Draft RPF III

Biocontrol II. (813)

Development of consortium of bio inoculants for management of pests, diseases and nematodes in spices

FINAL REPORT

Submitted by

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CONTENTS

PART - I: GENERAL INFORMATION

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800 Project Code :

8002 ICAR Project Code No. : Biocontrol II. (813)

801 Name of the Institute and Division :

8011 Name and address of Institute : Indian Institute of Spices Research, Calicut- 673 012, Kerala

8012 Name of Division/Section : Crop Protection/ Pathology

8013 Location of the Project : Indian Institute of Spices Research, Calicut & Cardamom Research Centre, Appangala

802 Project Title :

Development of consortium of bio-inoculants for management of pests, diseases and nematodes in spices

803 Priority Area : 01, 03 & 04

8031 Research Approach :

Applied Research	Basic Research	Process/Technology development	Transfer of Technology
01	02	03	04

804 Specific area : Spices Research

805 Duration of Project : 4 Years

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(Give reasons for variation, if any from original estimated cost)

807 EXECUTIVE SUMMARIES

Management of soil borne diseases continues to be a real challenge to researchers due to interaction with many biotic and abiotic factors. Though chemical methods offer stop-gap arrangement of suppressing the pathogens temporarily often, pathogens and pests cause irreparable losses due to faulty and untimely pesticide application methods. Further, pesticide residue problems particularly in spices is a serious concern for exporters, traders and consumers. Biological options offer ecological safe alternatives and many potential bio-inoculants have been identified and tested against soil borne pathogens of spices. To have comprehensive management of soil borne fungal, nematode and insect problems of black pepper, cardamom, vanilla and other spices, bio-consortium approach was tried at Indian Institute of Spices Research, Calicut and Cardamom Research Centre, Appangala for four years. Since *Phytophthora* foot rot of pepper is the major problem across black pepper growing areas, the emphasis was given to all possible aspects of biocontrol strategies to contain this disease.

Biocontrol organisms comprising of antagonistic fungi and bacteria effective against *Phytophthora capsici* and plant pathogenic nematodes *Radopholus similis* and *Meloidogyne incognita* were identified and evaluated individually against soil borne pathogens of black pepper, cardamom and vanilla. The compatibility test indicated that out of 37 bacterial isolates, 12 were inhibitory to *T. harzianum*, the antagonist used against *P.capsici*.

Black pepper

In vitro compatibility of *Pseudomonas bacterial* strains viz. *P. aeruginosa* (IISR 6, 13,51, 853), *P. mentosa* (IISR 8), and *Alcaligens faecalis* (IISR 859) revealed that only three of the isolates tested namely *P. mentosa*, *P. aeruginosa*, and *Alcaligens faecalis* were compatible with *T. harzianum*, and *Alcaligens faecalis* is compatible with *Pochonia chlamydosporia* also.

The percentage of vines showing yellowing was significantly lower in treatments involving the isolates *Alcaligens faecalis* where the nematode population was also reduced. Compared to bacterial consortium, individual inoculation of *T. harzianum* recorded better growth of black pepper cuttings in nursery.

A consortium of 12 rhizobacteria (IISR 6, 151, 522, 527, 528, 532, 641, 658, 853, 857,859, and 865) were evaluated in a newly established black pepper plot (variety Sreekara) at

Peruvannamuzhi, which was heavily infested with *R. similis*. The establishment of the treated cuttings (56%) was superior to untreated ones (24%)

Antagonistic potential of bioagents effective against *P. capsici* of black pepper were tested against other pathogens viz. *Pythium aphanidermatum, P. vexans, Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *vanillae*

The isolate IISR 853 which is antagonistic to both *R. similis* and *M. incognita* was found antagonistic to all other targeted pathogens giving more than 50% inhibition.

The compatibility among the bacterial isolates was tested *in vitro*. All the bacterial isolates tested were compatible with each other. But to *T. harzianum*, IISR 8 , 11SR 13, PB 21C and P1AR- 6 were compatible whereas IISR 13, IISR 859 and IISR 51 are not compatible .*P. chlamydosporia* is inhibitory to all the bacterial antagonists and *T.harzianum.*

A pot culture trial with black pepper was laid out with 10 treatments including short listed promising bioagents individually and in combinations keeping recommended chemical control as check. The result showed that among the bioagent combinations, *T. harzianum* and *P. aeruginosa* (IISR 6) were on par with chemical treatments in reducing the disease incidence followed by IISR 859 + *P. chlamydosporia.*

The promising nematode antagonistic fungal isolate *P. chlamydosporia* and bacteria viz. IISR-6, IISR-859, and IISR-853 were evaluated under green house conditions against *P. capsici* in comparison with recommended fungicides (copper oxychloride, potassium phosphonate) and biocontrol agents viz.*T. harzianum* and *P. aeruginosa*. The study indicated that IISR-853 and IISR-859 were promising and were at par with copper oxychloride.

The field trial to evaluate the effect of consortia of rhizobacteria on *Phytophthora* foot rot and slow decline diseases of black pepper was concluded after 5 years of imposing treatments. Among the eight treatments, the mortality of vines was lower in the treatment where the consortia of IISR-6, 8, 13, 51, 151, 853 (rhizobacteria) + PB-21C (P-solubilizer) were applied.

Cardamom

In vitro evaluation of two fungal bioagents viz., *T. harzianum* and *T. hamatum* and two bacterial antagonists viz., IISR – 6 and IISR – 853 was undertaken to test their efficacy against rhizome rot pathogens of cardamom (*P.vexans* and *R. solani*). *T. harzianum* was more effective on both test pathogens compared to other bioagents. IISR – 853 and IISR – 6 were not effective on both *P. vexans* and *R. solani*. Observations on hyphal interaction

between *R. solani* and *T. hamatum* revealed the movement of *T. hamatum* hyphae towards *R. solani* followed by penetration and parasitization.

An experiment was also conducted to study the effect of promising biocontrol agents of IISR on rhizome rot pathogens of cardamom in secondary nursery. The pathogens namely *P. vexans* and *R. solani* isolated from severely infected rhizome rot affected plots of Appangala, were used in this study. All the test biocontrol agents were superior over untreated control and effectively reduced mortality of secondary cardamom seedlings. However, among the treatments *T. harzianum* (P 26) is superior followed by IISR 6.

Vanilla

In vanilla, 11 rhizosphere bacteria, short listed as promising bioagents against pathogens of vanilla viz. *Fusarium oxysporum* f. sp *vanillae* and *P. meadii* causing fruit rot and stem rot were tested for their mutual antagonism/ synergism with *T. harzianum* under *invitro* conditions and found that isolates such as IISR 6, IISR 13, IISR 51, IISR 152, IISR 853, IISR 859, and IISR 909 were antagonistic to *T. harzianum* with an inhibition range of 15.55% -72.2%

The field trial for the evaluation of bioconsortium for root rot (*F. oxysporum* f.sp *vanillae*) disease of vanilla showed that the consortium containing IISR 6, 8, 13, 51, 151 P1AR6 followed by, consortium 3 (IISR 6, 8, 13, 51, 151, 853), consortium 4 (IISR 6, 8, 13, 51, 151, 859) and consortium 6 (P-26 alone) were effective in reducing the disease incidence and were at par.

From the different experiments it is inferred that inclusion of any bacterial isolates in consortia require compatibility testing before formulation of commercial products. Similarly *T.harzianum* cannot be used along with bacterial consortia because most of the bacterial compontents were antagonistic to *T.harzianum* except IISR 6. So in an integrated disease management system, a study on the compatibility of individual components, either chemical or bioagents, is a prime requirement before being applied to the host plant rhizosphere.

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PART II: INVESTIGATOR'S PROFILE

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PART III: TECHNICAL DETAILS

820 Introduction and Objectives

8201 Project objectives

To study the compatibility of various short listed biocontrol organisms and to develop a consortium against major diseases of black pepper (foot rot and slow decline), cardamom (rhizome rot) and vanilla (root rot).

8202 Back ground information and importance of the project

Spice crops such as black pepper, cardamom, ginger and turmeric are affected by soil borne diseases caused by the oomycetes fungi belonging to the genera *Phytophthora, Pythium* and fungi *Rhizoctonia* besides *Ralstonia solanacearum* causing bacterial wilt. Plant pathogenic nematodes *Meloidogyne incognita* and *Radopholus similis* are also common pathogens in these crops. The root mealy bugs (*Planococcus*) are increasingly becoming serious insect pests of black pepper especially at higher altitudes. Antagonistic fungi such as *Trichoderma harzianum* and *Pochonia chlamydosporia* have been selected as effective biocontrol agents for managing *P. capsici* and nematodes. Several bacterial isolates promising against these pathogens have also been short listed. The main objective of this project is to develop a consortium of biocontrol agents for managing pests, pathogens and nematodes in cropping system involving several crops.

(a) International status

Biocontrol agents effective against various groups of pests and diseases have been developed and used for managing pests and pathogens. Various commercial formulations are also being marketed. The potential of biocontrol in crop disease management has been well documented (Baker and Cook, 1974; Cook and Baker, 1983; Papavizas, 1985). Also the bio-efficacy of suppression of soil borne pathogens such as *Fusarium, Rhizoctonia, Sclerotium, Phytophthora* and *Pythium* by species of *Trichoderma, Gliocladium, Laetisaria, Coniothyrum, Sporidesmium* and, non pathogenic *Fusarium* and *Pythium ultimum* have also reported (Adams, 1990) . Bio-products based on *Trichoderma* and *Gliocadium* like Trichodex, Binab-T, AG-2, Gliogard, Trichoject, Tricho mini dowels etc. are commonly available for plant disease management (Samuels, 1996). Biological control of soil borne plant pathogens by bacteria is known for long time. Biocontrol potential has been reported in the genera of *Actinoplanes, Agrobacterium, Arthrobacter, Azotobacter, Bacilus, Enterobacter, Erwinia, Flavobacteium, Hafnia, Micromonospora, Pseudomonas, Pasteuria,*

Serratia, *Rhizobium*, and *Bradyrhizobium* (Weller, 1988). Control of root rot of soybean caused by *Phytophthora megasperma* by rhizobacteria is reported by Lifshitz *et al* (1986). Suppression of *Phytophthora cactorum*, the root rot pathogens of apple by *Enterobacter aerogens* and *Bacillus subtilis* and its mode of action has been established (Van Loon, 1998, Uthkede, 2000) Bacteria based bio-products like NoGall, Dagger-G, Quantum-4000 etc are available commercially for the management of diseases caused by fungal and bacterial plant pathogens.

Strains of fluorescent pseudomonad's have been demonstrated to reduce plant diseases by suppressing soil borne pathogens (Pieterse *et al*, 2000). This biological control activity is effective under field conditions and in commercial glass houses (Wei, *et al*, 1991) and can be the result of competition for nutrients and space, siderophore mediated competition for iron (Kloepper, *et al* 1980) or antibiosis through inhibitory chemicals and antibiotics viz. pyrrolnitrin, pyocyanin, 2-4 diacetyl phloroglucinol (Raaimakers *et al*, 1999). HCN production (Kloepper *et al* 1991) and degradation of toxins produced by pathogen (Defago *et al* 1990). Inductions of systemic resistance by fluorescent pseudomonads have been well documented. Induced systemic resistance (ISR) against different pathogens by PGPR strains have been achieved in tobacco (Maurhofer, *et al.*, 1994), cucumber (Liu, *et al.*, 1995), tomato, Radish (Leemann, *et al.*, 1995) and sugarcane (Ramamoorthy, *et al.*, 2001).

No reports are available on infestation of *Planococcus* sp. on roots of black pepper vines. However, *P. citri* which infests the aerial parts of coffee also infests the roots resulting in wilting and mortality of young plants especially in Africa. Various management schedules including biological methods have been suggested against mealy bugs infesting the aerial parts, but against root mealy bugs only drenching with insecticides have been suggested (Clausen, 1978; Pelley 1968).

Bacterial control of nematodes was successful in several crops like lettuce, grapes, tomato, banana, carrot, mustard, rice etc. Some of the rhizobacteria that reduce nematode populations are *Agrobacterium, Alcaligenes, Bacillus, Clostridium, Desulfovibrio, Pseudomonas, Serratia and Streptomyces* (Zavaleta-Mejia & Van Gundy , 1982; Becker, *et al.*, 1988; Spiegel *et al.*, 1991; Kloepper *et al.*, 1992; Neipp & Becker 1999). Rhizobacteria in combination with fungal bioagents or mycorrhizae are excellent for consortia approach.

Information on biological control agents against individual pests and pathogens are available. However, the mutual compatibility of promising isolates, their ecological fitness and mutual antagonism / synergism has not been studied. Management of pests and diseases

through non-chemical means and exploiting the potential of biocontrol organism for effective management of pests and pathogens is one of the major priorities of the Indian Institute of Spices Research, Calicut.

b) National status

Microorganisms effective against individual pests and pathogens have been reported for various spice crops.

Status of biocontrol on disease management of soil borne diseases of spice crops in general has been reviewed by Sarma *et al* (1996). The epidemiology, the ecology of *P.capsici* and the integrated approaches towards disease management of foot rot of black pepper has been well brought out (Anandaraj *et al*, 1991, Ramachandran *et al*, 1988, Sarma *et al*, 1991). The importance of *Verticillium chlamydosporium*, *V. tenerum*, *T.harzianum* and *G.virens* on disease suppression of foliar and rot infection by *P.capsici* in black pepper have been reported. The role of *Glomus fasciculatum* in suppression of root rot caused by *P.*capsici in black pepper and its utility in the nursery management has been reported. (Anandaraj *et al*, 1994, Anandaraj *et al*, 1996).

The potential of agro wastes like coffee pulp, tea wastes and coir pith etc. for large-scale multiplication and as carrier media for *Trichoderma* and *Gliocladium* have been studied (Prakash *et al*, 1997). Colonization of decomposed coconut coir pith by *Trichoderma viride* is reported by Kumar and Marimuthu (1997). Suppression of root rot in black pepper caused by these organisms has been evaluated in field under large-scale demonstration in farmer's field, to varying degrees. However the compatibility of antagonists in mixtures both *in vitro* and *in vivo* their mutual antagonism and their stability in the ecosystem in relation to pathogen population need to be studied. This remains as major gap in developing an effective biocontrol strategy against this major pathogen of black pepper. Very little has been done on cataloguing and conservation of these organisms, and their shelf life in carrier media. These are some of the priority programs that need immediate attention.

Several predacious, obligate and facultative fungal parasites are effective against root-knot nematodes but not so effective against *R. similis* (Eapen, 2004). Recently endophytic fungi have been reported to effective in suppressing *R. similis*. Endo mycorrhizal fungi are another group that are promising in the management of the nematodes.

Identification of ideal biocontrol consortium, its stability and effectiveness in disease suppression and standardization of their multiplication would be of utmost practical value for the plantation in disease management in view of the export potential of the crop.

Conservation and maintenance of biocontrol agents would be of great value for preserving the microbial diversity for posterity.

The root mealy bug was first reported on black pepper from Wyanad District in Kerala and was identified to be an undescribed species of *Planococcus* (Koya et al. 1996). In coffee, *P. citri* has also been reported to infest the roots. Drenching of affected vines with chlorpyriphos 0.075% was effective for the management of *Planococcus* sp. on black pepper (IISR 2004). In coffee, an integrated schedule including removal of alternate host plants management of ant colonies and drenching with insecticides has been recommended for the management of root mealybug (Reddy and Naidu 1999).

(Literature cited is given in Annexure II)

821 Project Technical Profile:

8211 Technical Programme

(Indicate briefly plan of procedure, techniques, instruments and special materials, organisms, special environments etc.)

1. Evaluation of promising isolates against *Phytophthora capsici. Pythium, Rhizoctonia* and *P. meadii* and studying their mutual antagonism / synergism
2. Evaluation of promising isolates against nematodes and insect pests.
3. Formulating biocontrol consortium and testing in the field.

The procedure adopted for fulfilling the technical programme were given below

Evaluation of promising isolates against P. capsici. Pythium, Rhizoctonia and P. meadii and studying their mutual antagonism / synergism

Thirty seven bacterial isolates which were inhibitory to pathogens of black pepper (*P. capsici* (19, *M. incognita* 17 and *R. similis* 1) were tested for their compatibility with antagonistic fungus *T. harzianum* (P26/IISR-1369) that has been identified as a biocontrol agent for P. *capsici*. Dual plate culture and co culture method was adopted for evaluating the mutual antagonism of the bacterial biocontrol organisms among themselves and towards *T. harzianum*.

Evaluation of promising isolates/consortia against soil borne pathogens (Black pepper, Cardamom and Vanilla)

The promising biocontrol isolates were multiplied in broth culture and inoculated to potted plants sufficiently well before the inoculation of the pathogen under green house conditions. The pathogen (*P. capsici*) multiplied in agar and inoculum discs of (10mm size) of 10

numbers were incorporated into soil in each pot. The soil was baited periodically for the presence of *Phytophthora* for black pepper, *Pythium* and *Rhizoctonia* for cardamom and *Fusarium* in case of vanilla and mortality of the plants were monitored.

Field evaluation of bio consortia against soil borne pathogens (Black pepper, Cardamom and Vanilla)

Field trial in RBD was laid out with two varieties of black pepper namely Panniyur 1 and Subhakara at IISR farm Peruvannamuzhi. Farm. The solarization of the soil was done before planting. The planting was done simultaneously with the imposition of differential treatments. The survival of the plants, disease incidence and yellowing due to nematode infestation was recorded at periodic intervals. The population of *P.capsici* and nematodes were also monitored periodically. Yield data was also recorded.

The trial on the development of biocontrol consortium for managing *P. capsici* and plant parasitic nematodes in black pepper has been laid out at IISR farm Peruvannamuzhi with the following treatments.

Treatment details

T1 → IISR-6, IISR-8, IISR-13, IISR-51, IISR-151 and PB21C*
T2 → IISR-6, IISR-8, IISR-13, IISR-51, IISR-151 and P_1AR_6
T3 → IISR-6, IISR-8, IISR-13, IISR-51, IISR-151 and IISR-853
T4 → IISR-6, IISR-8, IISR-13, IISR-51, IISR-151 and IISR-859
T5 → IISR-6, IISR-8, IISR-13, IISR-51, IISR-151 + PB21C + P_1AR_6 + IISR-853 + IISR-859
T6→ P.26 (*T.harzianum*)
T7→ Media (Nutrient Broth) alone
T8→ Chemical control (copper oxychloride)
T9→ Absolute control

*(IISR-6, IISR-8, IISR-13 and IISR-51 are *Pseudomonas aeruginosa* and IISR-151 is *Bacillus* pumilus, PB21C is phosphate solubilizing bacteria *Serratia marscens*, P_1AR_6 is *Rhizobium rhizogenes*

IISR-853 (*Pseudomonas aeruginosa)* and IISR-859(*Alcaligens faecalis*) were found to be effective against nematodes *Meloidogyne incognita* and *Radopholus similis* in the earlier researches of IISR, Calicut.

Influence of soil *p*H and moisture on the biocontrol potential of *Trichoderma* on *Phytophthora capsici* in Black pepper system

The experiment was conducted at Indian Institute of Spices research Calicut during 2007-08. Initially the effect of *p*H on the growth of *Trichoderma* and *P. capsici* was studied *in vitro*.

Further, conducive soil was adjusted to different pH and moisture levels and supplemented with the biocontrol agent and planted with rooted cuttings of black pepper to study the effect *in vivo*. Conducive soil was collected from IISR experimental farm Peruvannamuzhi. Rooted cuttings of black pepper variety Panchami having 3-4 leaf stage raised in potting mixture (1:1:1) in Polythene bags were used for the experiment. These plants were transplanted in 12" x12" size earthenware pots as per the experimental requirement.

Preparation of Biocontrol agent -*Trichoderma harzianum*

T. harzianum (MTCC 5179), being used for the biocontrol of foot rot disease of black pepper and maintained in the biocontrol repository of Indian Institute of Spices research, Calicut, Kerala, was used. The isolate was grown on 100ml of Potato Dextrose Agar (PDA) in 250 ml conical flask for 7 days. The spores formed on the surface of the medium was made into a suspension by adding of 300 ml of sterile distilled water and filtered through three layers of sterile muslin cloth. After filtration, the spore load ml^{-1} was calculated using Haemocytometer. Spore suspension @ 5ml containing 7 log cfu ml^{-1} was injected to 1 kg moistened and sterilized coir compost packed in polypropylene bags and mixed thoroughly. The polybags were incubated at room temperature for 10 days and the cfu g^{-1} of the coir compost was determined by serial dilution plate method using *Trichoderma* Selective Medium (TSM). This multiplied media contained a cfu of log 8 cfu g^{-1} was used as *Trichoderma* inoculum @ 100g pot^{-1}.

Effect of pH on the growth *T. harzianum* (MTCC 5179) and *P. capsici* in vitro

The effect of *pH* on the growth *T.harzianum* (MTCC 5179) was studied on PDA. The PDA was prepared and adjusted to *p*H ranging from 4.0 to 7.0 using 1N NaOH and 1N HCl after checking the initial *p*H of PDA (Sid Ahmed *et al.* 1999). The media was autoclaved at 121°C at 15 lbs pressure for 20 minutes. 15ml of the media was poured into 90mm Petri dishes and inoculated with 5mm mycelial plugs of *T. harzianum* cut from the edge of 72h old actively growing culture and incubated at 25°C. The diameter of the colony was measured at 48 h. Similarly 5mm mycelial plugs of *P.capsici* from 72h old actively growing culture was inoculated at the centre of the 90 mm Petri dish containing carrot agar adjusted to different pH and incubated at 25°C under laboratory conditions. The diameter of the colony was measured at 72h.

Effect of pH and moisture on the growth and proliferation of *T. harzianum* (MTCC 5179) and *P. capsici* in vivo

The experiment was designed in CRD in a split-split plot design. The main plot consisted of five pH regimes viz. 1) 4.5-5.0 2) 5.1-5.5 3) 5.6-6.0 4) 6.1-6.5 and 5) 6.6-7.0. The sub plot treatments were four moisture levels viz.1) 35-40% 2) 30-35% 3) 20-25% 4) 10-15%. The sub-sub plot consists of two treatments namely, conducive soil supplemented with *T.harzianum* and conducive soil without *Trichoderma* (control)

Adjustment of soil pH and moisture

The initial pH of the conducive soil was measured, and then adjusted to different pH levels by the addition of lime. To standardize the amount of the lime required to get the desired pH, different quantities of lime viz. 1 g, 3 g, 5g, 10g, 15g, 20g and 25g were mixed thoroughly with 12 kg soil in 12" x12" size earthenware pots in triplicate and saturated with water. The soil was incubated for 3 days. After 3 days, the *p*H of the soil was measured using a pH meter. The quantity of lime that gave the desired *p*H was selected for adjusting the soil *p*H for the experiment

Soil of about 12 kg each was filled in earthenware pots of 12" x12" size and adjusted to five different *p*H ranging from 4.5-7.0 viz. 4.5-5.0 (control), 5.1-5.5, 5.6-6.0, 6.1-6.5, 6.6-7.0. The soil moisture was adjusted by adding measured quantity of water at regular intervals to maintain a moisture level of 35-40%, 30-35%, 20-25%, and 10-15% respectively. The moisture level was adjusted in such a way that 35-40% is obtained by irrigating the pots twice daily, 30-35% by irrigating once daily, 20-25% by irrigating once in two days and 10-15% by irrigating once in 7 days.

The pots already adjusted to different moisture and *p*H levels were supplemented with *Trichoderma* multiplied in coir compost @ 100g/pot in the respective treatment.

Enumeration of *T. harzianum* from the inoculated soil was done by soil dilution plating.Soil was collected from respective treatments at weekly intervals and air dried. This was suspended in 90 ml sterile distilled water taken in a 250 ml Erlenmeyer flask and stirred well for 20 min. From this serial dilutions were made up to 10^{-5} and 1ml of this was plated using *Trichoderma* selective medium (TSM). The plates were incubated at 25°C for five days and enumerated the colony forming units.

Quantification of *P.capsici* from the inoculated soil was done out using the soil dilution baiting technique using leaflets of *Albizzia falcataria* (Anandaraj and Sarma 1990). Soil was diluted up to 10^{-10} (1/1024) dilution and the disease potential index (DPI) was calculated. The data were analyzed statistically using windowstat package.

Evaluation of bio consortia against soil borne pathogens of cardamom in secondary nursery

Trial in RBD was laid out with promising biocontrol agents on rhizome rot pathogens of cardamom in secondary nursery. The pathogens namely, *P. vexans* and *R.solani* isolated from severely infected rhizome rot affected plots of Cardamom Research Centre, Appangala were used in this study. Chemical fungicide was also included as one of the treatment for comparison. Observations were recorded periodically for root and rhizome infection and vigor of inoculants.

Evaluation of bacterial isolates against root mealybug

Twelve bacterial isolates (promising against *P. capsici* and nematodes) were evaluated against root mealybug in laboratory bioassays. The bacterial cultures were grown in Nutrient broth and tested against mealy bug under laboratory conditions by atomizing the bacterial suspension on the mealy bug population. The mortality of root mealy bugs was recorded at 15 and 30 days after spray.

Evaluation of entomopathogens against root mealybug

Commercial formulations of *Metarrhizium anisopliae* and *Nomuria rileyi* and *Verticillium lecani, Aspergillus* sp. and *T. harzianum* were evaluated against root mealybug in laboratory bioassays. The fungal cultures were grown in Potato Dextrose Agar medium and tested against mealy bug under laboratory conditions by atomizing the fungal suspension on the mealy bug population. The mortality of root mealybugs was recorded at 15 and 30 days after spray.

Evaluation of bioconsortia against root mealy bugs

The incidence of root mealy bugs (*Planococcus* sp.) was recorded three months after application of bioconsortia in various treatments in the field.

Total man months involvement of component project workers

M. Anandaraj	(2004-2006)	- 4 man months
M N Venugopal	(2004-2008)	- 8 man months
S. Devasahayam	(2004-2006)	- 4 man months
K M Abdulla Koya	(2004-2006)	- 4 man months
R Suseela Bhai	(2004-2008)	- 6 man months

Santhosh J Eapen	(2004-2008)	- 8 man months
A Kumar	(2004- 2008)	- 6 man months
C N Biju	(2007 - 2008)	- 3 man months
TOTAL		- 43 man months

822 Final Report on the Project

Detailed report containing all relevant data with a summary of results

8221 Achievements in terms of targets fixed for each activity:

Please See **Annexure 1**

8222 **Questions Answered**

Whether the already developed T. *harzianum* can be used in consortium?

All the bacterial strains in the consortia are not compatible with *T. harzianum* suggesting that these could not be used in a consortium. But IISR 6 can be used along with *T. harzianum*.

Is it possible to evolve management strategies using consortium of microorganisms for growth promotion and disease suppression in black pepper?

Yes, it is possible .The bio consortium containing IISR 6, 8, 13, 51, 151and 853 is effective in maintaining the plant stand and vigor by reducing the disease severity to 59% in case of black pepper variety Subhakara whereas in case of cardamom bioconsortium is less effective compared to individual inoculation of *T. harzianum* and *P. fluorescens*.

What is the role of soil factors in the population dynamics of pathogens and the introduced biocontrol organisms?

The result of the combined inoculation showed that when a *Phytophthora* conducive soil is supplemented with *T. harzianum*, there is a disease reduction of about 27.5-63.75% in 20 days of application over similar soil without supplementing with *T. harzianum* at pH 4.5-6.0. Hence for the biological control of *P. capsici* in black pepper system, it is inferred that the pH of the soil is to be maintained between 4.5-6.0 with irrigation on alternate days in order to facilitate the growth and proliferation of *T. harzianum* which in turn will reduce the population of *P. capsici* and thereby reduce the disease incidence.

Whether IISR 6, P-26 are effective in controlling on rhizome rot of cardamom?

Yes. IISR 6 and P 26 (*T. harzianum*) are effective in reducing rhizome rot in secondary nursery of cardamom. P-26 (*T. harzianum*) alone is also very effective and promoted better root biomass.

Whether the bacterial and fungal antagonists are compatible with each other?

All the bacterial bioagents short listed in the present study are compatible with each other. But *P. chlamydosporia* is inhibitory to all the bacterial and fungal bioagents tested.

8223 **Process/Product/Technology/Developed**

Identified a bioconsortium containing IISR 6, 8, 13, 51, 151, and 853 for effective management of foot rot and slow decline disease of black pepper.

Identified a bioconsortium containing IISR 6 and *T. harzianum* against rhizome rot of cardamom

Identified a bioconsortium containing P. *fluorescens*, *Bacillus* sp. and *B. polymixa* against root rot of vanilla caused by *F. oxysporum* f. sp.

8222 **Practical Utility (Not more than 150 words)**

The bacterial isolate IISR-6 which has been recommended for black pepper nurseries could be used along with *T. harzianum* for *Phytophthora* disease suppression and growth promotion The bio consortium (IISR 6, 8, 13, 51, 151, PB21C and 853) can be utilized to reduce foot rot damage in black pepper.

IISR 6 and P-26 (*T. harzianum*) are effective in reducing rhizome rot in secondary nursery of cardamom. P-26 (*T. harzianum*) is very effective and promoted better root biomass. Pretreated suckers and standard seedlings can be taken to field planting followed with application of respective biocontrol agents at field level to manage rhizome rot of cardamom.

Root rot of vanilla is a serious problem in all vanilla growing tracts. The consortium of biocontrol bacteria viz. IISR 6, 8, 13, 51, 151 P1AR6 developed can be recommended for field application in the initial stages of the disease so that the disease spread can be minimized.

8225 **Constraints, if any** : Nil

823 PUBLICATIONS AND MATERIAL DEVELOPMENT

8232 **Popular articles**

Anandaraj, M., 2004 Ecofriendly management of diseases of spice crops. Planters' chronicle 100(12): 27-34.

Research Articles

Bhai R S, Kumar A 2008. Effect of rhizobacteria on *Phytophthora meadii*, *Fusarium oxysporum* f.sp. *vanillae* and Colletotrichum *vanillae* infecting vanilla. *J. Biol.Control* 22 (1) 33-41.

Sangeeth K P, Bhai R S & Srinivasan V 2008. Isolation and selection of indigenous *Azospirillum* inoculants for their influence on growth and nutrient uptake of rooted cuttings of black pepper. J. *Spices and Aromatic Crops* 17(2): 128-133.

Bhai R S, Remya B, Jithya Danesh and S J Eapen 2009. *In vitro* and *in planta* assays for biological control of *Fusarium* root rot disease of vanilla (*Vanilla planifolia* Andrews). *J. Biol. Control*, 23(1): 83-86.

Papers Presented in Symposia/Seminars

Anandaraj, M. 2004. Potential of Plant Growth Promoting Rhizobacteria for Plant Disease Management. Invited talk at the XIII Southern Regional Conference on Microbial Inoculants, at University of Agricultural Sciences, Dharwar at Bijapur from 3-5 Dec 2004.

Anandaraj, M. 2004. Use of Mycorrhiza for Managing Soil borne Diseases in Spice crops. Presented at the short term training programme on Mycorrhiza organized by CPCRI from 1-10 Dec 2004.

Thankamani, C. K. Sreekala, K. and Anandaraj, M. 2004. Effect of *Pseudomonas fluorescens* (IISR-6) and *Trichoderma harzianum* on growth of black pepper varieties in the nursery. Presented at the Symposium on Spices and Aromatic crops, 1-2 Nov 2004, Indian Institute of Spices Research, Calicut.

Devasahayam, S, 2005 Prospects and emerging trends in biological control of insect pests of spices. Invited talk at Symposium on Biological control of pests of horticultural crops: New thrusts, Project Directorate of Biological Control, Bangalore, 19 March 2005.

Sangeeth K.P, Suseela Bhai R, Srinivasan V, and Venugopal M.N, 2007. Comparative Efficacy of Indigenous Microbial Inoculants in Improving Seedling Growth of Cardamom (*Elettaria cardamomum*. Maton): National Seminar on Plant Protection Association of India .14-17th March, NBPGR, Hyderabad.

Sangeeth K P and Bhai R S. 2009 In *vitro* plant growth-promoting and antagonistic effect against *Phytophthora capsici* by indigenous N fixers P solubilizers and K

mobilizers of black pepper (*Piper nigrum* L). IPS Ab. NO. 649 In Souvenir & Abstracts, 5th International Conference, "Plant Pathology in the Globalized Era" Nov 10-13, 2009 IARI, New Delhi pp. 348

8233 **Reports**

Annual report 2004 -2005, 2005-2006, 2006-2007, 2007-2008

Technical bulletins/books

Suseela Bhai. R 2008. Biological Control of fungal diseases. In "Organic Spices" (Eds. V. A Parthasarathy, K. Kandiannan, & V Srinivasan) New India Publishing Agency. Pitam Pura, New Delhi-110 088 pp 153.

Suseela Bhai R. 2009 Biological Control of Soil Borne Plant Pathogens with Special Reference to Major Spice Crops " In Role of Biocontrol Agents for Disease Management in Sustainable Agriculture (eds. P. Ponmurugan and M. A. Deepa) RESEARCH INDIA PUBLICATIONS, B-2/84, Ground Floor, Rohini Sector-16, Delhi-110085, INDIA PP.231—245.

M.Sc Project work

Sithara Raj 2008. Effect of pH and moisture on the survival of *Trichoderma* and *Phytophthora* with reference to Black pepper- - Hindustan College of Arts & Science Coimbatore Pp.

8234 **Seminars, Conferences and workshops (relevant to the project) in which them scientists have participated (List of abstracts forwarded)**

Anandaraj, M. 2004 XIII Southern Regional Conference on Microbial Inoculants, at University of Agricultural Sciences, Dharwar at Bijapur from 3-5 Dec 2004 and gave an invited talk on 'Potential of Plant Growth Promoting Rhizobacteria for Plant Disease Management'.

Anandaraj, M. 2004 Short term training programme on Mycorrhiza organized by CPCRI from 1-10 Dec 2004. Presented a paper on the 'Use of Mycorrhiza for Managing Soil borne Diseases in Spice crops'.

Suseela Bhai, R., 2004 Symposium on Spices and Aromatic crops, 1-2 Nov 2004, Indian Institute of Spices Research, Calicut. Presented a paper on "Efficiency of rhizobacterial cultures in managing soft rot disease of ginger".

Anandaraj M 2004 Symposium on Spices and Aromatic crops, 1-2 Nov 2004, Indian Institute of Spices Research, Calicut. Presented paper on "Effect of *Pseudomonas fluorescens* (IISR-6) and *Trichoderma harzianum* on growth of black pepper varieties in the nursery".

Devasahayam, S. 2005 Symposium on Biological control of pests of horticultural crops: New thrusts, Project Directorate of Biological Control, Bangalore, 19 March 2005. Gave an invited talk on "Prospects and emerging trends in biological control of insect pests of spices"

Anandaraj, M. 2005 National Symposium on Biotechnological interventions for Improvements of Horticultural crops: Issues and Strategies. 10 – 12 January 2005 (eds)G.S.L.H.V.P. Rao, Nazeem, P.A., Girija, Keshavachandran, L. Joseph and John, P.S. Kerala Agricultural University, Thrissur, Kerala, PP. 355-358.

Sangeeth K.P, 2007 National Seminar on Plant Protection Association of India .14-17th March, NBPGR, Hyderabad. Presented a paper on "Comparative Efficacy of Indigenous Microbial Inoculants in Improving Seedling Growth of Cardamom (*Elettaria cardamomum*. Maton)"

Suseela Bhai, R. 2009 5th International Conference, on "Plant Pathology in the Globalized Era" Nov 10-13, 2009 IARI, New Delhi and presented papers.

Suseela Bhai, R. 2008 National Conference on Organic farming in Horticultural Crops with special reference to Plantation Crops- promoting organic agriculture for safe food and healthy environment and presented paper on "Evaluation of antagonistic potential of *Trichoderma* isolates against *Pythium aphanidermatum* (Edson) Fitz. causing rhizome rot in Turmeric (*Curcuma longa* L")

Anandaraj, M. 2005 National Workshop on Microbial Inoculants for Crop Nutrition and Health, 30 Sep 2005, Kerala Agricultural University, Vellayani, Trivandrum. Lead paper presentation "*Pseudomonas fluorescens* for Disease Management and Growth enhancement of Crop Plants".

Anandaraj, M. 2006 34th Pepper tech meeting at Earl's Regency, Kandy, Sri Lanka 4-7 Sep 2006, Organized by International Pepper Community, Jakarta Key note address on "Implementation of IPM for Managing pests and diseases of black pepper"

824 **Infrastructural facilities developed**

(Details of field, laboratory, note books and final material and their location)

8241. **Infrastructure developed** : Nil

8242 **Details of field, laboratory books-**

Field books - 3 nos. Work book -3 Registers -3 Nos. Available in pathology section at IISR Calicut and 1 Field book and 1 work-book at CRC Appangala

8243 **Comments/suggestions of project leader regarding possible future line of work that may be taken up arising out of this project**

Toxicological data has to be generated for individual candidates in the consortium.

PART IV: PROJECT EXPENDITURE (Summary)

Year 2004-2008

830 Total Recurring Expenditure

8301 **Salaries**: (Designation with pay scale)

I) Scientific		
Principal Scientist (16400-450-2090-500-22400):	Rs.	6, 48, 000
Sr. Scientist - 12000-420-18300)	: Rs.	5, 50,000
Scientist (8000-)	: Rs.	1, 80,000
ii) Technical (T3) 4500-125-7000	: Rs.	1, 39, 626
iii) Supporting	: Rs.	52, 476
iv) Wages	: Rs.	40,000
Sub Total	: Rs.	16, 10,102

8302 **Consumables**

i) Chemicals	: Rs.	30, 000
ii) Glass wares	: Rs.	40, 000
iii) Others	: Rs.	20, 000
Sub Total	: Rs	90, 000

8303 **Travel** : Rs 50, 000

830 **Miscellaneous** (Other costs) : Rs 35, 000

8305 **Sub total** (Recurring) : Rs 1, 75,000

831 Total Non-recurring Expenditure (Equipments & works) : Nil

ii)

iii)

832 Total (830 and 831) : Rs. **17, 85,102**

PART IV: PROJECT EXPENDITURE (Summary)

Year 2004-2008

830 Total Recurring Expenditure

8301 **Salaries**: (Designation with pay scale)

I) Scientific		
Principal Scientist (16400-450-2090-500-22400):	Rs.	6, 48, 000
Sr. Scientist - 12000-420-18300)	: Rs.	5, 50,000
Scientist (8000-)	: Rs.	1, 80,000
ii) Technical (T3) 4500-125-7000	: Rs.	1, 39, 626
iii) Supporting	: Rs.	52, 476
iv) Wages	: Rs.	40,000
Sub Total	: Rs.	16, 10,102

8302 **Consumables**

i) Chemicals	: Rs.	30, 000
ii) Glass wares	: Rs.	40, 000
iii) Others	: Rs.	20, 000
Sub Total	: Rs	90, 000

8303 **Travel**	: Rs	50, 000
830 **Miscellaneous** (Other costs)	: Rs	35, 000
8305 **Sub total** (Recurring)	: Rs	1, 75,000
831 Total Non-recurring Expenditure (Equipments & works) ii) iii)	:	Nil
832 Total (830 and 831)	: Rs.	**17, 85,102**

PART-V: DECLARATION

This is to certify that the final report of the project has been submitted in full consultation with the project workers as per the approved objectives and technical programme and the relevant records, note books; materials are available for the same.

Signature of the Project Investigator:

M Anandaraj

M N Venugopal

Co Investigators:

S. Devasahayam

Abdulla Koya

R. Suseela Bhai

Santhosh J Eapen

A Kumar

C N Biju

Signature and comments of the Head of the Division/Section All the objectives of the project have been fulfilled. Consortium of biocontrol agents have been identified for the management of Phytophthora foot rot and nematodes of black pepper, rhizome rot of cardamom in the nursery and root rot of vanilla in the field. All registers and records pertaining to the project are available in the Division.

S Devasahayam
22/04/10

Signature and Comments of the Joint Director (Research)

Signature and Comments of the Director

24/5/10

ANNEXURE 1

ACHIEVEMENTS

I. Evaluation of promising rhizobacterial biocontrol isolates against *Phytophthora capsici. Pythium species, Rhizoctonia solani* and *P. meadii* and studying their mutual antagonism / synergism

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A. Black pepper

In vitro* assayfor the compatibility and evaluation of biocontrol agents against *P. capsici R. similis* and *M. incognita

Thirty seven rhizo bacterial isolates which were inhibitory to pathogens of black pepper (*P. capsici* 19, *M. incognita* 15 and *R. similis* 1) were tested for their compatibility with antagonistic fungus *T. harzianum* (IISR-1369) that has been identified as promising biocontrol agent for *P. capsici*. Among this, 12 isolates of bacteria inhibited the growth of *T. harzianum*. The per cent inhibition of *T. harzianum* varied from 13.3 to 76.3 by various isolates (Table.1a&b).

Table. 1a. Compatibility of *bacterial* biocontrol agents (against *P. capsici)* to *T. harzianum*

Sl. No.	Bacterial isolate number	Inhibition of *P. capsici* %	Inhibition of *T. harzianum* %
1	IISR- 290	65.0	0.0
2	IISR-289	62.0	0.0
3	IISR-293	56.0	0.0
4	IISR-292	51.0	0.0
5	IISR-40	50.0	0.0
6	IISR-6	69.0	0.0
7	IISR-51	71.2	0.0
8	IISR-26	51.0	66.6
9	IISR-18	58.3	66.6
10	IISR-13	52.6	66.3
11	IISR-15	41.5	63.3
12	IISR-287	60.5	0.0
13	IISR-8	56.0	70.8
14	IISR-44	41.2	76.3
15	IISR-35	48.2	72.6
16	IISR-11	58.3	0.0
17	IISR-56	44.0	0.0
18	IISR-3	55.0	0.0
19	IISR-10	58.8	73.1

Table. 1b. Compatibility of bacterial biocontrol agents (against nematodes) with *T. harzianum*

Sl. No.	Bacterial Isolate number	Inhibition of *T. harzianum* %
1	IISR-549	0.0
2	IISR-551	0.0
3	IISR-510	0.0
4	IISR-663	0.0
5	IISR-655	0.0
6	IISR-641	0.0
7	IISR-526	0.0
8	IISR-527	0.0
9	IISR-658	0.0
10	IISR-38	70.0
11	IISR-695	13.3
12	IISR-533	0.0
13	IISR-642	69.8
14	IISR-554	0.0
15	IISR-536	0.0
16	IISR-528	0.0

Assay for multiple antagonistic potential against soil borne pathogens of Black pepper

Antagonistic potential of bioagents viz. IISR 6, 8, 13, 51, 151, PB 21C, P1AR6 853, 859 & P 26 effective against *P. capsici* of black pepper were tested against other pathogens viz. *P. aphanidermatum, R. solani* and *F. oxysporum* f. sp. *vanillae* The isolate IISR 853 which is antagonistic to both *R. similis* and *M. incognita* is found antagonistic to all other targeted pathogens giving more than 50% inhibition (Table 2)

Table 2 Multiple antagonistic potential of short listed bioagents

	Bioagents	**inhibition of Pathogens (%)**		
		P. aphanidermatum	*F. oxysporum f.* sp *vanillae*	*R. solani*
1	IISR 6	0	66.66	88.88
2	IISR 8	0	0	0
3	IISR 13	0	20.83	0
4	IISR 51	0	0	0
5	IISR 859	0	0	0
6	IISR 853	87.77	50.0	83.33
7	IISR 860	0	0	0
8	IISR PB21C	0	46.66	0
9	IISR P1AR6	0	5.8	0

Compatibility of short listed antagonists

The compatibility among the bacterial isolates were tested *invitro* using the spray inoculation technique. All the bacterial isolates tested are compatible with each other. But to *T. harzianum*, IISR 8, 11SR 13, PB 21C and P1AR 6 are compatible whereas IISR 13, IISR 859 and IISR 51 are not compatible. *P. chlamydosporia* is inhibitory to all the bacterial antagonists and *T.harzianum*.

In vitro compatibility of *P. fluorescence* strains IISR 6, 8, 13, 51, 853, and 859 revealed that only three of the isolates tested namely IISR 8, 51, and 859 are compatible with *T. harzianum* and IISR 859 alone is compatible with *P. chlamydosporia*.

Evaluation of bacterial isolates against root mealy bug

Twelve bacterial isolates (promising against *P. capsici* and nematodes) were evaluated against root mealy bug in laboratory bioassays. None of the isolates were promising in reducing the population of root mealy bug.

Evaluation of entomopathogens against root mealybug

Commercial formulations of *M. anisopliae* and *N. rileyi* and *V. lecani*, *Aspergillus* sp. and *T. harzianum* were evaluated against root mealybug in laboratory bioassays. However, none of the fungal isolates/ formulations was promising in reducing the population of root mealybug.

B.Cardamom

***In vitro* evaluation of promising biocontrol agents on rhizome rot pathogens of Cardamom**

In vitro evaluation of two fungal bioagents viz., *T. harzianum* and *T. hamatum* and two bacterial antagonists viz., IISR – 6 and IISR – 853 was undertaken to test their efficacy against rhizome rot pathogens of cardamom (*P. vexans* and *R. solani*). *T. harzianum* was more effective on both test pathogens compared to other bioagents. IISR – 853 and IISR – 6 were not effective on both *P. vexans* and *R. solani*. Observations on hyphal interaction between *R. solani* and *T. hamatum* revealed the tropical movement of *T. hamatum* hyphae towards *R. solani* followed by penetration and parasitization.

B. Vanilla

Studies of mutual antagonism/ synergism of bioagents with *Trichoderma*

Sixteen rhizosphere bacteria, short listed as promising bioagents against pathogens of vanilla were tested for their mutual antagonism/ synergism with *T. harzianum* under *invitro* conditions. The result showed that isolates such as IISR 6, IISR 13, IISR 51, IISR 152, IISR 853, IISR 859, and IISR 909 were antagonistic to *T. harzianum*. The inhibition ranged from 15.55-72.2% (Table 3)

Table-3 Antagonism of effective bioagents for vanilla with *Trichoderma*

Sl. No	Isolate	% inhibition
1	IISR 6	72.2 a
2	IISR 8	0 e
3	IISR 13	15.55 d
4	IISR 51	71.66 a
5	IISR 147	0 e
6	IISR 148	0 e
7	IISR 149	0 e
8	IISR 151	0 e
9	IISR 152	38.8 c
10	IISR 906	0 e
11	IISR 907	0 e
12	IISR 909	67.7 ab
13	IISR 853	67.2 ab
14	IISR 859	0 e
15	IISR P1AR6	0 e
16	IISRPB21C	0 e

Mutual compatibility of rhizobacteria

In order to make a consortium of rhizobacteria for pathogen and pest management in spice crops, mutual compatibility of the rhizobacteria was studied as a prerequisite. The study indicated that certain rhizobacteria IISR 6, 8, 13, 51, 853, 859, PB 21C and P1AR 6 from the repository could co- exist in the common nutrient media sharing the common nutrient resources which were confirmed by microbiological and PCR assays.

Development of bioconsortium for the management of pests and disease of spices

Biocontrol consortium on *R. similis* infestation

A consortia of 12 rhizobacteria namely IISR 6, 151, 522, 527, 528, 532, 641, 658, 853, 857, 859, and 865 were evaluated against *R. similis* in an infested plot. The mortality was 44% in treated vines as compared to 76% in untreated. The population of *R. similis* was 449.78/g soil in treated as against 178.23/g soil in untreated check. So it is inferred that the bio consortium is ineffective in suppressing *R. similis* soil population.

II. Evaluation of promising biocontrol agents individually and in combination under greenhouse conditions

A. Black Pepper

Evaluation of promising biocontrol agents individually and in combination under greenhouse conditions against *P. capsici.*

A pot culture trial was laid out with 10 treatments including short listed promising bioagents individually and in combinations keeping recommended chemical control (Bordeaux Mixture Spray+ Copper oxychloride drenchingl as check. The treatments were challenged with *P.capsici.* Black pepper variety Subhakara was used for the experiment. The result showed that among the bioagents combinations. P26 and IISR 6 is comparatively better in reducing the disease incidence followed by 859 + *P. chlamydosporia* and is on par with chemical treatments. (Table 4)

Table-4 Evaluation of promising isolates under greenhouse conditions against soil borne pathogens

Sl No	Treatments	Mortality (%)
T1	BM Spray+ COC drenching	0.70
T2	Pot. Phosphonate spray+ drench	15.05
T3	*T.harzianum*+ IISR 859	37.27
T4	*T.harzianum*+ IISR 6	18.52
T5	*P. chlamydosporia* + IISR 859	22.45
T6	*T.harzianum*+ 859+IISR 6	33.33
T7	*T.harzianum*	44.44
T8	Control (*Phytophthora* inoculation alone)	81.48
T9	Absolute control (Untreated)	0.70
T10	*P. chlamydospori*a	25.92
CD at P=0.05		20.59

Evaluation of promising isolates under green house conditions against soil borne pathogens (Black pepper)

The promising nematode antagonistic isolates viz. *Pochonia chlamydosporia*, IISR-859, IISR-853 were evaluated under green house conditions in comparison with recommended fungicides viz. copper oxychloride (0.25%) and potassium phosphonate (0.3%) and biocontrol agents viz.*T. harzianum* + *Pseudomonas fluorescens* against *P. capsici*. The study was conducted for two years. The study showed that IISR-853 and IISR-859 were effective in checking *P. capsici* infection and were at par with copper oxychloride and P. chlamydosporia followed by Potassium phosphonate 0.3% (Table 5).

Table 5 Evaluation of promising isolates under green house against *P. capsici*

	Treatments	Mortality (%)	% control
T1	Copperoxychloride 0.25%	0. 00	100.00
T2	Potassium phosphonate 0.3%	14. 81	81.82
T3	*T.harzianum*(P26) 50g/pot	44. 44	45.46
T4	*P. chlamydosporia*	11.11	86.78
T5	IISR 853	0 .00	100.00
T6	IISR 859	7. 41	90.91
T7	*T.harzianum*(P26)+ IISR 6 (1:1)	18.52	77.27
T8	Control (*P. capsici* alone)	81.48	-
	LSD 0.05%	12.71	

Effect of pH and moisture on the survival of *Trichoderma* and *Phytophthora* with reference to Black pepper rhizosphere

A pot culture study was carried out to evaluate the effect of pH and moisture on the survival of *T. harzianum* and *P.capsici* under black pepper system. The study showed that pH 4.0- 5.5 supported the growth of *T. harzianum* whereas the same pH was suppressive to *P.capsici*. Disease Potential Index of the soil was reduced by 34% with the addition of *T. harzianum* in the soil. Moisture levels made no significant difference in the population of either *T. harzianum* or *P. capsici* under test conditions of experiment (Table-6-8)

Table 6. Effect of pH on *T. harzianum* and *P. capsici* (*in vitro*)

pH levels	Growth (mm)	
	T.harzianum (48h)	*P.capsici* (72h)
4.0	90.00	56.00
4.5	86.67	66.33
5.0	82.33	68.17
5.5	78.67	72.33
6.0	73.67	71.00
6.5	79.00	69.17
7.0	85.67	64.833
LSD	**4.77**	**2.085**
CV (%)	**4.98**	**2.68**

Table 7- *Trichoderma* population at different pH and moisture levels (pH x Moisture)

			Cfu x 10^4			
Sl No	pH	Moisture level (%)	7dai	14 dai	21 dai	28 dai
1	4.5-5.0	35-40	43.67 A	9.17 D	4.333 D	78.00 AB
2		30-35	41.83 A	20.33 BC	9.667 D	74.00 B
3		20-25	17.00 DE	18.33 C	61.33 B	50.33 C
4		10-15	32.83 B	26.17 BC	84.00 A	94.33 A
5	5.0-5.5	35-40	26.50 BC	28.50 AB	39.67 C	62.17 BC
6		30-35	16.17 DE	20.33 BC	34.17 C	95.50 A
7		20-25	11.33 DEFG	25.83BC	67.00 AB	65.83 BC
8		10-15	19.67 CD	36.00 A	77.50 AB	93.33 A
9	5.5-6.0	35-40	6.500 FGH	0.6333 E	0.5333 D	5.133 D
10		30-35	5.167 GH	0.7000 E	0.9667 D	6.467 D
11		20-25	8.833 EFGH	0.4000 E	2.200 D	2.767 D
12		10-15	14.33 DEF	0.5000 E	3.533 D	5.133 D
13	6.0-6.5	35-40	3.500 GH	0.5000 E	1.067 D	3.633 D
14		30-35	4.000 GH	0.4667 E	1.017 D	3.150 D
15		20-25	1.667 H	1.000 E	1.500 D	3.000 D
16		10-15	6.167 FGH	0.5000 E	1.967 D	3.167 D
17	6.5-7.0	35-40	4.833 GH	0.3667 E	1.400 D	3.167 D
18		30-35	3.000 GH	0.8000 E	1.133 D	6.267 D
19		20-25	4.167 GH	0.6333 E	1.200 D	5.067 D
20		10-15	5.000 GH	0.3333 E	1.500 D	4.500 D

Table 8. Effect of *T. harzianum* on the survival of *P. capsici*

Treatments	*DPI (1st sampling)	**DPI (2nd sampling)
T. harzianum + *P capsici*	394.67	337.07
P capsici alone	597.33	646.40
Initial DPI	1024	1024

The results of the overall survival of black pepper plants showed that the plants could survive at different pH.from 4-7. However it is comparatively better at pH 5.0 -5.5 or 6.5-7.0 almost the same as for *T. harzianum* survival (Table 7 & 8). But moisture level is playing a crucial role in the survival of plants. Irrigating the plants once in two days or a moisture content of 20-25% is ideal for the survival of the plants which is also favorable for soil population of *T. harzianum* . When conducive soil is supplemented with *Trichoderma,* there is a survival of 78%. at pH 4.5 -5.5 when moisture is maintained at 20-25% i.e. by irrigating the plants once in two days. Interaction of pH and *T. harzianum* on the survival of plants clearly indicated that *T. harzianum* applied at pH 4.5-5.5 is effective in reducing the mortality of the plants due to *P capsici* infection. Hence from the result of the present experiment on survival of *T. harzianum* and *P. capsici* and mortality due to *P. capsici*, it is inferred that pH of the soil should be kept at 4.5-5.5 with irrigation on alternate days along with fortification of *T. harzianum* helps in reducing the infection of *P capsici* .

Effect of biocontrol agent (*T. harzianum*) under varying levels of pathogen

The disease potential index of the soil treated with different doses of the pathogen was standardized. It is found that 20 nos. of 10mm discs from 72hr old sporulated culture could give a disease potential index (dpi) of 256 (9 dilutions) within 72 h of inoculation, 40 numbers give a DPI of 4096 (13 dilutions), 80 nos. give dpi of 32768 (16 dilutions)and 160 nos. give a dpi of 167777216 (i.e. more than 25 dilutions) . When these pots were planted with rooted cuttings of black pepper, the plants died in 7-11days in all the treatments. The dead plants were removed and the same pots were fortified with T. *harzianum* multiplied in

cowdung @ 250g /pot having a cfu of $6x10^8$/g and again baited for DPI after 10days.The DPI was found reduced to up to 16 (5^{th} dilution) in 10 days of *T. harzianum* application and reduced to 2 (2^{nd} dilution) in 60days.The pots were replanted with rooted cuttings of black pepper after 60^{th} day (2008). No mortality could be observed till date (2009).

B.Vanilla

Root rot and Stem rot

Root rot and stem rot disease caused by *F. oxysporum f. sp. vanillae* (*Fov*) (and in certain cases *P. meadii* also) is an economic constraint which affects vanilla production. At present only chemicals are being recommended for the control. Being export oriented crop it becomes imperative to search for bio control agents also for combating the diseases. Hence rhizosphere and phyllosphere organisms of vanilla were isolated and screened against *F. oxysporum f. sp. vanillae* (*Fov*) causing root rot of vanilla.

Twenty rhizobacterial isolates viz. *P. fluorescens* (5 isolates), *Enterobacter agglomerans* (one isolate) and *Bacillus* spp. (14 isolates) were screened for growth promotion and against rot pathogens of vanilla such as *Phytophthora meadii*, *F.oxysporum* and *Colletotrichum vanillae*. Under *invitro* conditions, all of the rhizobacterial isolates tested except *Bacillus polymixa (* isolate IISR 909) and one *Bacillus* sp. (isolate IISR 915) were inhibitory to *P. meadii* to an extent of 74 percent while *F. oxysporum* was highly inhibited (91.0%) by *Bacillus polymixa* (isolate IISR 909). The maximum growth promotion in terms of shoot length (27cm) in vanilla was observed in plants treated with *P. fluorescens isolate* (isolate IISR 13). The *P.fluorescens* isolates (IISR6, IISR 853), *B. lentus* (IISR 906), *B. polymixa* (IISR 909), *E. agglomerans* (IISR 912), *Bacillus* species (IISR 910, IISR 913, IISR 914, IISR 915 and IISR 149) were found promising for growth promotion and also for suppressing the rot pathogens viz. *Fov* and *P. meadii*..

P. fluorescens (isolates IISR 6, IISR 51, IISR 853), *Bacillus* species (isolates IISR 147, IISR 148 and IISR 152), were made into different combinations and tested against root rot of vanilla caused by *F. oxysporum* f. sp. *vanillae*. Four different consortia of rhizo bacterial isolates viz. **1)**- *P.fluorescens* (isolates IISR 13, IISR51), *Bacillus* sp.(IISR 152) and *B. polymixa* (IISR 909); **2)**- *P.fluorescens* isolates IISR 13, IISR 51), *Bacillus* sp .isolates (IISR 148, IISR 149, IISR 152, IISR 907) , *B. polymixa* (IISR 909) and *B. lentus* (IISR 906); **3)**

P. fluorescens isolates IISR 13, IISR 51, IISR 6, *Bacillus* sp. isolates (IISR 152, IISR 147, IISR 151 , IISR 153) and *B. polymixa* (isolate IISR 909); **4)** *P.fluorescens* isolates (IISR 6, IISR 51, IISR 147, IISR 148, IISR 149 and IISR 907) and *B. lentus* (IISR 906) were evaluated against root rot which gave significant disease reduction (88.22- 92.85%) when compared to control. However, among the four rhizobacterial consortia, the consortia T3 showed the maximum disease reduction of 92.9% and were at par with other treatments except control. (Table 9).

Table 9 Effect of mixture of rhizobacteria on Fusarium infection & growth promotion

Sl. No.	Treatment	*Fusarium* infection (%)	Shoot length (cm)
T1	*P.fluorescens* (IISR 13, 51), *Bacillus* sp.(IISR 152) and *B. polymixa* (IISR 909)	8.6 (88.3)	83.6
T2	T1+ *Bacillus* sp (IISR 148, 149, 907) and *B. lentus* (IISR 906)	8.1 (88.9)	71.9
T3	T1+ *P.fluorescens* (IISR 6), *Bacillus* sp. (IISR 147, 151 and 153)	5.2 (92.9)	84.3
T4	*P.fluorescens* (IISR 6, 51, 147, 148, 149 and 907), *B. lentus* (IISR906)	7.9 (89.2)	60.6
T5	Control – (only pathogen)	73.3 (0.0)	77.0
	LSD	24.3	NS

(Values in parenthesis are percentage disease reduction)

III. Field evaluation of bio consortia against soil borne pathogens (Black pepper, Cardamom and Vanilla)

A. Black Pepper

A field trial to evaluate the effect of five different combinations of rhizobacteria, in the form of consortia, against *Phytophthora* foot rot and slow decline diseases of two varieties of black pepper, was conducted for five years from 2003-2007 in comparison with *T. harzianum* alone and Copper oxychloride . The pooled data after 5 years indicated that the rhizobacterial consortium containing IISR-6, 8, 13, 51, 151, 853 (rhizobacteria) was effective in reducing the disease incidence in the variety Subhakara as well as increasing the survival of the plants. Among the eight treatments, the mortality of vines was lower in this treatment. But no significant difference was observed in Panniyur 1 (Table 10). Among the treatments drenching with COC 0.3% was found comparatively better when compared to other treatments.

Table 10. Disease Severity (%) in different biocontrol consortia

Treatment	Subhakara	Panniyur1	Mean
IISR 6, 8, 13, 51, 151, PB21C	27.16*	41.87	34.51
IISR 6, 8, 13, 51, 151, P1AR6	35.14	41.22	40.18
IISR 6, 8, 13, 51, 151, 853	**14.98**	60.57	37.78
IISR 6, 8, 13, 51, 151, 859	33.03	54.20	43.62
All together	33.97	43.24	39.11
T.harzianum	40.79	33.98	37.38
COC 0.3%	36.29	24.56	30.43
Control	36.75	32.71	31.73
Gen. Mean	32.39	42.05**	NS
LSD 0.05	Variety x Treatment = 22.51		

* Pooled data for the last three years (arc sine transformed)

In a separate comprehensive trial with different biocontrol consortia the health of the vine was scored and the yellowing was indexed. The percent vines showing yellowing were significantly lower in treatments involving the isolates IISR 859 and the nematode population

was also reduced. The population of root mealy bugs was also reduced in all the treatments compared to untreated control (Table 11)

Table 11 Efficacy of consortia on Yellowing and nematode population in black pepper

Treatments	Components	Yellowing(%)	Nematode population/g root	
			R. similis	*M incognita*
T1	IISR 6, 8,13, 51, 151+ PB 21C	24.9	0.00 a	214.77 a
T2	IISR 6, 8,13, 51, 151+ P1AR6	33.33	7.97 a	172.38 a
T3	IISR 6, 8,13, 51, 151+ 853	27.8	0.00a	33.36 ab
T4	IISR 6, 8,13, 51, 151+ 859	19.6	0.00a	2.18b
T5	IISR 6, 8,13, 51, 151+ PB 21C+ 853+859	28.0	1.04a	9.16 ab
T6	*T. harzianum*	13.8	1.06 a	43.45 ab
T7	Chemical Control	25.0	7.47a	43.77 ab
T8	Medium control	16.6	1.44 a	224.94a

Evaluation of bioconsortia against root mealy bugs

The incidence of root mealy bugs (*Planococcus* sp.) was recorded three months after application of bioconsortia in various treatments. The root mealy bug population was observed in all the treatments and it varied from 11.1% to 33.3%.

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T3	IISR 6, 8,13, 51, 151+ 853	27.8	0.00a	33.36 ab
T4	IISR 6, 8,13, 51, 151+ 859	19.6	0.00a	2.18b
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B. Cardamom

Effect of consortium on rhizome rot of cardamom and *Phytophthora* infection of black pepper in nursery

The consortium was applied to the root zone of three month old pepper cuttings and five month old cardamom seedlings. The pathogens- *P. capsici*, *P. vexans* and R. *solani* were released to the root zone of respective hosts after fifteen days of inoculation with biocontrol agents.

The mortality, yellowing, extent of collar rot and root rot were assessed in various treatments up to thirty days of challenging with pathogens . In cardamom, the root tip rot and mortality was less with *T.harzianum* alone (P 26) followed by consortium of rhizobacterial isolates viz.IISR 6, 13, 51, 151, 853 and 859 and standard control (drenching with 0.2% COC).

In pepper there were no significant differences between the consortium combinations. However, *T.harzianum* and also biocontrol consortium were better over control with regard to extent of root decay and yellowing.

Testing of promising biocontrol agents in secondary nursery of cardamom

Separate experiment was conducted to study the effect of promising biocontrol agents of IISR on rhizome rot pathogens of cardamom in secondary nursery. The pathogens namely *P. vexans* and *R. solani* isolated from rhizome rot affected specimens of Appangala, were used in this study. The treatments were IISR 6, IISR 853, P 26, COC (0.2%) and untreated control. Unsterilized but solarized potting mixture was used for raising secondary seedlings. The biocontrol agents were incorporated separately at the time of transplanting and tillering stage. After 7 days of second inoculation with biocontrol agents, the pathogen *P. vexans* were inoculated in the form of aqueous suspension (7-10 days old culture; 50 mg/plant). Finger millet grains colonized for 14 days *R. solani* was inoculated (100mg/plant) separately and also in combination with *P. vexans*. The treatments are replicated four times and 20 plants were maintained for each plot. The mortality was recorded up to 3 months of inoculation and

root rot index was recorded for only combined inoculation with *P. vexans* + *R. solani* after 3 months of releasing pathogens (Table 13).

Table 13. Evaluation of biocontrol agents on rhizome rot pathogens of cardamom.

Treatments	Mortality(%)			
	P. vexans	*R. solani*	P. v + R. s	Root rot index
IISR 6	12.5 (20.56)	8.25 (16.59)	21.5 (27.58)	1.25
IISR 853	19.75 (26.08)	14.00 (21.95)	30.25 (33.31)	2.5
P26	5.25 (12.91)	3.5 (10.64)	7.25 (15.49)	1.0
COC (0.2%)	2.75 (8.78)	4.75 (12.3)	5.5 (13.32)	1.0
Untreated control	41.5 (40.09)	23.5 (28.94)	58.5 (49.92)	4.75
LSD (P=0.05)	**6.046 (4.889)**			

Among the biocontrol treatments P-26 is superior and was on par with chemical control of drenching COC (0.2%). All the test biocontrol agents are superior over untreated control and effectively reduced mortality of secondary seedlings. However, P 26 (*T. harzianum*) is superior followed by IISR 6.

C. Vanilla

Evaluation of bio-consortium on root diseases of vanilla (Field trial)

A field trial was laid out in RBD at IISR farm at Peruvannamuzhi to study the effect of bioconsortium on root diseases of vanilla. The trial consisted of nine treatments including control. There were three replications /treatment and 6 plants/ replication. Two rounds of treatments were given at one month interval. The trial was conducted for two years. The pooled data for the two years showed that the Treatments 2 containing the isolates IISR 6, 8, 13, 51, 151 P1AR6), and Treatment 3 containing the isolates IISR 6, 8, 13, 51, 151 , 853) , are effective in reducing the disease incidence . (Table 14)

Table-14 Effect of bioconsortia on root disease of vanilla

Sl.No	Treatments	Disease incidence (%) (2005-2006)	Disease incidence (%) (2006-2007)
T1	IISR 6, 8, 13, 51, 151, PB21C	22.22 bc	44.44
T2	IISR 6, 8, 13, 51, 151, P1AR6	11.11 c	22.46
T3	IISR 6, 8, 13, 51, 151, 853	22.22 bc	16.67

T4	IISR 6, 8, 13, 51, 151, 859	16.67 bc	50.00
T5	All together	38.89abc	38.89
T6	*T. harzianum (p-26)*	22.22bc	28.01
T7	COC 0.3%	33.33abc	55.57
T8	Control -Nutrient broth	50.00ab	27.77
T9	Absolute control	61.11a	38.89
	LSD (p=0.05)		19.42

IV .Demonstration of technologies for management of foot rot and slow decline

The different management strategies adopted for foot rot and slow decline diseases of black pepper were demonstrated in IISR Experimental Farm- Peruvannamuzhi in a split plot design with variety Sreekara. The different treatments included

1. COC 0.25% drenching, + BM spray , 2. IISR 6+ P-26 , 3. Potassium phosphonate 0.3% (Spray + drenching) , 4. Metalaxyl mancozeb (Spray + drenching) and 5. Control without any treatments. Two separate sets were maintained with Phorate and without Phorate. Initial establishment of the plants were 95.83% with Phorate and 85.53% without Phorate. No disease incidence was noticed in any of the treatments so far (2009 monsoon season). The plot is being maintained.

Evaluation of promising lines under Biocontrol management system

A field trial was taken up with three tolerant lines viz, IISR Shakti (tolerant to *P. capcisi*), hybrid HP 39 (tolerant to *nematode*) and cultivar C 1090 (tolerant to both *P.capsici* and nematodes) in comparison with Sreekara. Four promising biocontrol agents viz. IISR 853 (antagonistic to nematodes and *P. capcisi*), *T. harzianum* (antagonistic *to P. capcisi), P.chlamydosporia (*antagonistic to nematodes) and *P.fluorescens* IISR 6 (antagonistic *to P. capcisi*) were evaluated to study the effect of biocontrol agents in combination with field tolerant selections. The biocontrol agents were applied along with vermicompost and farmyard manure. The initial observation on plant height and no. of leaves showed significant difference in bioagents treated plants when compared to untreated susceptible control (Table 15).This trial is being continued in the Project Crop. Prot. 1.5 (813): Integrated management of *Phytophthora* foot rot and slow decline diseases of black pepper.

Table 15. Evaluation of promising lines under Biocontrol management system

Sl.No	Tolerant lines	C 1090		Hp 39		IISR Shakti		Sreekara	
	Bioagents	Total leaves	Total length	Total leaves	Total length	Total leaves	Total length	Total leaves	Total length
T1	*T. harzianum*	24.33	171.33	3.3	21.22	16.67	145.56	13.22	113.89
T2	IISR 853	11.44	103.44	10.11	77.33	15.44	112.67	19.22	167.67
T3	P. chl.	17.78	145.56	8.22	57.56	9.00	67.67	10.56	82.67
T4	IISR 6	20.56	178.67	3.33	17.78	19.22	149.78	10.78	111.67
T5	Control	5.22	44.22	1.33	8.00	1.77	6.00	4.44	41.78
	Lsd	7.970	66.39	7.970	66.39	7.970	66.39	7.970	66.39

ANNEXURE II

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