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THE POTENTIAL OF PGPRS IN DISEASE MANAGEMENT OF SPICE CROPS

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INTRODUCTION

India is known as the "Land of Spices" as spices are grown over an area of 25 lakh ha and producing about 26 lakh tonnes. Out of these, about 8-10% are exported. The major spices exported from India are black pepper (*Piper nigrum* L.), cardamom (*Eletteria cardamomum* Maton), ginger (*Zingiber officinale* Rosc.) and turmeric (*Curcuma longa* L.). The production and productivity of these crops are limited by several soil borne diseases. Some of these diseases become complex with infestation by plant parasitic nematodes. Often these organisms are carried inadvertently from the nursery to the main field along with planting materials. Biological control methods have been developed for managing the diseases in black pepper, cardamom and ginger (Anandaraj and Sarma, 1994, Sarma et al 1998, Anandaraj 2000). Soil borne diseases such as foot rot and slow decline in black pepper (Sarma, 2003), clump rot and capsule rot of cardamom, rhizome rot of ginger and Turmeric and wilt in cumin and coriander are the major production constraints. To avoid pesticide load in to the environment and also pesticide residues in the produce, major thrust was given to biological control as a component of Integrated Disease Management. In this context, the role of PGPRs has become all the more important and the studies carried out so far on foot rot and slow decline of black pepper and rhizome rot of ginger have indicated the potential both in growth promotion and disease suppression.

Isolation and Maintenance of Plant growth promoting Rhizobacteria

Fluorescent pseudomonads are some of the effective candidates for biological control of soil borne plant pathogens owing to their rhizosphere competence (Kloepper, *et al*, 1981 & 1980). These bacteria are termed as Plant growth Promoting Rhizobacteria (PGPRs) because of their ability to improve plant growth through suppression of deleterious root colonizing microorganisms and by production of plant growth regulators (Suslow *et al*, 1982) such as gibberellins, cytokinins and indole acetic acid (Krause *et al*, 1992). Several antagonistic *Trichoderma* and other beneficial organisms such fluorescent pseudomonads were isolated from roots and rhizosphere soil of spice crops collected from different places in the states of Kerala, Karnataka, Tamil nadu, Andhra Pradesh and Sikkim. The Bacterial strains were screened against the pathogens and those that showed significant inhibition are retained for further studies. A repository of rhizobacteria is being maintained at IISR, Calicut with a collection of over one thousand isolates. The bacterial cultures are stored at -80°C (Sanyo medical Freezer) with glycerol as cryoprotectant.

In vitro screening of bacteria

The antagonistic potential of bacterial isolates was evaluated for both pathogenic fungi and nematodes. Dual culture technique followed to test against *P.*

capsici, the foot rot pathogen of black pepper and the efficient bacterial strains that showed up to 70% inhibition of *P.capsici* were short listed (Minimol, 2002, Diby et al 2001). Bacterial isolates were screened against plant parasitic nematodes by the buffer culture filtrate assay method and assessed their nematode suppressing ability. Most of the bacterial isolates caused negligible mortality of nematodes in this test. Bacterial isolates were selected for further *in vitro* evaluation using different methods like, direct assay of bacterial suspension and assay of volatile and non-volatile metabolites. Culture filtrates of 77 bacterial isolates were studied for their nematode toxic activity. Out of these, 22 isolates caused >90% mortality to root knot nematodes while another 40 isolates possessed high (>50% mortality) nematicidal property. Metabolites (volatile and non volatile) of 67 bacterial isolates were also tested for their nematicidal activities. Bacterial isolates that showed in *vitro* mortality of nematodes up to 50% and 90% were short listed. The short listed organisms were also tested in green house experiments and finally in the field. Under green house 175 isolates were tested against *Meloidogyne incognita* with tomato as test crop and with 30 isolates with black pepper as test crop. Twenty five isolates showed 100 % nematode suppression. The most promising isolates for *Radopholus similis* suppression have also been identified (Anandaraj and Eapen 2003).

Evaluation of PGPRs in green house

The selected isolates of PGPR were evaluated in green house for their efficiency in growth promotion, biomass production and foot rot and root rot suppression in black pepper. Bacterial cultures were multiplied in nutrient broth and at late log phase of growth, the cells were washed and the harvested cells were re-suspended in sterile 10mM MgSO4. Uniform size rooted black pepper cuttings of were selected and the roots were dipped in bacterial suspension for 30 minutes and planted in sterile soil. The height of the plant and number of leaves were recorded periodically. A second application of the bacteria was given after four months of planting. The isolates showing enhanced growth and root rot suppression were short-listed. In the green house assay performed, the bacterial strain, IISR-51 promoted growth of the black pepper up to 57.15% (Lisha et al 2002)

For testing nematode suppression 75 bacterial isolates were tested in four different experiments. All the isolates caused significant reduction in nematode development and 100% nematode suppressions was obtained with three isolates (Beena et al., 2001; Ramana et al., 2002). Though a number of fluorescent pseudomonads were evaluated, none of them had any significant influence on any of the plant growth characters or in reducing the plant damage due to root-knot nematode infestation. Many of them were good growth promoters with an increase in growth ranging from 26.7 to 55.6% even when the plants were affected by root-knot nematodes (Eapen et al., 1997). Five isolates of rhizobacteria (IISR 522, IISR 528, IISR 658, IISR 853 and IISR 859) having dual nematicidal action (suppressing both *R. similis* and *M. incognita*) were short-listed from a collection of 291 isolates.

Biocontrol Consortium

The consortium approach for disease management in plantation and spice crops was suggested earlier (Sarma and Anandaraj, 1998). Mutual compatibility of fungal & bacterial antagonists viz. *Trichoderma harzianum* & fluorescent *Pseudomonas* were studied in order to establish an efficient consortium for the

management of foot rot of black pepper caused by *Phytophthora capsici*. The study revealed that the fungal and bacterial antagonists are compatible (Jisha *et al*, 2002).

Compatibility of two *Pseudomonas fluorescens* (IISR - 51) and *Trichoderma harzianum*, IISR-1369)

Mutual proliferation of fungal & bacterial antagonists, *Trichoderma harzianum* & fluorescent *Pseudomonas* were studied in order to establish an efficient consortium for the management of foot rot of black pepper caused by *Phytophthora capsici*. Experiments were conducted to study the population dynamics of fungal and bacterial antagonists in co-inoculated liquid medium and soil, using plate assay for a week. The two-biocontrol agents were found to be compatible with each other and successfully colonized black pepper rhizosphere. The population of fluorescent *Pseudomonas* in broth increased from 10^6 to 10^9 cfu/g after seven days when inoculated alone. In all the combinations, the CFU was in the range of $10^6 - 10^7$ cfu/ml. The fluorescent *Pseudomonas* population in sterile soil changed from 10^6 to 10^9 cfu/g whereas unsterile soil recorded 10^4 cfu/g after seven days the same as the initial level. Fluorescent *Pseudomonas* population rose from the initial population (10^6 cfu/g) reached a maximum of 10^{11} cfu/g then started declining to 10^7 cfu/g in sterile soil amended with *P. capsici*. In unsterile soil amended with *P. capsici*, the population of fluorescent *Pseudomonas* was static i.e. 10^4 cfu/g. There was no significant difference among the treatments, whether *T. harzianum* was inoculated alone or in combination with fluorescent pseudomonas. In broth, after seven days the population of *T. harzianum* rose to 10^5 cfu/ml , in sterile soil it increased to 10^7 cfu/g, in unsterile soil and in sterile soil amended with *P. capsici* it was 10^4 cfu/g , and in unsterile soil amended with *P.capsici* it decreased to 10^3 cfu/g , from the basal population of 10^4 cfu/g in all the treatments. The pepper rhizosphere maintained the fluorescent *Pseudomonas* population to about 10^5 cfu/g and the *T.harzianum* population in the range of 10^2 to 10^3 cfu/g. It can be concluded that the ability of *T.harzianum* and fluorescent pseudomonas to colonize the rhizosphere soil were essentially similar when the two strains were applied singly or co inoculated in soils, in the presence or absence of the pathogen. In unsterile soil, the behavior of fungal, bacterial and actinomycetes population were determined in order to study the changes in the indigenous micro flora, by the introduced organisms. The total bacterial population was in the range of 10^5 cfu/g and that of fungal and actinomycete population was in the range of 10^4 cfu/g

Efficiency of bacterial consortium in suppressing root rot.

Five bacterial strains, which had been proved efficient in suppressing *P.capsici,* were made into consortia in different combinations and its effect in disease suppression & growth promotion in black pepper were evaluated using black pepper seedlings. The experiments proved that there was synergistic effect when the strains were used in combination.

Efficiency of rhizobacteria in protecting black pepper from its three root pathogens viz. *R. similis, M. incognita* and *P. capsici.*

An experiment on the efficiency of *Bacillus* (7 strains), fluorescent pseudomonads (3 strains) and two strains of bacteria isolated from silent valley in protecting black pepper from three pathogens viz. *R.similis, M.incognita* and *P.capsici* were conducted. The root rot was indexed as 1 to 5 (No root rot was

indexed as 0, 0-25 % root rot as 1, 25-50 % as 3, 50-75% as 4 and 75-100% as 5). The fluorescent pseudomonads protected black pepper significantly from all the pathogens (IISR 2001, IISR 2002).

Rejuvenative capacity of fluorescent pseudomonads in black pepper

Five efficient strains of rhizobacteria which were short listed in earlier experiments were tested for the rejuvenation of black pepper cuttings infected with *P.capsici* either alone and in combinations with themselves and with a systemic fungicide metalaxyl (Ridomil – Mancozeb RMZ 72 wp). It was found that the strains IISR-8, IISR-51 and IISR-11 could effectively rejuvenate the black pepper cuttings when treated alone and also in combination. However, the percent rejuvenation of the infected cuttings increased when the bacterial treatments were with metalaxyl (Data unpublished).

Experiments on mode of action of PGPRs

Understanding the mechanisms through which the biocontrol of plant diseases occurs is critical to the eventual improvement and wider use of biocontrol methods. In addition to competition for limited carbon sources in the rhizosphere, antagonism can be mainly attributed to the production of secondary metabolites like antibiotics (Garner, *et al* 1984, Weller, 1988,) siderophores and cyanides. (Kloepper *et al* 1980). The short listed strains of fluorescent pseudomonads in black pepper were found to produce various volatile and non-volatile metabolites against the fungal pathogen (Lisha *et al*, 2002). HCN produced by the bacteria limited the growth of *P.capsici* (Diby *et al*, 2001). The inhibitory chemicals released by the strains also included antibiotics viz. pyoluteorin, and pyrrolnitrin as evidenced by TLC. TLC separation of the metabolites of the strain, IISR-6 yielded a fraction, which had high level of inhibition of growth of *P.capsici in vitro*. Kloepper *et al*, (1980) has clearly demonstrated the role of siderophores in rhizobacteria-mediated antagonism. Siderophore mediated antagonism is implicated in *P. capsici* - *P. fluorescens* antagonistic system (Diby Paul *et al*, 2001). The culture filtrate of the bacteria not only inhibited the mycelial growth of *P. capsici*, but also the explosive asexual phase, sporangial production, release of zoospores and germination of zoospores.

Radial growth inhibition by volatiles

The short listed strains of rhizosphere pseudomonads were screened based on their *in vitro* efficiency to suppress the growth of *P.capsici* by the production of inhibitory volatiles against *P.capsici* using standard methods. Isolates, IISR-51 & IISR-13 significantly inhibited the radial growth of *P.capsici* maximum being 22.4% inhibition over control.

Production of HCN

Strains of *Fluorescent pseudomonads* were characterized for the production of HCN against *P.capsici.* Production of HCN was determined using a modification of the procedure of Wei *et al* (1991). Bacteria were grown on King's B medium amended with of glycine in a petriplate, then filter paper strips soaked in picric acid solution was placed in the lid of each petriplate. Dishes were sealed with Para film and incubated for 2-4 days. HCN production was indicated by the change in colour of the filter paper strips from yellow to brown to red. Reaction was scored as low, medium and high, depending on the intensity of the colour. Different strains produced varying levels of HCN. Black pepper specific bacteria and fungi were isolated from

Silent valley biosphere reserve and were short-listed based on antagonistic properties on *P.capsici in vitro.* The bacterial strain IISR-310 was found to produce maximum level of HCN. The percentage inhibition of growth of *P.capsici* by the inhibitory volatiles produced by the isolates IISR-25 and IISR-36 were 39.7. The Volatile metabolites play a crucial role in killing the nematodes. Besides, the production of HCN and H_2S by these bacteria were also monitored. Out of the 98 isolates screened, only 6 isolates produced HCN. H_2S production was observed in another 6 isolates among the 50 tested (IISR, 2002).

Production of siderophores

Isolates were characterized for iron dependant siderophore production following standard methods (Loper, 1988 & Kloepper, 1980). The iron dependant production of siderophores by the bacterial strains was studied *in vitro*. It was found that the production of siderphores comes down as the concentration of $FeCl_3$ increases. All the strains, which inhibited *P.capsici in vitro*, were found to produce siderophores in varying degrees.

Phosphate solubilization potential

The phosphate solubilization capacity of the PGPRs was tested *in vitro* using Pikovskaya's agar as well as broth. In the agar plates, diameter of clearing zone indicated the phosphate solubilization. The broth assay gave the micro grams of phosphates released to the medium by the strains from tri-calcium phosphate, which was read spectrophotometrically. The intensity of colour of the solution is directly proportional to the amount of phosphate present or released by the bacterial strains. In agar plates, IISR-400 showed the maximum diameter of clear zone when compared to the others. In broth assay its efficiency got confirmed as it could release 0.7 µg of phosphate per ml of the culture media. *P. fluorescens* strain IISR –51 followed Phosphobacteria in its efficiency in phosphate solubilization.

Induction of mycolytic enzymes

The efficient strains of *Pseudomonas fluorescens* viz. IISR-13, IISR–51, IISR-8, ISR-11 & IISR-6 and *Trichoderma* spp. viz. Isolates, P-26, P-12, GV-19, Tav-25 & Th-39 were tested for their mycolytic potential on *P.capsici*. Isolates were tested for its efficiency in lysing the cellulose and lipid components of the hyphal wall of the pathogen. The cell free culture filtrate obtained from *P.fluorescens* strains and *Trichoderma* spp. in minimal media with four carbon sources viz. glucose, live mycelium, autoclaved mycelium and the hyphal wall components of (HWC) *P. capsici*, was assayed for β-1,3 glucanases, β-1,4 glucanases and lipases. The amounts of β-1,3 glucanases, β-1,4 glucanases and lipases released by the biocontrol agents were negligible when glucose served as the carbon source whereas significant amount of hydrolytic enzymes could be estimated when mycelium of *P.capsici* served as the carbon source. *Pseudomonas* strain, IISR-6 was found to produce highest levels of all the three enzymes, followed by IISR-51. The enzymatic induction by the strains was highest when HWC was supplemented as carbon source. In the case of *Trichoderma*, isolate Th-39 produced highest levels of the mycolytic enzymes. The activities of β-1,3 glucanases, β-1,4 glucanases and lipase produced by fluorescent pseudomonads were 60.35 GU, 1839 GU and 62.4 LU and that produced by *Trichoderma* isolates were 5.46-25.68 lipolytic units, 72.79 –471 GU and 1050 – 4763 GU respectively. The *in vitro* studies performed earlier had proved the multi-modes of antagonism

performed by the bacterial strains viz. siderophore production, production of volatile and non-volatile inhibitory compounds including HCN. The mycolytic potential is yet another mode of action found in these biocontrol agents. Enzymatic degradation of the cell wall of fungal pathogens by biocontrol agents is reported (Fridlender *et al.*,1999). The lytic extra cellular enzymes Chitinase and β-1,3 glucanase were found capable of degrading *Sclerotium rolfsii*, *Rhizoctonia solanii* and *Pythium aphanidermatum* cell walls. (Lorito *et al*, 1998). Kumar *et al.*, (1999) have demonstrated the production of mycolytic enzymes such as β-1,3 glucanase, β-1,4 glucanase and chitinase by fungal biocontrol agent *T. viride* against *Macrophomina phaseolina*. Strains of *Pseudomonas fluorescens* caused cytoplasmic coagulation in the mycelium of *P. capsici* when they were cultured together. The lytic activity of these biocontrol agents indicated their hyper-parasitic activity. These strains of Fluorescent pseudomonads were proved to be efficient in inhibiting different species of *Phytophthora* inhabiting various crops viz. *P.meadii* of Cardamom (*Eletteria cardamomum*), *P. parasitica* of Betelvine (*Piper betle*), *P. palmovora* of Coconut (*Cocos nucifera* L.), *P.palmivora* of Cocoa, *P. heveae* of Rubber (*Heavea brasiliensis*) and *P. meadii* of Areca. (data unpublished)

Characterization of Bacterial isolates

Characterization of 25 efficient strains of bacterial biocontrol agents were performed based on carbon utilization, antibiotic sensitivity, and utilization of succinic acid. Based on antibiotic sensitivity these were grouped in to 5 clusters and 14 clusters based on carbon source utilization. Few strains were found to utilize succinic acid as carbon source

Antibiotic resistance screening (Nalidixic acid (40µg/ml), Ampicillin (100µg/ml), Tetracycline (15µg/ml), Kanamycin (50µg/ml), Gentamycin (10µg/ml), Chloramphenicol (30µg/ml), Streptomycin (100µg/ml), Cycloheximide (100µg/ml), Rifampicin (100µg/ml) Penicillin(100µg/ml), polymyxin(100µg/ml), Spectinomycine, Bacitracin and Trichloro Tiphenyl Tetrazolium chloride TTC)) of efficient fluorescent pseudomonad isolates were performed for the identification of markers. King's B plates were prepared amending the antibiotics. The plates were inoculated with 5µl of the 24 h old culture of selected strains of Fluorescent pseudomonads cells, which had been washed free of media and incubated for 24 hrs. Colonies appeared on plates which were comparable to non-amended plates were scored as resistant types.

The strains also were evaluated for its efficiency to grow on different carbon sources, which in turn represent the efficiency of the strains to adapt to varying agro climates. The carbon sources tested were Glucose, Lactose, Maltose, Galactose, Mannose, Sorbitol, Dulcitol, and Sucrose at e percent.

Endophytic localization of PGPRs in black pepper vascular tissues

Black pepper cuttings were treated with *P.fluorescens* strains viz. IISR-6 and IISR-51 and allowed to root in sterilized coconut coir husk medium. After 4 months of growth the plants were removed and the root, stem and leaves were separated. It was surface sterilized by dipping in 0.1% mercuric chloride for I min, 2 min in 70% ethanol followed by 3 washes with sterile water. The samples were macerated in sterile pestle and mortar ascetically and the tissue was extracted in 10mM MgSO4 and the dilutions were plated on to King's B agar media amended with Nalidixic acid

(40µg/ml), Ampicillin (100µg/ml) and Kanamycin (50µg/ml). In order to make sure the sterility of the sample surface, one set each of leaf, root and stem were plated as such to nutrient agar plates after surface sterilization of the samples. The population of strains IISR-395, IISR-51 and IISR-396 in the root was found to range from 10^3 to 10^4 cells per gram of the root tissue. The shoot tissue contained up to 10^3 cells of the introduced *Pseudomonas fluorescens* cells per gram. There were only 10^1 to 10^2 cells of these cells per gram of the leaf tissue as endophyte. Among the three strains of *Bacillus* spp. tested, only two could reside in the plant system and one was not even detected in the roots. Phosphobacteria strain IISR-400 could be detected in the root, stem and leaf of the plant system in the level of 10^2.

The endophytic colonization of the bacteria was located by fluorescent microscopy. The stem samples were made in to thin sections and stained with Nile blue and observed under UV light. The localization of the bacteria was observed as orange fluorescence around the vascular bundles. All the four efficient strains of fluorescent pseudomonads tested for its endophytic nature in black pepper, were found to reside inside the root of the plant.

The efficient strains of fluorescent pseudomonads were found to be endophytic in black pepper, root, stem and leaves (Diby Paul *et al*, 2000). The population of *P.fluorescens* strains IISR-395, IISR-51 and IISR-396 in the root was found to range from 10^3 to 10^4 cells per gram of the root tissue. The shoot tissue contained up to 10^3 cells of the introduced *P. fluorescens* cells per gram. There were 10^1 to 10^2 cells of these strains per gram of the leaf tissue as endophyte. The endophytic colonization of the bacteria was also located by fluorescent microscopy after staining the cells with Nile blue. MclnRoy, and Kloepper, (1995) have shown that biological control by endophytic bacteria is possible and can involve induced resistance to soil borne pathogens.

Rhizosphere Competence

The more a biocontrol agent compatible to the rhizosphere of the host plant more can be the protection offered against soil borne fungal pathogens. In a view to study the rhizosphere competence of 7 strains biocontrol bacteria, black pepper plants were treated with the bacterial strains by rot bacterization and the population succession in the rots were studied upon destructive sampling. The study was conducted in sterile as well as non-sterile soil. The total microbial populations, fungi, bacteria and actinomycetes present in the non-sterile soil on every 5th day, from 0-25th day were enumerated. The total bacteria obtained were classified based on colony morphology and its response to Gram's reaction. The total fungi obtained from the non-sterile soil were identified. The introduced PGPR were enumerated, exploiting their intrinsic antibiotic resistant markers. It was found that the bacterial population maintained a minimum level of 10^6 through out the period of study. It was also found that the population of the introduced bacteria maintained on roots of black pepper cuttings planted in sterile as well as in non-sterile soil. The similar trend was observed with the introduced bacteria isolated from the rhizosphere soil too. The results implied that the selected biocontrol bacteria are compatible with the rhizosphere of black pepper. It even maintains a threshold level of population when applied in non-sterile soil after combating with other resident microorganisms.

Growth Promotion

The growth promoting strains of Fluorescent pseudomonads were found to synthesize phytohormones viz. IAA and GA as detected in TLC. The other determinants for growth promotion in black pepper were enhanced production of feeder roots in the plant and also the increased absorptive surface area of the roots as detected upon scanning and analysis by GS-Root software.

ISR

Pseudomonas fluorescence has been demonstrated to induce systemic resistance to a variety of pepper by the strains exploring the prevention of even foliar infection by the pathogen, *Phytophthora capsici*. The increase in production of defence enzymes upon challenge were higher in the non bacterized plants compared to the bacterized plants, indicating the lesser requirement of defence enzymes in the bacterized plants upon encounter with the pathogen. There also found a relatively higher quantity of lignification (30 – 100% over control) in the bacterized roots compared to the plants untreated. This was correlated with the lesser root rot in the bacterized plants upon challenge inoculation with the pathogen (Diby Paul and Sarma, 2003 with present workshop). Systemic increase in the PAL activity upon treatment with PGPRs has been reported in several crops including pigeon pea and rice. (Podile and lami, 1998, Meena *et al*, 1999).

Nutrient Regulation

Glick *et al*, (1995) reported that P solubilization, biological nitrogen fixation, improvement of other plant nutrients uptake and phytohormone production like IAA is some examples of mechanisms of PGPRs that directly influence plant growth. Strains of *P.fluorescens* viz. IISR-6, IISR-8 IISR-11, IISR-13 and IISR-51 obtained from black pepper rhizosphere released inorganic phosphate from tricalcium phosphate. The role of *P. fluorescens* mediated nutrient flux in the soil microcosm in plant growth promotion was confirmed with the higher uptake of nutrients by the bacterized plants. Significant uptake of nitrogen (N) and potassium (K) was noticed in the treated black pepper. Since these strains were found producing siderophores, the release of available P may be by chelating metal ions that are associated with complex forms of P, in addition to the other mechanisms as production of phosphatase enzymes and release of organic acids. The uptake of K also was found to be higher in bacterized plants. Thus enhanced nutrient mobilization was effected in the rhizosphere of black pepper with PGPR treatment, which resulted in enhanced plant vigor (Diby Paul *et al*, 2003 with present workshop).

Application of rhizobacteria and *T. harzianum* resulted in enhanced growth of black pepper, which resulted in increased number of nodes and consequently cuttings. The increase was 51.6% over control for *P.fluorescens* strain, IISR-51 and 38.9% for the treatment with consortium of *P. fluorescens* strain, IISR-6 and *T. harzianum* (IISR-1369). The treatment with consortium of *P. fluorescens* strain, IISR-6 and *T.harzianum* was superior to IISR-6 alone by producing 28% more cuttings. The root system of the PGPR and the consortium treated plants were disease free. The combination of *Trichoderma* spp (IISR-1369) and *P. fluorescens* (IISR-6) in combination was found to decrease the root rot disease besides increasing the yield in the field trials conducted.

Sarma *et al*, (2000b) has established the rhizobacterial mediated biocontrol strategies for Black pepper, Ginger and Cardamom. Biocontrol agents *P. fluorescens* and *T. harzianum* isolated from rhizosphere of black pepper, ginger and cardamom were evaluated for their efficiency on growth promotion and disease suppression in three major spice crops viz. black pepper, ginger and cardamom. The maximum disease suppression (63%) obtained by the treatment combination, *T. harzianum* isolate, IISR - 1369 and *P. fluorescens* strain, IISR–6 in black pepper and in cardamom, it was 36% over control. The same treatment could impart 66.2 % survival of ginger tillers after challenge inoculation with *P. aphanidermatum*. The combination of *T. harzianum* isolate, IISR-1369 and *P. fluorescens* strain, IISR –11 could improve the vigour of the plant both in black pepper and ginger. The same treatment combination imparted maximum yield in ginger and cardamom. When these biocontrol agents were applied in combination there was synergistic effect both for growth promotion and disease suppression. The efficient isolate from black pepper can be used in a cropping system involving black pepper, ginger and cardamom.

The PGPR strains were tested for their ability in rejuvenating *Phytophthora* infected black pepper cuttings. *P. fluorescens* strain, IISR-6 ensured survival of 70% of the infected cuttings where as only 30 % of the untreated plants survived 3 months after treatment. The fungicide, Metalaxyl - Mancozeb was found to have additive effect in suppressing the pathogen and the bacteria was compatible with the fungicide. There were no synergistic effects when combination of bacterial strains was used for treatment of the treated cuttings. Apart from rejuvenation of the infected cuttings, PGPRs also enhanced growth of the plant. Production of more roots was evident in the PGPR treated cuttings, compared to control.

The nematode pathogens of black pepper viz. *Meloidogyne incognita* and *Radopholus similis* also were inhibited by these strains of *P. fluorescens*. An economical and ecofriendly multiplication medium was developed for the large-scale production of this biocontrol agent. The industrial waste, molasses (0.1%) supported growth of the bacteria to log 14 cfu/ml in 32h. The Indian Institute of Spices Research has recommended this *P.fluorescens* strain IISR-6 for release to the benefit of farmers.

Field trial with consortium of biocontrol agents

An experiment was conducted at Vythiri (Wynad district in Kerala) in RBD with three replications using the following bio-control agents: *Trichoderma aureoviride, Trichoderma harzianum, Trichoderma virens, Pseudomonas fluorescens* (IISR-6), *Pseudomonas fluorescens* (IISR-11). *Trichoderma* was multiplied in sorghum grain (10^9 propagules/g) and Bacterial isolates were grown in Nutrient broth, pelleted, washed and resuspended in 10 mM MgSO4. Copper oxychloride (0.4%) drenching (1L/vine) served as control. The best treatment was found to be treatment number 11 where in combination of *Trichoderma* spp (Is. No. IISR-143and IISR-369) *P.fluorescens* (IISR-6) in combination was found to decrease the root rot disease besides increasing the yield.

The strains also were evaluated for its efficiency to grow on different carbon sources, which in turn represent the efficiency of the strains to adapt to varying agro climates. The carbon sources tested were Glucose, Lactose, Maltose, Galactose, Mannose, Sorbitol, Dulcitol, and Sucrose in one percent.

Effect of PGPRs on rejuvanation of slow decline in the field

The bacterial isolates which were short listed based on *in vitro* and green house experiments both for suppression of *P. capsici* and nematodes were tested on a standing crop of black pepper showing slow decline symptoms at IISR experimental farm Peruvannamuzhi. The evaluation on the disease severity was made based on foliar yellowing. There were 34.7% of plants were showing yellowing before the application of PGPRs. After 60 days of application of PGPRs, it was found that all the bacterial treatments significantly reduced the foliar yellowing. The intensity of yellowing was graded based on visual observations. There were three grades (upto 25%, upto 50% and greater than 50% yellowing). In all the PGPR treated vines the grades have come down and the vines produced new foliage. The recovery of vines was total in vines treated with IISR-853, followed by IISR-51. Where as the untreated vines deteriorated in health as the intensity of yellowing has increased. Among the strain IISR-853 and IISR-51 were found to be most effective in reducing the disease (Sabir, 2002).

Use of a single efficient strain in a cropping system

In order to test the strain specificity and possibility of using a single strain in a cropping system involving more than one crop the following experiment was conducted. Efficient isolates of biocontrol agents, *Pseudomonas fluorescens* and *Trichoderma harzianum* identified for black pepper, ginger and cardamom were used against the pathogens of respective crops. The efficient isolates of *Trichoderma* used were: IISR-1369, IISR-1370 from black pepper, IISR-1371 from ginger and IISR-1292 from cardamom. These isolates used in to different combinations and tested, along with *P.fluorescens* strains from black pepper (IISR-11) and ginger (IISR-6) for their ability in disease suppression and growth promotion in these crops. The experiments on black pepper and ginger were conducted in the green house and that of cardamom was carried out in already existing field. Out of the 22 different treatments, 3 treatments were found to be effective in suppressing root rot (*Phytophthora capsici*) disease in black pepper, soft rot (*Pythium aphanidermatum*) in ginger and clump rot (*Pythium vexans*) in cardamom. The best biocontrol agents included *T. harzianum* isolate, IISR - 1369 and *P. fluorescens* strain, IISR –11 or IISR-6 in common. The maximum disease suppression obtained by the treatment combination, *T.harzianum* isolate, IISR - 1369 and *P.fluorescens* strain, IISR–11 in black pepper was 63 and for cardamom, it was 36% over control. The same treatment could record 66.2 % survival of ginger tillers after challenge inoculation with the pathogen. The combination of *T.harzianum* isolate, IISR-1369 and *P. fluorescens* strain, IISR –11 could improve the vigour of the plant both in black pepper and ginger. The same treatment combination recorded maximum yield in ginger and cardamom. Our earlier studies had proved the mutual compatibility between *T.harzianum* and *P.fluorescens.* When these biocontrol agents were applied in combination there was synergistic effect both for growth promotion and disease suppression (Diby et al 2001, Minimol 2002, IISR 2002)

Mass multiplication of PGPRs

Growth of *P. fluorescens* in varying concentrations of molasses ranging from 0.05%, 0.1% and 0.2% indicated that even at 0.05 % molasses supported 10^{13} cells /ml after 48h of growth. The same concentrations also supported good growth of *T. harzianum* (Dhanya 2002). After multiplication in liquid fermentation using molasses

the organisms were mixed independently with various forms of coir pith in the proportions of 1:10, 1:20 and 1:40. The population of *T. harzianum* in these media was estimated immediately after inoculation and every 15 days interval up to 45 days. The population of *P. fluorescens* was also found to increase from 10^6 to 10^{12} within 40 days (Saluja, 2002, Dhanya 2002).

Conclusion

In spice crops the major diseases are soil borne and infestation with plant parasitic nematodes further complicate the management. A greater thrust is given for development of biological consortia with multiple modes of action. Efforts are also made to identify organisms which are efficient in suppressing both fungal pathogens and nematodes and also promote growth. The mass multiplication of PGPRs in inexpensive media and use of agricultural waste products such as coir pith as carrier medium offers tremendous scope for adoption of this technology in farmer's fields.

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