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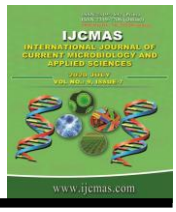

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Growth Promotion in Chilli (*Capsicum annuum* L.) on Inoculation with Co-cultured *Piriformospora indica* and *Pseudomonas fluorescens*

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A B S T R A C T

Application of consortium of microorganisms as bio fertilizers increases its efficiency. In the present study co-culturing of root endophytic fungi *P. indica* and the rhizobacterial strains of *P. fluorescens* and its application in chilli is described. Prior to the coculturing of the fungi and bacteria, the bacterial strains were tested against the fungi for direct antagonism by dual culture plate assay in both PDA and coconut water agar (CWA). Indirect antagonism was also checked using the culture filtrate by agar well diffusion method and paper disc diffusion method. The strain *P.fluorescens* PN026 showed no antagonism against the *P. indica* in CWA whereas the strain *P.fluorescens* AMB8 showed a reduced antagonism in CWA compared to PDA. During coculture, bacterial strains showed a similar growth rate as that of monoculture, when grown in autoclaved coconut water (ACW), where as in PDB, there was a declining population of the bacteria was observed. So ACW was selected for coculturing of the fungi and bacterial strains. When the microorganisms were applied individually and as consortium (both in the form of mixed inoculum and cocultured inoculum) to chilli enhanced vegetative growth, early flowering, increased yield and highest root colonization percentage were observed in the plants applied with consortium of *P. indica* and the strain *P.fluorescens* PN026.

Introduction

Chilli, known as wonder spice is one among the most important commercial spice crop around the world and is best known for its hot, pungent flavor. It is mainly raised by seedling using plug trays (pro-trays). This system helps the grower for establishing seedlings with perfect stands, uniform physiological plant age and optimal spacing during transplanting and thus enable quicker re-establishment and less transplanting shock when the seedlings are transferred to the field from nursery. The use of biological agents at the nursery production stage is advantageous as many of them enhance better rooting and health of the seedlings (Vavrina, 1998).

Fluorescent Pseudomonads are considered to be the most promising group of plant growth

promoting rhizobacteria. Various studies revealed that this PGPR is able to substitute the use of chemical fertilizer to a greater extent by various mechanisms like production of plant hormones, siderophore production etc. (Gamez *et al.,* 2015; Karnwal *et al.,* 2009; Saranraj *et al.,* 2013; Linu *et al.,* 2019).

Piriformospora indica is a wide host range root colonizing endophytic fungus which allows the plant to grow under extreme physical and nutrient stress condition. The fungus can be cultivated on complex or mineral substrate. It belongs to the Sebacinales in Basidiomycota (Verma *et al.,* 1998; Weiss *et al.,* 2004; Franken 2012; Varma *et al.,* 2012). Root colonization of *P.indica* has resulted in increased nutrient uptake, temperature and salt stresses, and confers systemic resistance to pathogenic organisms, insects, toxins and heavy metals. It enhances biomass production, stimulate early flowering and seed production. It is used as a potential microorganism for biological hardening in tissue culture raised plants (Verma*et al.,* 1998; Yadav *et al.,* 2010; Das *et al.,* 2012).

For increasing the spectrum of action and efficiency of bio-inoculants, they can be used as mixed inoculum or consortium with more than one bio-agents as a formulation (Vidyasekarn and Muthamilan 1995; Schisler *et al.,* 1997; Janisiewicz 1988; Slininger *et al.,* 2010). Synergetic effect of co-inoculation of micro-organisms resulted in increased dry root and shoot weight, enhanced productivity, faster seed germination etc. in plants (Meena *et al.,* 2010; Sarma *et al.,* 2011; Kumar *et al.,* 2012; Saxena *et al.,* 2015)

Co-culturing requires a single medium that could support the growth of the component microbial strains. It should be rich with various source of sugars, amino acid,enzymes etc. for growing a variety of organisms like bacteria and fungi.A number of studies have suggested coconut water, naturally available and cheap product, as highly potential medium for growing microorganisms. Multiplication of PGPR in coconut water makes bio-fertilizer production more farmer friendly (Anith 2009).

The present study was undertaken considering these key points, with an objective of assessing the compatibility of *P. indica* along with two *Pseudomonas fluorescens* strain and evaluate their effect on growth promotion in chilli.

Materials and Methods

Cultivation of fungal and bacterial strain

Piriformospora indica was obtained from Dr. Ajit Varma, former Professor, Jawaharlal Nehru University, New Delhi, India. It was cultivated on Potato Dextrose Agar (PDA; pH 6.5) at 28°C (Fakhro *et al.,* 2010; Kumar *et al.,* 2011). The fungus was mass multiplied in 100 ml Potato Dextrose Broth (PDB; pH 6.5) in 250-ml Erlenmeyer flasks after inoculating with a mycelial disc from a freshly grown PDA plate, and incubating at 28°C for 15 days with constant shaking in a rotary shaker (Scigenics, India) at 90 rpm (Anith *et al.,* 2011). This root endophytic fungus was also grown on coconut water agar (CWA) and autoclaved coconut water (ACW) during the course of present study providing similar incubation temperature asin the case of PDA and PDB. For this coconut water collected from a local coconut processing unit was filtered using muslin cloth to avoid dirt and debries.100ml of it was transferred to a 500 ml Erlenmeyer flask, the pH was adjusted to 6.5 and the solution was sterilized by Autoclaving at 121 °C for 20 minute. For preparing CWA, 2% agar was added before autoclaving (Anith *et al.,* 2015).

Bacterial strains used in the study were *Pseudomonas fluorescens* PN026 and *Pseudomonas fluorescens* AMB8, PGPR strains available at Department of Agricultural microbiology, College of Agriculture, Vellayani. Both of them were grown in King'S medium B agar and broth at 28°C (Anith *et al.,* 2015).

Testing *In vitro* antagonism between *piriformospora indica* and bacterial strains

Direct Antagonism

Compatibility of *Piriformospoara indica* with *Pseudomonas fluorescens* strains was evaluated by dual culture plate assay in PDA and coconut water agar (CWA). For this single colonies of *Pseudomonas* strains were obtained by streak plating on King's medium B agar. Mycelial disc (8 mm dia) was cut from the 10 day old culture of *P.indica* grown on PDA plates and transferred to the centre of fresh PDA plate and CWA plate. When the fungal growth reached a diameter of 5 cm, each of the test organism each of the test organism was streaked as a band (5cm) separately on two sides of the PDA plates and CWA plates at a distance of 2 cm away from the periphery. Control plates were also maintained with *P.indica* alone. Plates were incubated at 28°C for seven days. Observations were recorded by measuring the inhibition zone if any (Anith *et al.,* 2015).

Indirect antagonism

Antagonistic effect of the culture filtrate of the bacterial bioagents against the endophytic fungus was assessed by agar well diffusion method and disc diffusion method. For the preparation of culture filterate, a loopful of bacterial cells was transferred to King's medium B broth from the pure culture of the bacterial bioagents. It was incubated overnight in incubator shaker (110 rpm) at 28°C. Ten ml of the broth culture from each of the strain was centrifuged at 10,000 rpm for five minutes in sterile polypropylene tube. The supernatant was aseptically collected and filter sterilized using a 0.2 µ nitrocellulose bacteriological filter. The filtrate was aseptically collected and stored at 4°C for further use.

Agar well diffusion method

Mycelial disc (8 mm dia) was cut from the 10 day old culture of *P. indica* grown on PDA plates and transferred to the centre of fresh PDA plate. Wells (8 mm dia) were cut at two opposite edges of the plate using a sterile cork borer. The wells were partially filled with 100 *µl* of molten agar. Once the well was sealed properly, 100 *µl each of the* culture filtrate of each test organism was added to the wellsand incubated for48 h at 28°C. Three replications were maintained for each of the organisms. The inhibition zone from the well was measured *(*Balouiri *et al.,* 2016*).*

Disc Diffusion Method

Mycelial disc (8 mm dia) was cut from the 10 day old cultureof*P. indica*grown on PDA plates and transferred to the centre of fresh PDA plate. Sterile filter paper discs (5 mm dia) were soaked with *ten* µl of the culture filtrate. The discs were dried in a laminar air flow chamber and placed at two opposite edges of the Petri plate containing *the fungus.* Plates were then incubated for aperiod of 48 h at 28°C. Three replications were maintained for each of the organism. Inhibition zone from the filter paper disc was measured (Nawangsih *et al.,* 2011).

Co-culture experiment

100 ml of ACW was sterilized in 250 ml Erlenmeyer flask and both the media were inoculated with two mycelial plugs (8 mm

dia) of *Piriformospora indica* obtained from PDA plates previously grown for 10 days. Bacterial strains were streaked out for single colonies on King's medium B agar plate. Cells from a single colony were pooled in one ml of sterile distilled water and 200 µl of the bacterial suspension was aseptically added to flasks of PDB and ACW wherein *P. indica* had been growing since 10 days. The initial population of the bacteria added to the flasks was determined by dilution plating on King's B agar medium immediately after inoculation. The flasks were further incubated under agitation (150 rpm) for 48 h and the population of the bacteria was determined at 24 h intervals by dilution plating on King's medium B agar. The bacterial population from five flasks was independently assessed for both growth media. Growth of the bacteria in fresh PDB, ACW and King's B broth was taken as baseline to determine the efficiency of the co-culture in supporting bacterial growth (Anith *et al.*, 2015).

Plant growth experiment

Vermiculite was used as planting medium in the pro-trays. It was sterilized by autoclaving at 121^0C for 1 h each for three consecutive days. Pro-trays (50 cells; each cell having a dia of 5 cm) were filled with the sterile potting mixture. Seeds of chilli were surface sterilized in one percent sodium hypochlorite aqueous solution for 3 minutes in a laminar air flow chamber. The seeds were further washed thrice with sterile distilled water.

For inoculaton of bacteria alone *Pseudomonas* strains were heavily cross streaked on King's medium B agar. After 24 h of incubation, the plates were drenched with 10 ml sterile distilled water and the bacterial growth was suspended in it by using a sterile glass spreader. The suspension was collected aseptically in sterile glass vials. The OD of the suspension was adjusted to 0.6 at 660 nm using sterile distilled water so that the suspension contains approximately 10^8cfu ml^{-1}. Bacterization was done by soaking the surface sterilized seeds in bacterial cultures for 20 minutes prior to seeding.

P. indica mycelium was incorporated into the planting medium before filling the pro-tray cavities. For this, mycelium of the fungal endophyte grown for 15 days in a 250 ml flask containing 100 ml PDB medium was collected by filtering the contents of the flask through a muslin cloth. The same was weighed and mixed thoroughly with sterile planting medium @ one percent (w/v).

For mixed inoculation of the fungus and bacteria, after incorporating the fungal mycelium in the planting medium seeds for which bacterization was done by soaking the surface sterilized seeds in bacterial cultures for 20 minutes prior to seeding were used for planting.

Co-culturing of the fungus and the bacteria was done as described earlier. Forty-eight hours after the addition of the bacteria to the flask containing the fungus grown in ACW, the mixture was filtered through muslin cloth and the fungal mycelium was collected and its fresh weight was determined. The incorporation of the fungal-bacterial mixture in vermiculite (1 % w/v) was performed as described above. No further supplementation of bacterial culture was done, as the cocultured fungal mycelium contained bacterial cells.

Two seeds were planted per cavity of pro-tray and further thinned to single seedling after germination. Plants were grown in a net house with natural ventilation, sunlight as light source with 50 percent shade. Seedlings were irrigated with tap water twice daily. Once in 10 days, fertigation was provided by pouring 10 ml of one per cent water soluble fertilizer

solution (N:P:K - 17:17:17) per cavity starting from first week after seeding. Plants were kept for 25 days in the nursery. Twenty five day-old seedlings were transplanted to pots (15 cm dia) filled with one kg each of potting mixture (soil, sand and cow dung in the ratio 2:1:1).

Root colonization by *Piriformospora indica*

The plants treated with *Piriformospora indica* were assessed for root colonization by the endophytic fungus. 30 days after plant growth, five plants from each treatment were uprooted without damaging the roots (Anith *et al.,* 2015). The root system was washed in running tap water to get rid of the adhering planting medium. They were then cut into small bits of one cm length. The bits were softened by boiling in 10 per cent potassium hydroxide (KOH) for five minutes. KOH was removed by washing with distilled water. Roots were then acidified with 1N HCl for five minutes and directly transferred to the staining solution, lactophenol-tryphan blue for 10 minutes. Destaining with lactophenol solution for 10 minutes was done prior to examination under a compound bright field microscope. Presence of chlamydospores was taken as a positive indication of root colonization. The percentage root colonization was calculated using the formula;

$$\text{Percentage root colonization} = \frac{\text{No. of root bits with chlamydospores}}{\text{Total number of root bits observed}} \times 100$$

Statistical analysis

Statistical analysis was done using the package available with the online portal of IASRI, New Delhi. The means were compared using Least Significant Difference (LSD) at 5 per cent level of significance using ANOVA.

Results and Discussion

Microbial technologies have been applied to various agricultural and environmental problems with considerable success in recent years. Biofertilizer and biopesticide containing efficient microorganisms improve plant growth in many ways compared to synthetic fertilizers, insecticides and pesticides by way of enhancing crop growth and thus help in sustainability of environment and crop productivity. Major objective of the current study was to assess the compatibility of the root endophytic fungus *Piriformospora indica* and two *Pseudomonas fluorescens* strains and to evaluate their effect on growth promotion in chilli.

Dual culture plate assay was done to test the compatibility of bacterial bioagents with *P. indica* on PDA and CWA plates as both the fungal endophyte and the bacterial agents could grow well on them. Compatibility was assessed by lack of any inhibition zone whereas, the non-compatible ones would develop zone of inhibition. In the present screening on PDA plates both *Pseudomonas fluorescens* PN026 and *Pseudomonas fluorescens* AMB8 were found to be incompatible with *P. indica* in which the former one developed a reduced zone of inhibition. When screening was done on CWA plates only the strain *Pseudomonas fluorescens* AMB8 developed zone of inhibition and the strain *Pseudomonas fluorescens* PN026 was compatable with the fungus. *P. indica* had varying reactions with different rhizobacterial isolates. When co-cultured on agar plates some of them displayed neutral response, however many displayed stimulatory to inhibitory responses (Varma *et al.,* 2012). In an experiment done by Anith *et al.,* (2015) dual culture assay between *P. indica* and two Bacillus strains showed differential response. Zone of inhibition was larger for *B. amyloliquefaciens*

whereas no antagonistic effect was seen with *B. pumilus* when the screening was done on coconut water agar medium. This implied that *P. indica* could be co-cultured with *B. pumilus*. Dual culture plate assay done by Varkey *et al.,* (2018) using *B. pumilus* VLY17 and *P. fluorescens* AMB8 with *P. indica* on PDA exhibited inhibition pattern and both the strains were incompatible with the endophytic fungus. In the former case however the screening was done in CWA medium. Differential inhibition pattern for the same bacterial strain on different media indicates the influence of the screening medium in determining the interaction between the microorganisms or variability among the isolates (Table 1, Figure 1).

Indirect antagonism by culture filtrate of bacterial strains against *P. indica* was done by Agar well difussion method and paper disc diffusion method. In both of these indirect methods, same trend was observed. In case of *P. fluorescens* PN026, the zone of inhibition was getting reduced rapidly and reached a negligibly small value in the third day of observation. In case of *P. fluorescens* AMB8 a higher zone of inhibition compared to *P. fluorescens* PN026 was observed and it was remaining as almost stable. It was reported that antagonistic activity of *Pseudomonas fluorescens* was tested successfully against various fungi and bacteria using these methods. Agarry (2005) tested the antagonistic activity of *Pseudomonas fluorescens* isolated from cassava rhizosphere against fungal pathogens like *Fusarium moniliforme* and *Aspergillus niger* efficiently by using the agar well diffusion method. Maji and Chakrabartty (2014) used culture filtrates of *Pseudomonas* spp. which exhibited zone of growth inhibition on *R. solanacearum*. Antifungal metabolites, antibiotics, enzymes etc. secreted by the bacteria are present in the cultural filtrate and play a major role in fungal suppression (Table 2; Figure 2)

Co-culturing of the bacterial bioagents with the endophytic fungus *Piriformospora indica* showed varying levels of population buildup of the bacteria after 24 h and 48 h of incubation. When 10 day-old cultures of the fungus in ACW and PDB were inoculated with the bacterial strains, the former medium supported the growth of the bacteria similarly to fungus free culture and King's medium B broth. Both monoculture and co-culture in ACW resulted in achieving a population of 10^{10} cfu/ml from an initial inoculum of 10^{5} cfu/ml. On the other hand co-cultivation PDB led to a decline in bacterial population. In ACW *Pseudomonas fluorescens* PN026 grew similar to that of the conventional medium King's medium B broth and when co-cultured with fungi the population build up was comparable with conventional medium and higher than that of the monoculture of the bacteria. In case of *Pseudomonas fluorescens* AMB8 same trend was observed except that the population in co-culturing was lower than that of the monoculture of the bacteria. Similar to the result obtained in the present study, it was reported that co-culturing of *Bacillus pumilis* with *P. indica* in PDB resulted in a decline in population buildup of bacteria (Anith *et al.,* 2015) (Table 3 and 4).

Statistical analysis of various growth parameters revealed that there was a significant enhancement in the growth of plants treated with combined application of *P. indica* and *P. fluorescens* PN026, both as coculture and mixed inoculum. The endophytic fungus has been reported to improve the uptake of nitrogen by plants through the enhanced expression of nitrate reductase in plant roots (Sherameti *et al.,* 2005). Various strains of *P. fluorescens* have been reported as effective as that of 100 percent fertilizer application (Gamez *et al.,* 2015). The improved vegetative growth can be attributed to the synergetic effect by combining the organisms in mixed application

which enhanced the growth promotion capacity of *P. indica* and *P. fluorescens*. Number of branches was significantly higher only from 60 days after transplanting. It may be due to the reprogramming of root exudation pattern of the host by endophytic fungus and thus increasing the population of PGPR in the rhizosphere of chilli as reported by Saxena *et al.*, (2015).

Application of co-cultured *P. indica* and *P. fluorescens* PN026 induced early flowering in the plants followed by the plants treated with mixed inoculation of *P. indica* and *P. fluorescens* PN026. Earliness in flowering in *P. indica* applied black pepper plants has been reported by Anith *et al.*, (2018). The medicinal plants *Spilanthes calva* and *Withania somnifera* were inoculated with *Piriformospora indica* and it was observed that number of inflorescences and flowers and seed production were all enhanced in the presence of the fungus (Rai *et al.*, 2014). A study conducted in the medicinal plant, *Coleus forskohlii*, *P. indica* colonized plants flowered at least 7 day earlier and more vigorously than the non-colonized plants. It was suggested that the increase in flower production may be caused by an increase in plant nutrient (especially K+ and P) uptake by the fungal endophyte, in combination with a possible hormonal effect. Hormones, such as gibberellins that induce the bud production could be transported in faster rates due to higher levels of K+ in the plant and phosphorus have a great impact on bud formation and development (Das *et al.*, 2011). Maximum number of fruits, fruit length and highest yield was recorded in the co-cultured *P. indica* and *P. fluorescens* PN026 treated plants (Figure 3).

Application of *P.indica* and *P. fluorescens* PN026 in combination resulted in the highest root fresh weight and dry weight which was at par with all other treatments including *P.indica* either applied individually or combined with bacterial strains both as co-cultured mixture and mixed inoculation (Figure 4). The possible reason may be due to the ability of *P. indica* to enhance the root growth and number of adventitious root as reported in many studies. Sirrenberg *et al.*, (2007) attributed the ability of this fungus to enhance root growth promotion to the production of auxin inside plant after the entophyte get established. Justice *et al.*, (2018) reported potential enhancement of adventitious root formation as well as increase in root weight in the flowering plants like crossandra, dahlia and poinsettia when the cuttings were planted in rooting medium amended with *P. indica* (Table 5)

Table.1 Mycelial growth inhibition of *P. indica* by bacterial strains in dual culture plate assay

Bacterial strain	Zone of inhibition (mm) *			
	PDA**		CWA**	
	5th DAY	7th DAY	5th DAY	7th DAY
***P. fluorescens* PN026**	4.34	1.16	0.67	nil
***P. fluorescens* AMB8**	5.67	1.50	2.34	0.67

*Mean of eight observations from four dual culture plates. Each plate represents single replication (n=4)
**PDA- Potato Dextrose Agar; CWA- Coconut Water Agar

Table.2 Mycelial growth inhibition of *P. indica* by bacterial strains in agar well diffusion method and paper disc diffusion technique

Bacterial strain	Zone of inhibition measured in 7th day (mm) *	
	Agar well diffusion method	Paper disc diffusion method
***P. fluorescens* PN026**	0.17	0.83
***P. fluorescens* AMB8**	0.84	3.00

*Mean of eight observations from four dual culture plates. Each plate represents single replication (n=4)

Table.3 Population buildup of *P. fluorescens* PN026 (cfu ml^{-1}) in different media and cultural conditions

Type of inoculation	Population buildup of *P. fluorescens* PN026 (cfu ml^{-1})		
	Time of population assessment		
	0 h	24 h	48 h
***P. fluorescens* PN026 alone**			
PDB	1.17 x 10^5	1.00 x 10^7	1.50 x10^6
ACW	1.84 x 10^5	5.57 x 10^7	1.45 x 10^{10}
KB	2.74 x 10^5	6.10 x 10^7	1.65 x 10^{10}
***P. indica* and *Pseudomonas fluorescens* PN026 coculture**			
PDB	3.30 x 10^5	8.60 x 10^4	6.60 x 10^4
ACW	1.18 x 10^5	6.1 x 10^7	1.65 x 10^{10}

Table.4 Population buildup of *P. fluorescens* AMB8 (cfu ml^{-1}) in different media

Type of inoculation	Population buildup of *P. fluorescens* AMB8 (cfu ml^{-1})		
	Time of population assessment		
	0 h	24 h	48 h
***Pseudomonas fluorescens* AMB8 alone**			
PDB	1.35x 10^5	1.60 x 10^6	4.40 x 10^6
ACW	4.40 x 10^5	5.57 x 10^7	1.45 x 10^{10}
KB	7.56 x 10^5	3.67 x 10^9	2.77 x 10^{10}
***P. indica* and *Pseudomonas fluorescens* AMB8 coculture**			
PDB	5.00x 10^5	4.97 x 10^4	5.30 x 10^4
ACW	1.74 x 10^5	1.77 x 10^7	1.05 x 10^{10}

*Mean of eight observations from four dual culture plates. Each plate represents single replication (n=4)

Table.5 Growth parameters in pot culture of chilli after 80 days of transplantation. *Each treatments were having 3 replication having 5 plants in each

Treatments*	Biometric observations												
	Height (cm)	No.of. leaves	No. of branches	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	No. of fruits /plant	Fresh fruit yield (g/plant)	Dry fruit weight (g/plant)	Fruit length (cm)	Days to first flowering	Days to fruit set
***P. fluorescens* PN026**	40.34^{a}	54.17^{a}	12.17^{abc}	45.92	7.93	12.78^{c}	3.54^{b}	8.71^{cd}	24.54^{b}	3.18^{bc}	7.75^{c}	35.67^{a}	70.58
***P. fluorescens* AMB8**	38.50^{a}	51.34^{c}	11.58^{bc}	39.93	6.06	15.32^{bc}	3.69^{b}	8.13^{de}	22.74^{b}	2.86^{bc}	7.72^{c}	38.25^{a}	68.00
P. indica	38.51^{a}	65.54^{a}	10.74^{cd}	42.23	7.16	19.73^{ab}	7.05^{a}	9.76^{bc}	34.08^{a}	3.49^{b}	9.74^{ab}	35.34^{a}	61.59
P. fluorescens* PN026 and *P. indica	41.88^{a}	68.34^{a}	15.75^{a}	49.91	8.71	21.14^{a}	8.26^{a}	10.25^{ab}	37.78^{a}	3.55^{b}	9.62^{ab}	34.88^{ab}	63.75
P. fluorescens* AMB8 and *P. indica	39.34^{a}	48.08^{c}	9.92^{cd}	39.62	6.54	18.31^{abc}	7.25^{a}	8.42^{de}	33.97^{a}	2.21^{c}	8.05^{bc}	36.50^{a}	66.17
Co-cultured *P. fluorescens* PN026 and *P. indica*	42.17^{a}	65.50^{ab}	14.67^{ab}	38.39	6.41	18.12^{abc}	7.45^{a}	11.25^{a}	37.95^{a}	4.76^{a}	10.12^{a}	31.75^{b}	62.25
Co-cultured *P. fluorescens* AMB8 and *P. indica*	36.08^{a}	48.50^{c}	10.08^{cd}	37.79	5.85	17.96^{bc}	6.52b^{b}	7.29^{e}	21.31^{b}	2.48^{bc}	7.53^{c}	35.33^{a}	66.34
Uninoculated control	38.34^{a}	45.92^{c}	7.25^{d}	32.40	6.33	13.18^{c}	3.84^{b}	8.09^{de}	21.61^{b}	2.62^{bc}	7.27^{c}	21.61^{a}	62.84
SEm (±)	2.03	3.79	1.32	7.348	1.27	1.92	0.71	0.40	1.73	0.62	1.55	1.20	2.20
CD (0.05)	NS	11.12	3.89	NS	NS	5.63	2.08	1.17	5.06	1.15	1.81	3.52	NS

Fig.1 *In vitro* assessment of compatibility between bacterial bioagents and *P. indica* in PDA plates and CWA plates

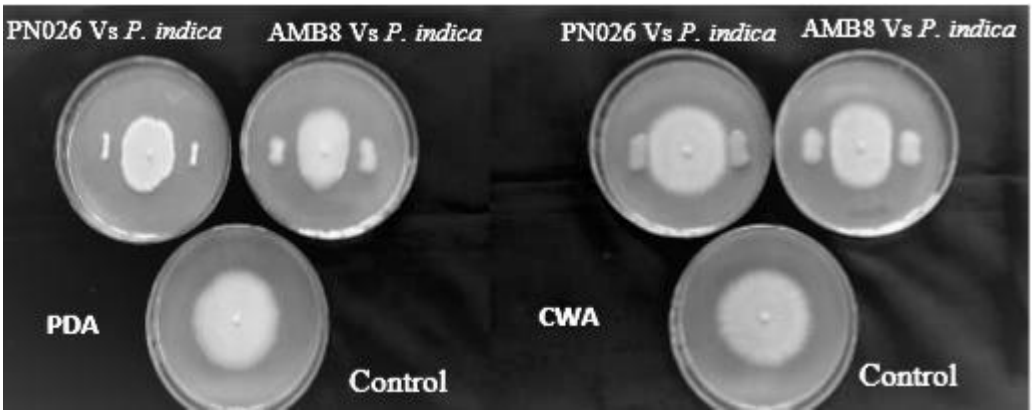

Fig.2 Indirect methods used for checking antagonism of bacterial bioagents against the fumgal endophyte *Piriformospora indica* A. Agar well diffusion method using *P. fluorescens* PN026; B. Agar well diffusion method using *P. fluorescens* AMB8; C: Disc diffusion method using *P. fluorescens* PN026; D: Disc diffusion method using *P. fluorescens* AMB8; E: Control

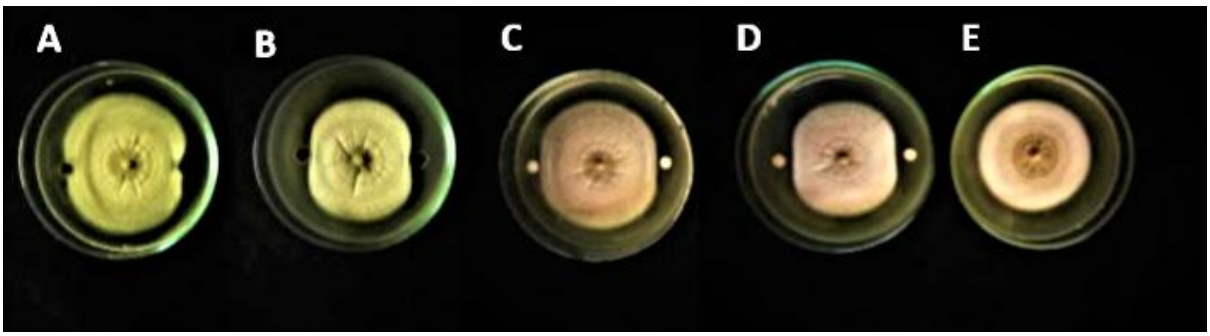

Fig.3 Comparison of the length of fruits from each treatment . plants treated with 1: *P. fluorescens* PN026 ; 2 : *P. fluorescens* AMB8; 3: *P. indica ;* 4: *P. fluorescens* PN026 and *P. indica*; 5: *P. fluorescens* AMB8 and *P. indica;* 6: Co-cultured *P. fluorescens* PN026 and *P. indica* ; 7: Co-cultured *P. fluorescens* AMB8 and *P. indica;* 8: Uninoculated control

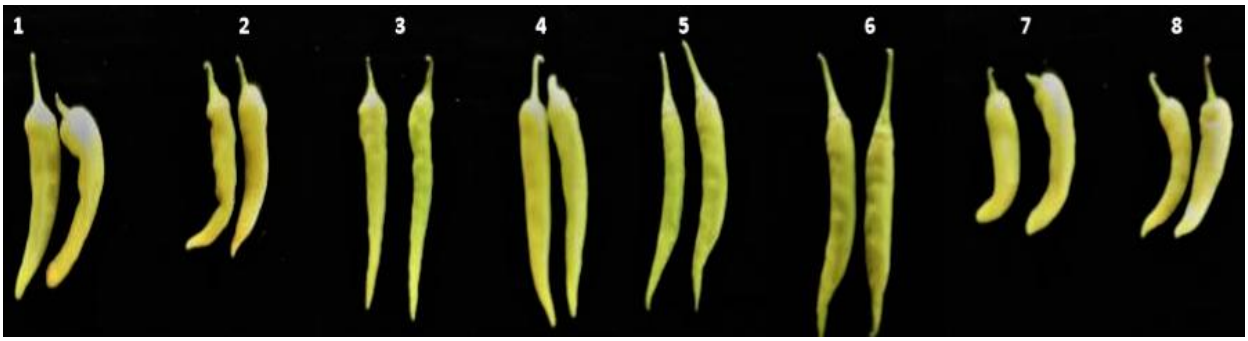

Fig.4 Comparison of roots of plants inoculated with *P. indica* with uninoculated control

Plants treated with co-cultured *Piriformospora indica* and *Pseudomonas fluorescens* PN026 showed highest root colonization. In a study conducted by Jayasinghearchchi and Seneviratne (2010) reported that an endophytic fungus *Pleurotus ostreatus* co-cultured with *Pseudomonas fluorescens* improved the endophyte colonization of tomato through biofilm formation. The same reason can be proposed for the enhanced root colonization by the co-cultured *Piriformospora indica* and *Pseudomonas fluorescens* PN026 in chilli.

Among all the parameter analysed, either the uninoculated control or the treatment with co-cultured *P. indica* and *P. fluorescens* AMB8 showed the lowest value and it is evident that this combination had no additional advantage in the plant growth promotion of chilli. The possible reason for this may be the antagonistic effect showed by the bacterial strain against the endophytic fungus. The fungal colonization percentage in the roots of plants treated with co-cultured *P. indica* and *P. fluorescens* AMB8 was the lowest and the growth promotion by the bioagent was not visible in the plants.

Results of the current investigation suggest that mixed inoculation and inoculation with the co-culture of *P. indica* and *P. fluorescens* PN026 are more efficient than single inoculation of the biological agents for improving plant growth in chilli. Previous reports involving other crops also supported the idea of co-inoculation of *P. indica* with beneficial bacteriata achieve greater plant growth (Meena *et al.*, 2010; Sarma *et al.*, 2011; Kumar *et al.*, 2012; Anith *et al.*, 2015). So application of consortium of microorganisms is advisable rather than application of a single organism as biortilizer in fields.

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