition zone when *P. indica* and bacterial isolates were cultured together in PDA. The non-compatible ones usually develop zone of inhibition. Results of dual culture plate assay revealed that growth of *P. indica* was not inhibited by the endophytic bacterial strain *Rhizobium radiobacter* PCRE10. However, *Bacillus velezensis* PCSE10 produced an inhibition zone of 4 mm. *Bacillus velezensis* PCSE10 was found to be incompatible with *P. indica* as it developed zone of inhibition whereas *R. radiobacter* PCRE10 was compatible with the fungus. Incompatible reaction suggests that there may be an inhibition in the growth of *P. indica* by the metabolites secreted by the endophytic bacteria when applied together in the rhizosphere. Dual culture plate assay done by Athira and Anith (2020) with the same bacterial isolates exhibited similar interaction pattern. Mixed inoculation of different biological agents on crop plants is not advisable when there is antagonism among them. However, incompatibility of the inoculants could be resolved by following a temporal separation in application to the root zone of the crop plants (Anith et al., 2011).

When tested against *P. capsici,* both the endophytic bacterial strains showed inhibitory activity against the foot rot pathogen as evidenced by the presence of inhibition zone (Fig 1). The zone of inhibition measured on 6th and 8th day after the bacterial inoculation showed that *P. capsici* growth inhibition by *R. radiobacter* PCRE10 was lesser compared to that by *B. velezensis* PCSE10 (Table 1). Competition, antibiosis and mycoparasitism are reported to be the biocontrol traits of antagonist in *in vitro* inhibition against plant pathogens (Narisawa et al., 2004; Bailey et al., 2008; Morath et al., 2012; Sreeja et al., 2016). The production of inhibitory metabolites may be the reason for *in vitro* inhibition of these isolates against *P. capsici*. However, results of dual culture assay may be contradictory with the *in vivo* performance as reported by several authors



PCRE10 PCSE10 CONTROL

Figure 1 *In vitro* inhibition of *Phytophthora capsici* by endophytic bacterial isolates on dual culture plate assay in potato dextrose agar (PDA) medium

Table 1. Antagonism of endophytic bacteria against *Phytophthora capsici* in dual culture plate assay on PDA medium

Isolate	Presence of inhibition zone	Inhibition zone (mm)*
PCSE10	+	4.67±0.333
PCRE 10	+	1.03±0.226

*Mean (+ SD) of five independent measurements. + Presence of inhibition, - Absence of inhibition.

(Baker, 1968; Schroth and Hancock, 1981; Wong and Baker, 1984; Anith et al., 2003). Presence of inhibition in the dual culture plate assay can be taken as an initial measure for assessing antagonistic potential of the isolates. However, there is absence of the host in the dual culture plate assay. An assay which resembles the field conditions in a better manner would be one that involves interaction of the pathogen, antagonist, and the host plant (Anith et al., 2003). Therefore, a detached leaf assay was done by inoculating both the antagonist and the pathogen on leaves of black pepper.

In the detached leaf assay, there was significant difference in lesion size on control leaves and leaves sprayed with the bacterial isolates after four days of pathogen inoculation. The minimum lesion size was observed on leaves treated with isolate *R. radiobacter* PCRE10 with a lesion size of 1.43 cm which caused 52.33 per cent disease suppression over control which was on par with leaves treated with isolate PCSE10 with lesion size of 2.18 cm and caused 27.33 per cent disease suppression over control. The maximum lesion size was observed in control leaves sprayed with sterile water (3.0 cm). The reduction in the lesion size indicated that the isolates have successfully suppressed the disease by direct antagonism.

The ability of endophytic bacterial strains to produce IAA was determined quantitatively. Both the bacterial isolates were found to produce IAA both in the presence and absence of L- Trypthophan. *Rhizobium radiobacter* PCRE10 was found to produce 4.616 µg/ml and 8.793 µg/ ml IAA without and with L- Trypthophan respectively. *B. velezensis* PCSE10 was found to produce 3.768 µg/ml and 4.920 µg/ml IAA in absence and with L- Tryptophan respectively. Indole Acetic Acid (IAA) is considered to be the most important native auxin and it belongs to the group of phytohormones (Strzelczyk and Pokojska, 1984). Endophytic bacteria within the plant release auxin which results in the formation of lateral, adventitious roots and root hairs thereby increasing the root surface area, thus promote plant growth (Hilbert et al., 2012). Bacterial IAA in conjugation with endogenous auxins produced by host plant stimulates growth. Detection of plant growth promoting beneficial microorganisms can be done by screening based on IAA production (Ali and Hasnain, 2007; Govindarajan et al., 2007; Jasim et al., 2013).

The bacterial and fungal endophytes were tested for their ability to promote the growth of bush pepper

Table 2. Plant growth promotion in bush pepper variety Panniyur 1 on inoculation with endophyte

	2 MAT		4 MAT		6MAT	
	Number of leaves per plant	Total leaf area per plant	Number of leaves per plant	Total leaf area per plant	Number of leaves per plant	Total leaf area per plant
P. indica	11.66±0.88^{bc}	660.00±94.73	26.00±0.57^{b}	1,650.66±251.33^{a}	48.33±1.76^{b}	2,474.33±777.48
B. velezensis PCSE10	9.33±0.88^{cd}	464.00±161.34	19.66±0.88^{bd}	884.66±99.15^{cd}	31.33±0.88^{c}	1,518.33±13.41
R. radiobacter PCRE 10	14.33± 1.45^{ab}	788.33± 90.12	26.00±1.00^{c}	1,194.66±142.75^{bc}	84.00±2.08^{a}	3,662.66±1,128.35
P. indica and *B. velezensis* PCSE10	11.66±1.45^{bc}	473.66± 36.37	23.00±2.51^{bc}	706.09±321.20^{d}	28.00±2.40^{c}	2095.34±484.23
P. indica and *R. radiobacter* PCRE 10	16.33±0.88^{a}	861.00±228.84	34.00±0.57^{a}	2,257.00±292.99^{cd}	69.66±1.52^{a}	2,442.33 ±556.83
Control	8.33± 0.66^{d}	515.00±125.04	19.66±1.76^{d}	1,302.00 ±430.86^{ab}	30.66±5.89^{c}	1,615±297.742
CD	3.36	NA	4.385	860.67	9.09	NA

*Mean (+ SD) of three replications. (n=12). Figures in a column followed by the same alphabet do not differ significantly according to DMRT ($p \leq 0.05$). MAT: Months after transplanting

plants *in vivo*. More number of leaves was recorded in plants treated with single or combination of the bio-agents. Observations on the second and fourth month after transplanting showed that maximum number of leaves was recorded in plants treated with combination of *P. indica* and *R. radiobacter* PCRE10. However, at sixth months after transplanting, more number of leaves was recorded in plants treated with *R. radiobacter* PCRE10 (Table 2). A notable increasing trend was observed in leaf area per plant of the plants treated with combined application of *P. indica* and *R. radiobacter* PCRE10 (Table 2). Maximum leaf area per plant was recorded in the plants treated with combination of *P. indica* and *R. radiobacter* PCRE10 from fourth month after transplanting and it was statistically on par with the treatment consisting of *P. indica* alone.

Observation on number of spikes harvested was taken eight months after transplanting (Table 3). There was no significant difference observed among the treatments. However, the highest value was recorded in the treatment with combination of *P. indica* and *R. radiobacter* PCRE 10. Mean spike length differed significantly among the treatments. Application of *P. indica* alone resulted in the highest mean spike length. When the berry fresh weight as well as dry weight was analyzed statistically, there was no significant difference observed among the treatments (Table 3). The highest value was recorded in the treatment involving single application of *P. indica*. Anith et al. (2011) reported positive effect on vegetative growth in tissue cultured black pepper on colonization of *P. indica*. Plants inoculated with *P. indica* in combination with *R. radiobacter*

Table 3. Yield parameters in bush pepper variety Panniyur 1 on inoculation with endophytes.

Treatments	Number of spikes harvested/plant*	Mean spike length* cm	Spike weight/plant (g)*	
			Fresh	Dry
Piriformospora indica	5.33 ± 0.66	11.38 ± 0.66^{a}	5.71 ± 0.74	2.13 ± 0.21
Bacillus velezensis PCSE10	3.33 ± 1.15	12.26 ± 0.43^{a}	5.26 ± 0.76	1.64 ± 0.19
Rhizobium radiobacter PCRE10	5.00 ± 0.66	12.02 ± 0.81^{a}	2.58 ± 0.10	0.75 ± 0.06
P. indica and *B. velezensis* PCSE10	4.66 ± 1.20	10.89 ± 1.15^{ab}	3.61 ± 0.79	1.25 ± 0.36
P. indica and *R. radiobacter* PCRE 10	6.00 ± 0.66	8.44 ± 0.61^{bc}	3.92 ± 1.87	1.28 ± 0.66
Control	2.00 ± 0.66	6.65 ± 0.27^{c}	3.00 ± 1.25	0.93 ± 0.44
CD	NS	2.58	NS	NS

*Mean (+ SD) of three replications. (n=12). Figures in a column followed by the same alphabet do not differ significantly according to DMRT ($p \leq 0.05$).

PCRE10 exhibited significant growth improvement. *P. indica* has been reported to have some close association with bacterial flora. These intimate bacterial species that are present within the hyphae of the fungus included a *Rhizobium radiobacter* strain as well (Sharma et al., 2008). *R. radiobacter* PCRE10 has been reported to be an efficient plant growth promoter and biocontrol bacterium in black pepper (Kollakkodan et al., 2021).

In the present study, root colonization pattern by *Piriformospora indica* in plants was analysed after one month of transplanting. Pear shaped chlamydospores were observed under 40X magnification of a compound bright field microscope within the cortical cells of root tissues of the inoculated bush pepper plants at one month after transplanting. No colonization was observed in uninoculated control plants and also in treatments with individual bacterial application. Plants treated with *P. indica* alone showed highest root colonization with 35.50 percent followed by a combination by *P. indica* and *B. velezensis* PCSE10 (31.11 %) and *P. indica* and *R. radiobacter* PCRE10 (19.35 %). Persistent nature of colonization in bush pepper plants after six months of inoculation was reported by the detection of chlamydospores (Anith et al., 2018). Treatments that involved combination of *P. indica* with bacterial bioagents had reduced colonization. Bacterial bioagents may have some adverse influence on the fungal root colonization, though the same has not been completely prevented. A reduction in the amount of root colonization by *P. indica* in tomato plants when combined inoculation was done with *Rhizobium radiobacter* PCRE10 and *Bacillus velezensis* PCSE10 has already been reported (Athira and Anith, 2020). Varkey et al. (2018) reported a reduction in the amount of root colonization by *P. indica* in tomato plants when combined inoculation was done along with *B. pumilus* VLY 17 and *P. fluorescens* AMB 8.

Biocontrol experiment showed difference in disease index between bush pepper plants treated with bacterial endophytes and the control plants. Lesion size showed significant difference at four days after inoculation, whereas observations after seven days of inoculation were non-significant. Seven days after inoculation, the minimum disease incidence was noticed in the plants treated with combination of *P. indica* and *R. radiobacter* PCRE10 (0.43 cm). Treatments had no significant effect on the disease index at seven days after inoculation. The lowest disease index was observed in plants treated with combination of *P. indica* and *R. radiobacter* PCRE10 (0.20) (Table 4, Fig 2). Data on foliar infection could not be taken after seven days as many of the infected leaves fell off the plants due to infection. *Piriformospora indica* improves defensive capacity of several crop plants against fungal, nematode and viral pathogens (Fakhro et al., 2010; Lakshmipriya et al., 2017; Varkey et al., 2018). *P. indica* more likely protects plants by inducing their systemic immunity (Serfling et al., 2007; Oelmuller et al., 2009; Molitor et al., 2011; Pedrotti et al., 2013; Narayan et al., 2017). In the present study disease suppression by *P. indica* was less than that with all other bioagents.

Table 4. Effect of endophytes application on bush pepper plantlets on lesion development by *Phytophthora capsici*

Treatments	Lesion size on inoculated leaves (cm)*		Disease index
	4 DAI	7 DAI	calculated on7 DAI*
Piriformospora indica	0.78 ± 0.30	2.96 ± 0.89	0.62 ± 0.08
Bacillus velezensis PCSE10	0.13 ± 0.06	0.99 ± 0.65	0.22 ± 0.13
Rhizobium radiobacter PCRE 10	0.08 ± 0.04	1.41± 0.55	0.45 ± 0.11
P. indica and *B. velezensis* PCSE10	0.09 ± 0.01	0.88 ± 0.58	0.22 ± 0.13
P. indica and *R. radiobacter* PCRE 10	0.03 ± 0.01	0.44 ± 0.42	0.20 ± 0.12
Pathogen control	0.01 ± 0.06	0.48 ± 0.35	0.30 ± 0.09
Chemical control (COC @0.2%)	0.00 ± 0.00	0.00 ± 0.00	0 ± 0.00
Absolute control	0.00 ± 0.00	0.00 ± 0.00	0 ± 0.00

*Mean of five replications. (n=15). Treatment means were statistically non-significant ($p \leq 0.05$). DAI: Days after inoculation

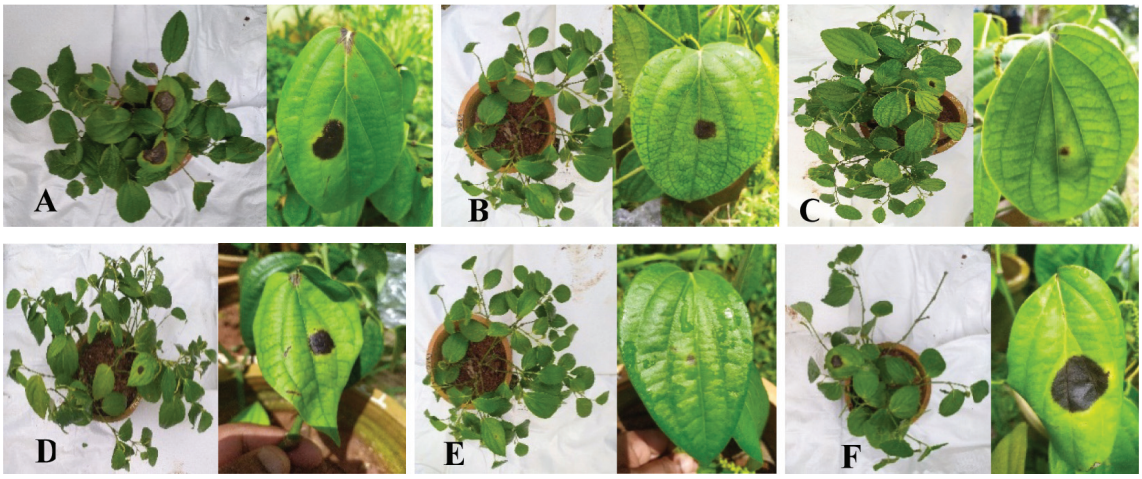


Figure 2. Bush pepper plants treated with endophytes showing foliar symptoms of infection six days after challenge inoculation with *Phytophthora capsici*. Representative leaves from the corresponding infected plants are also shown. A- *Piriformospora indica,* B- *Bacillus velezensis* PCSE10, C- *Rhizobium radiobacter* PCRE 10, D- *P. indica* and *Bacillus velezensis* PCSE10, E- *P. indica* and *Rhizobium radiobacter* PCRE 10, F- Pathogen control

Significant reduction in foliar infection by *P. capsici* has been reported when strains PCSE 10 and PCRE 10 were used for bacterization of black pepper variety Karimunda (Kollakkodan et al., 2021). In the present study the variety used was Panniyr 1. Varietal difference plays an important role when it comes to disease suppression brought about through induction of disease resistance. Direct antagonistic activity comes to play in disease suppression when the pathogen and the antagonist directly interact in the host system. In the present study, bacterization was done to the root region. Treated bacteria in the planting material may enter the plants and systemically colonize different parts (Leite et al., 2003). We have not attempted on deciphering the internal colonization of inoculated strains in black pepper plants. However, since the isolates were obtained from wild pepper, chances of them getting colonized within black pepper are high. Such colonization might have contributed to disease suppression by direct antagonism on the foliage. *Bacillus* spp. are also well known for inducing defense in several crop plants on inoculation (Kloepper et al., 2004; Choudhary and Johri, 2009). Since there was a spacial distance between the bacterized part and the site of disease suppression, induced resistance triggered by endophytes is supposed to have contributed to the disease suppression in black pepper. Further analyses are to be carried out to decipher the mechanism behind disease suppression by the endophytes in black pepper plants. Quantifying expression of defense related enzymes and genes is one way to understand the mechanism. Disease resistant varieties of crop plants may harbour potential endophytic antagonists against the pathogen within them as reported earlier (Feng et al., 2013; Upreti and Thomas, 2015). Antagonistic endophytes in the host plant would supplement their resistance against pathogens. It may be postulated that endophytes isolated from wild pepper might have successfully colonized in the treated black pepper plants and thus provided suppression against *P. capsici*. Endophytic bacteria can be delivered into the plant system prior to planting of cuttings in nursery in the case of black pepper. Endophytes were applied as bacterization of cuttings and also during transplanting into larger pots. Results of the present study shows that endophytic bacteria obtained from *Phytophthora* resistant pepper species could impart suppression of the disease in a susceptible variety of black pepper. The information generated in the present study about endophytic bacteria from *Piper colubrinum* and root endophytic fungus *P. indica*

will be helpful in evolving better plant health management strategies in black pepper.

The present study has established the beneficial effect of the use of a consortium of bacterial endophyte, *Rhizobium radiobacter* PCRE10 and root endophytic fungus *P. indica* in bush pepper plants. Though *P. indica* was not highly effective in suppressing the infection by *P. capsici*, it improved growth of bush pepper plants. Endophytic bacteria from the wild pepper, *P. colubrinum* showed both plant growth promotion and disease suppression. A formulated product of the consortium may be developed for the use in black pepper.

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