ICAR-INDIAN INSTITUTE OF SPICES RESEARCH
KOZHIKODE-673 012, KERALA
(Indian Council of Agricultural Research)

FINAL RESEARCH PROJECT REPORT (RPP- III)

1. Institute Project Code : : Path XXI (813)

2. Project Title :Diversity of rhizome- root rot pathogens and their antagonists in cardamom

3. Key Words :Cardamom, rhizome rot, root rot, *Rhizoctonia solani, Fusarium* sp*, Pythium* vexans, *Trichoderma* sp

4. (a) Name of Lead Institute : ICAR - Indian Institute of Spices Research, Kozhikode

(b) Name of Division/Regional Centre/Section: Regional Station, Appangala, Kodagu, Karnataka

5. (a) Name of the Collaborating Institute(s) : Nil

(b) Name of Division/Regional Centre/Section of Collaborating Institute(s): Nil

6. Project Team (Name(s) and designation of PI, CC-PI and Co-PIs, with time spent):

S. No.	Name, designation and institute	Status in the project (PI/CC-PI/ Co-PI)	Time spent (%)	Work components assigned to individual scientist
1.	Dr. Praveena .R Scientist, Division of Crop Protection, ICAR-Indian Institute of Spices Research, Kozhikode	PI	25 % (3 man months)	1. Survey in the major cardamom growing areas of Karnataka, Kerala and Tamil Nadu. 2. Surveys in the hot spots to study the temporal variation of pathogens. 3. Isolation of pathogens associated with rhizome - root rot diseases. 4. Isolation of the antagonists from cardamom growing areas. 5. Pathogenicity of the pathogens on susceptible cultivar/ variety. 6. Morphological characterization of the pathogens and antagonists. 7. *In vitro* evaluation of biocontrol agents against

				selected isolates of rhizome and root rot pathogens. 8. Evaluation of effective biocontrol agents against selected isolates of rhizome and root rot pathogens under glass house conditions. 9. Sequential inoculation studies to identify the primary causal pathogen of rhizome-root rot diseases. 10. Pot culture trials to identify the primary causal pathogen of rhizome and root rot diseases. 11. *In vitro* screening of chemicals against rhizome and root rot pathogens. 12. Pot culture trials to evaluate shortlisted fungicides against rhizome and root rot pathogens.
2.	Dr. Biju,C.N. Scientist, Regional Station, Appangala ICAR-Indian Institute of Spices Research, Kozhikode.	Co-PI	25% (3 man months)	1. Survey in the major cardamom growing areas of Karnataka, Kerala and Tamil Nadu. 2. Surveys in the hot spots to study the temporal variation of pathogens. 3. Isolation of pathogens associated with rhizome - root rot diseases. 4. Molecular characterization of the pathogens and antagonists. 5. *In vitro* screening of chemicals against rhizome and root rot pathogens. 6. Pot culture trials to evaluate shortlisted fungicides against rhizome and root rot pathogens.

7. Priority Area :Diagnosis and management

8. Project Duration: Date of Start: 01.04.2010 Date of Completion:31.03.2015

(a)Objectives:

- To collect, identify and characterize rhizome - root rot pathogens infecting cardamom by employing morphological and molecular tools.
- To study the diversity of rhizome - root rot pathogens and their antagonists.
- To evaluate potential biocontrol agents against rhizome - root rot pathogens.

(b) Practical Utility:

Identification of primary causal organism of rhizome - root rot diseases which will help in formulating integrated disease management strategies. Temporal variation studies of rhizome rot pathogens will also help to develop specific management schedules basedon the occurrence of pathogen during particular season. The potential antagonists and chemicals identified can be integrated in the disease management strategy against rhizome and root rot pathogens.

9. Final Report of the Project

(a) Materials and Methods :

- **Survey in the major cardamom growing areas of Karnataka, Kerala and Tamil Nadu. Isolation of pathogens associated with rhizome - root rot diseases. Pathogenicity of the pathogens on susceptible cultivar/ variety.**

Surveys were conducted in the major cardamom growing areas of Kerala, Karnataka and Tamil Nadu and rhizome and root rot infected cardamom samples were collected representing different geographical locations. The rhizome and root rot pathogens were isolated from the infected samples using standard isolation procedures on potato dextrose agar (PDA) medium supplemented with streptomycin sulphate, under aseptic conditions. The pathogen cultures were maintained on PDA medium amended with streptomycin sulphate to prevent bacterial contamination and used for further studies. Pathogenicity studies of organisms isolated were conducted on susceptible variety of cardamom, Appangala 1.

- **Surveys in the hot spots to study the temporal variation of pathogens.**

During the surveys, locations with severe rhizome rot incidence were identified as hotspots of the disease. These hotspots were surveyed during subsequent years to study the temporal variation in the occurrence of rhizome and root rot pathogens.

- **Morphological and molecular characterization of the pathogens and antagonists.**

Characterization of the isolates was done by employing morphological and molecular methods. The morphological characteristics of pure cultures of rhizome and root rot pathogens were studied at the temperature range of 21 – 25^{O}C for seven days. Observations on morphological characteristics like colony colour, colony diameter, Hyphal characters, sclerotial formation etc were recorded. For molecular characterization,100mg of mycelium of fungus was ground into a fine powder using pestle and mortar.This was transferred to a clean Eppendorf tube and 1ml of 2% CTAB extraction buffer was added.The sample was then incubated at 60^{0}C for 1h with occasional mixing by gentle swirling. The mixture was centrifuged at 12,000rpm at room temperature.The aqueous phase was transferred to a new tube and added equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and mixed well by inversion.The mixture wascentrifuged at 12,000rpm at room temperature.Then the aqueous phase was transferred to a new tube, added RNase-A (10mg/ml) and incubated at 37^{o}C for 30 min. Then added equal volume of chloroform:Isoamyl alcohol (24:1) to the same tube and mixed by inversion (10 min).The mixture was centrifuged at 15,000 rpm for 10 min at room temperature. The aqueous phase was transferred to a clean tube with the use of a pipette. 0.6 volume of isopropanol was added and mixed by gentle inversion and incubated for 1h30 min at room temperature. The sample was centrifuged at 10,000 rpm for 10 min. The pellet was washed with 70% chilled ethanol to remove the salts, air dried and 50ml of TE buffer was used to dissolve the pellet.

- **Isolation of the antagonists from cardamom growing areas. *In vitro* evaluation of biocontrol agents against selected isolates of rhizome and root –rot pathogens. Evaluation of effective biocontrol agents against selected isolates of rhizome and root –rot pathogens under glass house conditions.**

Surveys were conducted in the major cardamom growing areas of Kerala,Karnataka

and Tamil Nadu. Soil samples were collected from fields with healthy cardamom plants. Antagonists were isolated from soils by following specific isolation procedure. The potential isolates of *Trichoderma*were identified by assessing their antagonistic potential against the rhizome and root rot pathogens (*Pythium vexans, Rhizoctonia solani*and *Fusarium oxysporum*) under *in vitro* conditions using dual culture technique. The selected isolates of *Trichoderma* were further evaluated against rhizome rot pathogens under glass house and nursery conditions.

- **Sequential inoculation studies to identify the primary causal pathogen of rhizome/root rot diseases.**

(I) *R. solani* and *F. oxysporum*:Fresh young roots of small cardamom (variety: - Appangala 1) were collected and surface sterilized with 1% sodium hypochlorite solution. The roots were then rinsed with sterile water for 3-4 washings. These roots were placed in a glass Petri dish (15 cm diameter) lined with wet filter paper at room temperature. Mycelial discs from 5 day old culture of respective pathogens were placed on the surface sterilized root surface with the mycelial side facing the root, under high humid conditions. The roots were observed and sections were taken at every 2 hour (h) interval until 12 h and further at 16, 20, 24, 36, 48, 72, 96 and 120 h. The sections were stained in lactophenol cotton blue solution and observed under microscope.Observations on pre and post-penetration events were recorded.

(II) *P. vexans* :Fresh young root system of cardamom (variety:- Appangala 1) was collected and sterilized with 1% sodium hypochlorite solution. The roots were then rinsed with sterile water for 3-4 washings. These roots were placed inside beakers filled with sterile distilled water covered with parafilm and kept under room temperature (22-24^{o}C). Mycelial discs from 5 day old culture of the pathogen were placed on the surface of water, under high humid conditions. The roots were observed and sampled at every two hour (h) interval until 12 h and further at 16, 20, 24, 36, 48, 72, 96 and 120 h. Mycelial discs were also placed on the disinfected roots kept inside glass Petri dish (15 cm diameter) with the mycelial side

facing the root, under high humid conditions.

Glass house conditions**:-** In order to identify the primary causal pathogen of rhizome-root rot diseases, sequential inoculation studies with the pathogens *viz*., *P. vexans,R. solani* and *F. oxysporum* individually and in various combinations by employing two methods (with and without injury) were carried out on cardamom seedlings (Variety: Appangala 1) under glass house conditions.

Pot culture trials:-In order to identify the primary causal pathogen of rhizome-root rot diseases, sequential inoculation studies with the pathogens *viz*., *P. vexansR. solani* and *F. oxysporum* individually and in various combinations by employing two methods (with and without injury) were carried out on cardamom suckers (Variety: Appangala 1) under pot culture conditions.

- ***In vitro*** **screening of the chemicals against rhizome-root rots pathogens. Pot culture trials to evaluate shortlisted fungicides against rhizome and root rot pathogens.**

Sensitivity of rhizome-root rots pathogenswere tested against contact, systemic and combination fungicides *viz*., carbendazim + mancozeb, Fenamidone + Mancozeb, Isoprothiolane, Carbendazim, Hexaconazole + Zineb, Tebuconazoleand Kresoxim-methyl at recommended dosages under *in vitro* conditions by employing poisoned food technique. Fungicidal suspensions were prepared by dissolving requisite quantities of each fungicide in warm PDA. About 20 ml of the medium was poured into Petridishes and medium without fungicide served as control. Mycelial discs (5 mm diameter) from the advanced margin of five day old culture of *P. vexans, R. solani* and *F. oxysporum* were placed at the center of each Petri dish and each treatment was replicated thrice. The plates were incubated at 25^{o}C and observations on radial growth of the colony were recorded seven days after the incubation period.

The percent inhibition of the colony growth was calculated using the formula:

$$\frac{C - T}{C} \times 100$$

Where C = Growth of culture in control plate, T = Growth of culture in fungicide treated plate.

The most effective fungicides were tested against the pathogens under pot culture conditions using the suckers of cardamom variety, Appangala 1.

Data analysis

The *in vitro* experiments were laid out in completely randomized design (CRD) and the data recorded in percent were transformed to arc sine transformation. The transformed data were statistically analyzed using the software package AGRES version 7.01.

(b) Results and Discussion :

- **Survey in the major cardamom growing areas of Karnataka, Kerala and Tamil Nadu.** Surveys were carried out in Wayanad and Idukki districts of Kerala, Valparai in Tamil Nadu and Hassan and Kodagu districts of Karnataka. Thirty five locations were surveyed in which, Meppadi panchayat in Wayanad district was identified as a hot spot.
- **Isolation of pathogens associated with rhizome - root rot diseases.**

  Eighty five isolates of fungi were isolated from rhizome (Plate 1a) and root rot (Plate 1b) affected samples. The fungi included *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium* spp, *Colletotrichum* sp, *Pythium vexans*, *Botryodiplodia theobromae* and unidentified cultures (Plate 2a, b, c). Among the fungi isolated, *Pythium vexans, Rhizoctonia solani* and *Fusarium* species were found to be dominant. Prasath and Venugopal (2001) reported that the incidence of rhizome rot in all plantations of Kodagu district, Karnataka ranged from 0.2% to 47% .The severity of the disease was more in plantations located in high rainfall areas and also under irrigated conditions. Survey showed consistent association of *P. vexans*, *Fusarium* sp and *R. solani.*

Plate 1a Rhizome rot **Plate 1b Root rot**

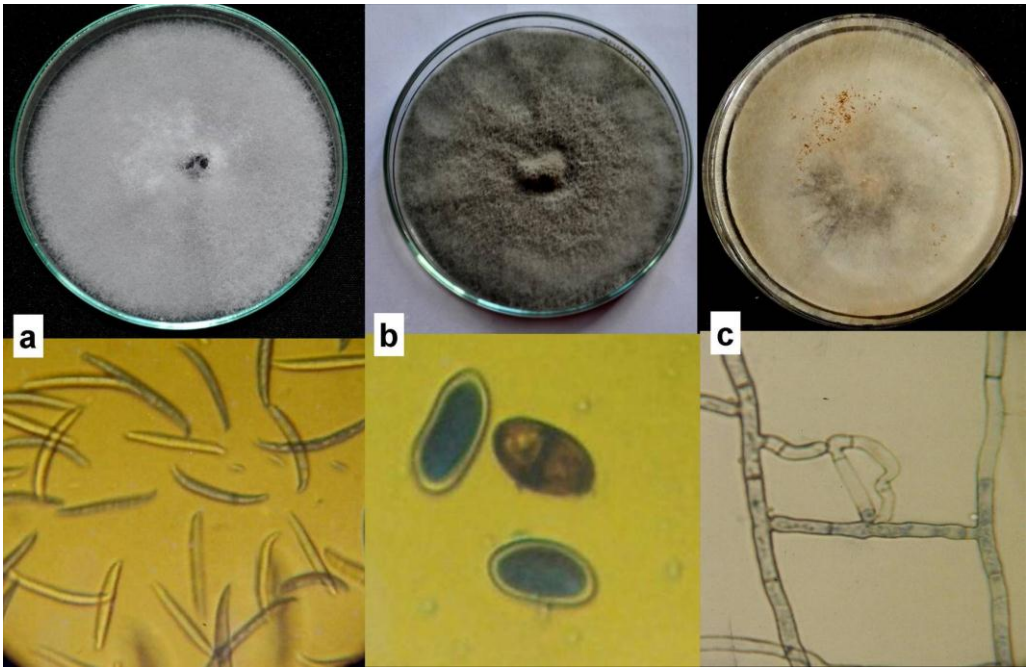


Plate 2a*Fusarium*2b *Botryodiplodia*2c *Rhizoctonia*

- **Pathogenicity of the pathogens on susceptible cultivar/ variety.**

Pathogenicity of 10 *Fusarium* isolates was tested on Appangala 1. (Table 1 and Plate 3). Among the isolates, Appangala isolate caused complete wilting of the seedling within six days after inoculation. Pathogenicity studies of 8 *Rhizoctonia* isolates were also carried out on Appangala 1 seedlings (Table 2).Pathogenicity trials were carried on the cardamom variety, Appangala 1 showed that the Appangala isolate of *Fusarium* and *R. solani* was the most virulent.

Table 1: Pathogenicity of *Fusarium* isolates on Appangala 1 seedlings

Isolate Code	Rhizome/Root	Location	Symptoms	Conidia
APR-2	Rhizome	Kodagu, Karnataka	Complete wilting and severe rotting	Very less macroconidia (2-3 Septa) and microconidia
VPR-2	Rhizome	Valparai, Tamil Nadu	Severe rotting, flaccidity of leaves	More macroconidia (5 septa)
BGR-1	Rhizome	Kodagu, Karnataka	Moderate wilting, Leaves flaccid	Microconidia and less macroconidia(3septa)
PYR-2	Rhizome	Kodagu, Karnataka	Complete wilting, flaccidity of leaves	Microconidia
BTR-1	Root	Kodagu, Karnataka	Mild rotting, no leaf symptom	Microconidia
SKR-1	Root	Sakleshpur, Karnataka	Mild rotting, no leaf symptom	Microconidia and Macroconidia (2-3 septa)
WYR-3	Root	Wayanad, Kerala	Moderate rotting, Leaves drying and flaccid	Small size macroconidia (1-2 septa) and microconidia
WYR-10	Root	Wayanad, Kerala	Moderate rotting, leaves drying and flaccid	Microconidia and less macroconidia (3 septa)
IDK-5	Root	Idukki, Kerala	Moderate rotting, and leaves flaccid	Macroconidia (4 septa)
IDK-6	Root	Idukki, Kerala	Mild rotting, No	Microconidia

			flaccidity	

Table 2: Pathogenicity of *Rhizoctonia* isolates on Appangala 1 seedlings

Isolate code	Rhizome/Root	Location	Symptoms
APR-1	Rhizome	Kodagu, Karnataka	Complete wilting and severe rotting
APR-3	Rhizome	Kodagu, Karnataka	Severe rotting, flaccidity of leaves
WYR-1	Rhizome	Wayanad, Kerala	Mild rotting, no leaf symptom
CHR-1	Rhizome	Kodagu, Karnataka	Mild rotting, no leaf symptom
CHR-2	Rhizome	Kodagu, Karnataka	Moderate rotting, Leaves flaccid
CHR-3	Rhizome	Kodagu, Karnataka	Mild rotting, no leaf symptom
IDK-4	Rhizome	Idukki, Kerala	Moderate rotting, Leaves drying and flaccid
SPR-3	Rhizome	Kodagu, Karnataka	Moderate rotting, leaves drying and flaccid

- **Surveys in the hot spots to study the temporal variation of pathogens.**

Surveys were repeated in Wayanad and Idukki districts of Kerala, Hassan and Kodagu districts of Karnataka to study the seasonal variation of rhizome and root rot diseases. Among the fungi isolated, *P. vexans, R. solani* and *Fusarium* species were found to be dominant and associated with rhizome rot disease. The root rot diseases were found associated with different species of *Fusarium viz*., *F. oxysporum, F. solani, F. pallidoroseum* and *F. verticillioides*, of which, *F. oxysporum* was the predominant species. Artificial inoculation studies proved that among the different fungi isolated, *P. vexans*, *R. solani* and *F.oxysporum* were found to be pathogenic to cardamom. Incidence and severity of rhizome rot disease was higher in Meppadi panchayat of Kerala and Appangala and Kadagudalu areas of Karnataka. Surveillance and isolation from diseased samples showed that, *P. vexans* and *R. solani* were present during the period August - September and June –

October respectively whereas *Fusarium oxysporum* showed continuous association with cardamom from May – January.

- **Isolation of the antagonists from cardamom growing areas.**

Surveys were conducted in the major cardamom growing areas of Kerala, Karnataka and Tamil Nadu and soil samples were collected from rhizosphere of healthy cardamom. The samples were serially diluted and plated in Rose Bengal Agar and Nutrient agar media. Forty five isolates of *Trichoderma* spp (Idukki – 6, Wayanad – 13, Valparai – 5, Kodagu- – 17 and Hassan – 4) were isolated from the soil samples.

- **Morphological and molecular characterization of the pathogens and antagonists.**

Morphological characterization of 10 *Fusarium* (Plate 4) and 8 *Rhizoctonia* isolates was completed. Different species of *Fusarium* isolated were identified as *F. oxysporum, F. solani, F. Pallidoroseum* and *F. verticillioides.* Vijayan (2009) surveyed in the high ranges of Idukki district and collected 20 different isolates of *F. oxysporum*. RAPD analysis of five isolates showed that the variability between the isolates is moderate. *Rhizoctonia* isolates from all the locations surveyed were identified as *R. solani only*.45 isolates of *Trichoderma* [20 isolates each from Karnataka and Kerala and 5 isolate from Tamil Nadu] have been characterized based on colony morphology, sporulation, colour of spores and colouration of media (Plate 5).

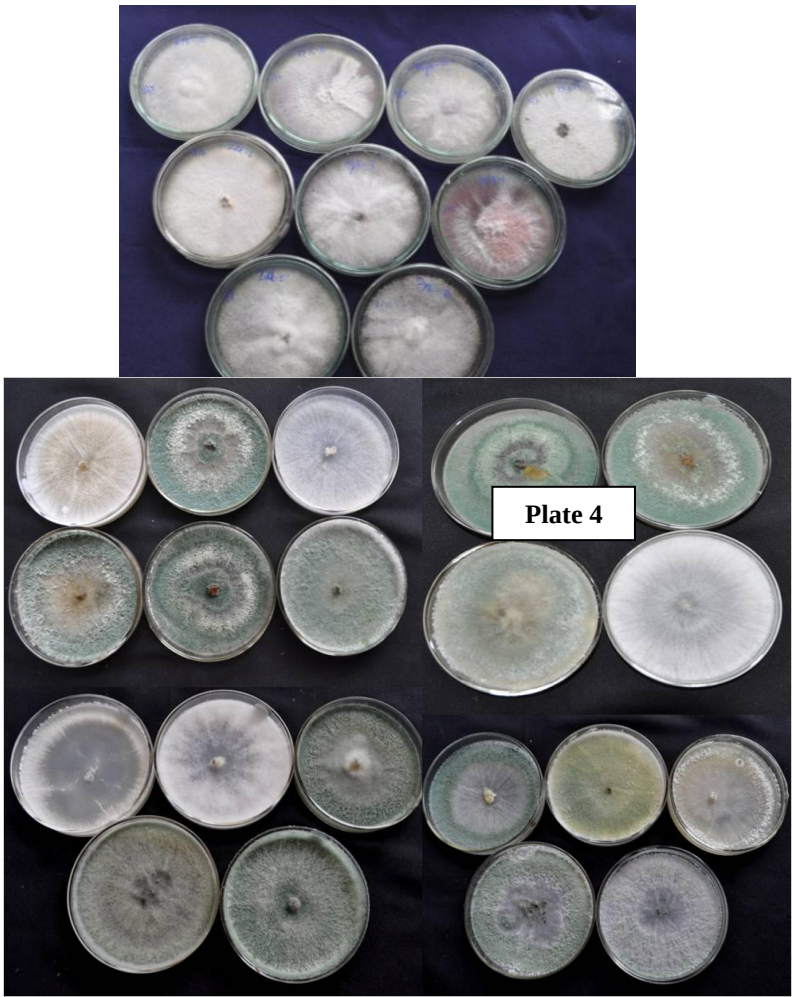


Plate 4

Plate 5

The methodology for molecular characterization of *Trichoderma* and *Fusarium* isolates were standardised. The shortlisted efficacious *Trichoderma* isolates KA-1, KA-3, KA-20 (Karnataka), KL-3, KL-10, KL-13, KL-17, KL-19 (Kerala) and TN-3 (Tamil Nadu) were characterized based on ITS r-DNA sequencing.

- **Sequential inoculation studies to identify the primary causal pathogen of rhizome/root rot diseases.**

In order to identify the primary causal pathogen of rhizome/root rot diseases, sequential inoculation studies with the pathogens *viz*., *P. vexansR. solani* and *F. oxysporum* individually and in various combinations by employing two methods (with and without injury) were carried out on cardamom seedlings (Variety: Appangala 1) under glass house conditions. Among the individual pathogen treatments, plants treated with *P. vexans* alone showed the 66.67 percent mortality of seedlings (Plate 6).When fungi were sequentially inoculated the treatment *P. vexans* followed by *R. solani* inoculation recorded 83.33% mortality of plants. Even though the plants treated with *F. oxysporum* showed severe root damage (Plate 7), new root initiation was also observed in affected plants (Plate 8) Preliminary observations of sequential inoculation studies revealed that *P. vexans* and *R. solani* are primarily involved in rhizome rot infection.

Plate6

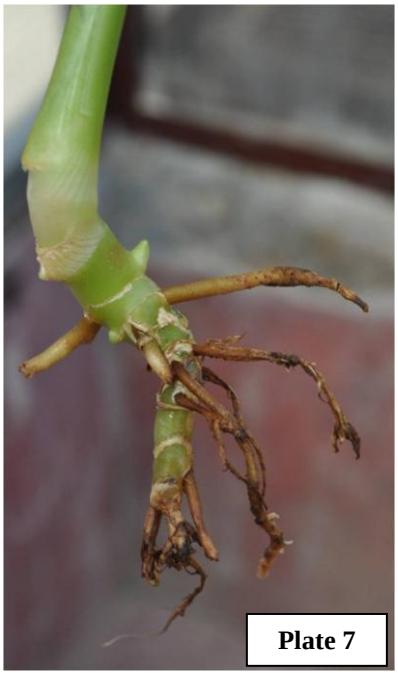


Plate 7

Plate 8

Infection process studies

Studies on infection process of *P. vexans,R. solani* and *F. oxysporum* showed that, *P. vexans* required only 4 hours to colonize the roots (Plate 9), whereas *R. solani* and *F. oxysporum* took 12 and 96 hours respectively (Plate 10, 11). Under high moisture conditions oospores of *P. vexans* were formed in large numbers and aggregated near the root tip region. Then the fungus entered the host system through the root tip, proliferated, causing rapid collapse and death of the rootlets.

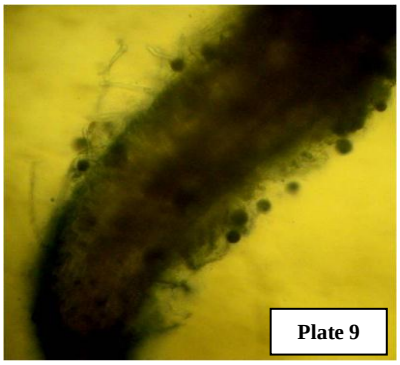


Plate 9

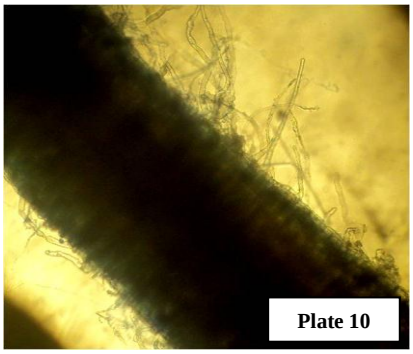


Plate 10

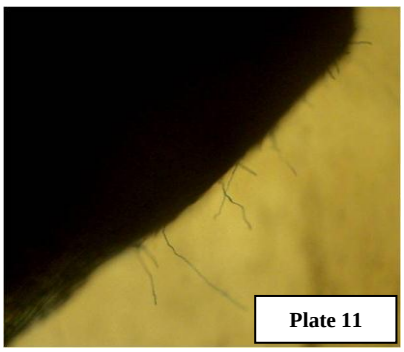


Plate 11

The fungus *P. vexans* produced unornamented spherical yellowish brown oospores which germinated directly by a germ tube at higher temperatures (> 22^0C) (Plate 12).

However at lower temperatures of 15-19^{0}C, the oospores were found to act as zoosporangia and formed vesicles in which zoospore differentiation took place (Plate 13). The process of vesicle formation, differentiation of zoospores, breakage of vesicle and zoospore liberation occurred within 15-18 minutes.

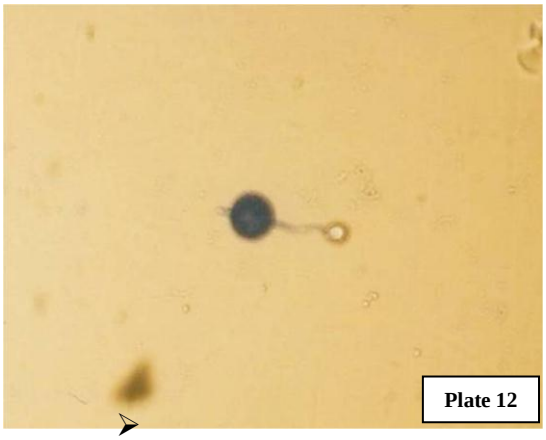


Plate 12

Plate 13

- **<u>Pot culture trials to identify the primary causal pathogen of rhizome/root rot diseases.</u>**

  In order to identify the primary causal pathogen of rhizome-root rot diseases, sequential inoculation studies with the pathogens *viz*., *P. vexans, R. solani* and *F. oxysporum* individually and in various combinations by employing two methods (with and without injury) were carried out on cardamom suckers (Variety: Appangala 1) under pot culture conditions. Pot culture trials using suckers proved that *R. solani* and *P. vexans* were primarily involved in rhizome rot diseases while *F. oxysporum* caused root rot in cardamom. Among the individual pathogen treatments, pots treated with *R. solani* alone showed the 100 per cent incidence of rhizome rot disease whereas *P. vexans* alone showed the 88.88 per cent incidence of rhizome rot disease. The pots treated with *F. oxysporum* did not show rhizome rot butshowed severe root damage in all treated pots. The pot culture experiment on inoculation studies revealed that, *R. solani* and *P. vexans*were primarily involved in rhizome rot infection (Plate 14 a, b,c,d).

Plate 14 a Control

Plate 14 b *Rhizoctonia solani* treated pots

Plate 14 c *Pythium vexans* treated pots

Plate 14 d *Fusarium oxysporum* treated pots

***In vitro* evaluation of biocontrol agents against selected isolates of rhizome and root –rot pathogens.**

Under *in vitro* conditions, nine isolates of *Trichoderma* namely, KA-1, KA-3, KA-20 (Karnataka), KL-3, KL-10, KL-13, KL-17, KL-19 (Kerala) and TN-3 (Tamil Nadu) were effective against *P. vexans* (21.48 – 67.77 %) (Plate 15), *R. solani* (44.44 – 60.74 %) (Plate 16) and *F. oxysporum* (49.62 – 77.40 %) (Table 3, 4, 5) .The shortlisted efficacious *Trichoderma* isolates were evaluated against *P. vexans*, *R. solani*and *F. oxysporum* under glass house conditions.

Table 3: *In vitro* evaluation of *Trichoderma* (Karnataka isolates) against rhizome and root rot pathogens of cardamom

***Trichoderma* Isolates**	***Rhizoctonia solani***	***Fusarium oxysporum***	***Pythium vexans***
KA-1	44.44	65.19	21.48
KA-2	44.81	62.22	27.4
KA-3	58.51	65.93	53.7
KA-4	43.33	57.78	42.22
KA-5	46.66	54.81	25.18

KA-6	44.44	50.00	22.59
KA-7	56.66	55.16	25.55
KA-8	49.62	55.56	28.14
KA-9	48.14	48.89	43.70
KA-10	47.77	57.41	37.77
KA-11	43.33	51.85	41.11
KA-12	44.44	55.93	34.81
KA-13	44.44	58.89	34.81
KA-14	44.44	52.96	41.85
KA-15	43.70	64.07	41.11
KA-16	46.29	51.85	41.85
KA-17	48.88	54.07	33.7
KA-18	48.13	50.74	36.29
KA-19	48.88	50.74	24.07
KA-20	56.66	59.63	67.77
CD (0.05)	3.99	4.10	3.98
CV (%)	5.54	5.08	6.55

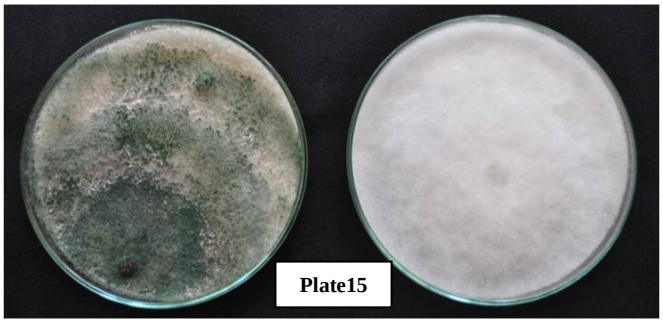
Table 4: *In vitro* evaluation of *Trichoderma* (Kerala isolates) against rhizome and root rot pathogens of cardamom

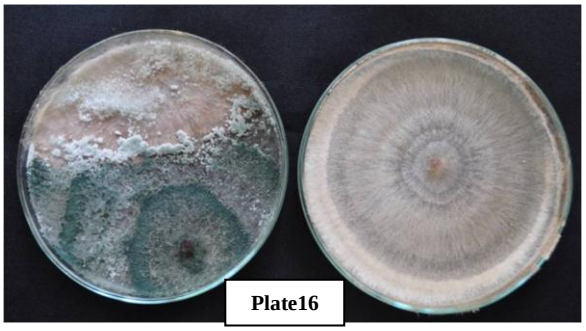
Trichoderma Isolates	*Rhizoctonia solani*	*Fusarium oxysporum*	*Pythium vexans*
KL-1	51.85	50.37	45.92
KL-2	46.66	64.44	47.77
KL-3	58.14	64.81	55.18
KL-4	51.85	68.14	49.26
KL-5	51.11	65.92	44.44
KL-6	51.48	54.44	49.62
KL-7	50.74	64.07	47.77
KL-8	48.14	55.18	52.96
KL-9	49.99	66.66	44.44
KL-10	57.03	52.96	52.59
KL-11	49.63	55.55	46.29
KL-12	49.63	68.14	48.15
KL-13	50.00	77.4	40.00
KL-14	49.63	52.22	45.18
KL-15	49.25	49.99	42.59
KL-16	49.26	68.88	45.18
KL-17	60.74	49.62	41.85
KL-18	49.25	58.88	49.25

KL-19	56.29	76.29	44.07
KL-20	46.66	49.99	47.40
CD (0.05)	3.09	1.28	5.05
CV (%)	4.11	1.51	7.03

Table 5 : *In vitro* evaluation of *Trichoderma* (Tamil Nadu isolates) against rhizome and root rot pathogens of cardamom

Trichoderma Isolates	*Rhizoctonia solani*	*Fusarium oxysporum*	*Pythium vexans*
TN-1	48.88	55.93	28.51
TN-2	49.26	56.30	23.33
TN-3	57.03	70.37	48.51
TN-4	48.14	54.81	35.55
TN-5	49.99	62.22	37.77
CD (0.05)	NS	1.86	3.01
CV (%)	-	2.01	4.45





- **Evaluation of effective biocontrol agents against selected isolates of rhizome and root –rot pathogens under glass house conditions.**

The shortlisted nine efficacious *Trichoderma* isolates were evaluated against *P. vexans*, *R. solani* and *F. oxysporum* under glass house conditions. Under glass house conditions, the isolate KA-3 was found to be the most effective isolate against *P. vexans* and *F. oxysporum*whereas KA-20 was effective in against *R. solani.*

Trichoderma* isolate (KA-3) against *Pythium vexans

Trichoderma* isolate (KA-20) against *Rhizoctonia solani

Trichoderma* isolate (KA-3) against *Fusarium oxysporum

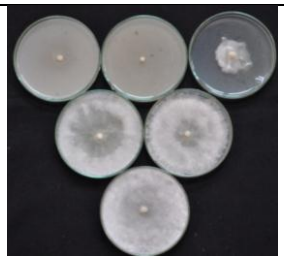
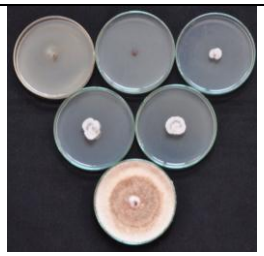
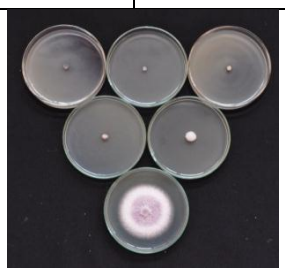
Thomas *et al*., (1991) reported that, recent attempts in the management of rhizome rot is by the use of *Trichoderma* sp. Application of biocontrol agents such as *Trichoderma harzianum* and *T. viride* were reported to reduce incidence of rhizome rot.

The seedling mortality caused by *P. vexans* and *R. solani* was reduced to the extent of 55-61% by the application of *T .harzianum* mass cultured on coffee husk medium. Soil drenching of *Trichoderma* sp. one week prior to transplanting of seedlings was effective in reducing the seedling mortality compared to *Trichoderma* application after transplanting or as seedling dip (Suseela and Thomas, 1998). In the present study also, application of *Trichoderma* to soil before transplanting was effective in reducing rot incidence and it also increased the total biomass of cardamom seedlings compared to control plants.

- ***In vitro* screening of the chemicals against rhizome-root rots pathogens.**

Selected chemicals *viz*., fenamidone +mancozeb, copper oxychloride, metalaxyl +mancozeb, captan + hexaconazole, carbendazim + mancozeb, isoprothiolane, carbendazim, hexaconazole + zineb, tebuconazole, kresoxim-methyl, copper hydroxide and chlorothalonil were evaluated against *P. vexans*, *R. solani* and *Fusarium* spp under *in vitro* conditions. Among the seven fungicides tested against *P. vexans,* fenamidone + mancozeb (0.2%) and captan + hexaconazole (0.2%) were effective under *in vitro* conditions. Fenamidone + mancozeb (0.2%) and tebuconazole (0.05%) were effective against *R. solani* whereas, tebuconazole (0.05%) was superior over other fungicides against *F. oxysporum*, under laboratory conditions (Table 6).

<table>
<tr><td rowspan="2">Table 6

Pythium vexans</td><td>  </td><td>  </td></tr>
<tr><td>Fenamidone + mancozeb</td><td>Captan + hexaconazole</td></tr>
<tr><td rowspan="2">Rhizoctonia solani</td><td>  </td><td>  </td></tr>
<tr><td>Fenamidone + mancozeb</td><td>Tebuconazole</td></tr>
<tr><td rowspan="2">Fusarium oxysporum</td><td colspan="2">  </td></tr>
<tr><td colspan="2">Tebuconazole</td></tr>
</table>

- **Pot culture trials to evaluate shortlisted fungicides against rhizome and root rot pathogens.**

The suckers of cardamom (variety: Appangala 1) were established in pots to evaluate shortlisted fungicides, fenamidone + mancozeb (0.2%), captan + hexaconazole (0.2%) and tebuconazole (0.05%) against *P. vexans*, *R. solani* and *F. oxysporum*. Among the three fungicides tested, tebuconazole (0.05%) was effective against *R. solani* and *F. oxysporum*, whereas, the tested fungicides were not effective against *P. vexans.*

(c) Objective-wise Achievements:

Survey in the major cardamom growing areas of Karnataka, Kerala and Tamil Nadu.	Surveys were carried out in Wayanad and Idukki districts of Kerala, Valparai in Tamil Nadu and Hassan and Kodagu districts of Karnataka. Incidence and severity of rhizome rot disease was higher in Meppadi panchayat of Kerala and Appangala and Kadagudalu areas of Karnataka.

Isolation of pathogens associated with rhizome - root rot diseases.	Eighty five fungal isolates were isolated from the samples of rhizome and root rot disease, of which *R. solani*, *P. vexans* and *Fusarium* species were found to be dominant.
Survey in the hot spots to study the temporal variation of pathogens.	*R. solani*, *P. vexans* and *Fusarium* spp were found to be associated with rhizome rot disease of cardamom. Of the different species of *Fusarium*, *F. oxysporum* was found to be the predominant species. Identified the primary causal pathogen of rhizome/root rot diseases by sequential inoculation studies.
Isolation of the antagonists.	45 isolates of *Trichoderma* sp were isolated from the soil samples collected from Karnataka, Kerala and Tamil Nadu.
Morphological characterization of pathogens and antagonists.	45 isolates of *Trichoderma, Fusarium* and *Rhizoctonia* isolates have been characterized based on morphological characters. Different species of *Fusarium* isolated were identified as *F. oxysporum, F. solani, F. pallidoroseum* and *F. verticillioides.* *Rhizoctonia* isolates from all the locations surveyed were identified as *R. solani*.
Molecular characterization of pathogens and antagonists.	Protocols for DNA isolation of *F. oxysporum* and *Trichoderma* have been standardized. The shortlisted efficacious *Trichoderma* isolates KA-1, KA-3, KA-20 (Karnataka), KL-3, KL-10, KL-13, KL-17, KL-19 (Kerala) and TN-3 (Tamil Nadu) were characterized based on ITS r-DNA sequencing.
*In vitro*screening of antagonists against rhizome/ root rot pathogens.	Under *in vitro* conditions, nine isolates of *Trichoderma* namely, KA-1, KA-3, KA-20 (Karnataka), KL-3, KL-10, KL-13, KL-17, KL-19 (Kerala) and TN-3 (Tamil Nadu) were effective against *P. vexans* (21.48 – 67.77%),*R. solani* (44.44 – 60.74%) and *F. oxysporum* (49.62 – 77.40%).
In vitro screening of chemicals against rhizome/root rot pathogens.	Among the seven fungicides tested, *P. vexans* - carboxin + thiram (0.2%) and captan + hexaconazole (0.2%), *R. solani*- carboxin + thiram (0.2%) and tebuconazole (0.05%) *F. oxysporum* - *tebuconazole* (0.05%) were effective under *in vitro* conditions.

Evaluation of potential antagonists under glass house.	Among the shortlisted nine efficacious *Trichoderma isolates,* the isolates KA-3 and KA-20 were effectiveagainst *P. vexans* and *F.oxysporum* and *R. solani respectively* under green house conditions.
Evaluation of chemicals under glass house.	Among the three fungicides tested, tebuconazole (0.05%) was effective against *R. solani* and *F. oxysporum*, under glass house conditions, whereas the tested fungicides were not effective against *P. vexans.* The average per disease incidence in *R. solani and F.oxysporum* inoculated pots treated with tebuconazole was 11.59 &10.50 per cent respectively.

(d) Conclusions:

Surveys were carried out in Wayanad and Idukki districts of Kerala, Valparai in Tamil Nadu and Hassan and Kodagu districts of Karnataka and Incidence of rhizome rot disease was higher in Meppadi panchayat of Kerala and Appangala and Kadagudalu areas of Karnataka. *R. solani*, *Pythium vexans* and *Fusarium* spp were found to be associated with rhizome rot disease of cardamom. Of the different species of *Fusarium, F. oxysporum* was found to be the predominant species. 45 isolates of *Trichoderma, Fusarium* and *Rhizoctonia* isolates were characterized based on morphological characters.Protocols for DNA isolation of *F. oxysporum* and *Trichoderma* have been standardized and the shortlisted efficacious *Trichoderma* isolates KA-1, KA-3, KA-20 (Karnataka), KL-3, KL-10, KL-13, KL-17, KL-19 (Kerala) and TN-3 (Tamil Nadu) were characterized based on ITS r-DNA sequencing. Under *in vitro* conditions, out of the 45 isolates, nine isolates of *Trichoderma* namely, KA-1, KA-3, KA-20 (Karnataka), KL-3, KL-10, KL-13, KL-17, KL-19 (Kerala) and TN-3 (Tamil Nadu) were effective against *P.vexans* ,*R. solani* and *F. oxysporum*.Among the shortlisted nine efficacious *Trichoderma isolates,* KA-3 and KA-20 were effective under green house conditions *against P. vexans* and *F.oxysporum* and *R. solani* respectively.Among the seven fungicides tested, 3 newer molecules were found effective against *P. vexans* (carboxin + thiram and captan + hexaconazole),*R. solani*- (carboxin + thiram and tebuconazole) ,*F. oxysporum* – (tebuconazole) under *in vitro* conditions.The three fungicides selected were further tested under pot culture studies and the fungicide tebuconazole (0.05%) was effective against *R. solani* and *F. oxysporum*, whereas the tested fungicides were not effective against *P. vexans.*

10. Financial Implications (Lakh Rs.): 18.212

11.1 Expenditure on

(a) Manpower :11,01,200

(b) Research/Recurring Contingencies :5,80,000

(c) Non-Recurring Cost (Including Cost of Equipment) :Nil

(d) Any Other Expenditure

incurred (Travel) : 1,40,000

11.2 Total Expenditure: 18,21,200

12. Cumulative Output :

(a) Special Attainments/Innovations :

Identified the primary causal organism of the rhizome rot disease.

(b) List of publications (1 copy each to be submitted) :

i. Research Papers

ii. Reports/Manuals

iii. Working and ConceptPapers

iv. Popular Articles:

- **Praveena, R.,**Biju, C. N. and Ankegowda, S. J. (2012) Strategies to curtail fungal diseases in cardamom plantations. *Spice India.* 25 (11): 20 – 23.
- **Praveena, R**. and Biju, C.N. 2012, **Pests of cardamom**, In: SADARAM-Cardamom, Sasikumar, B., Dinesh, R. and Anandaraj, M. (eds.), published by IISR, Kozhikode, pp 71-88.

v. Books/Book Chapters

- Kumar, A., C.N. Biju and **R. Praveena.** 2012. Diseases of Cardamom, Ginger, Turmeric and Others. In: Zingiberaceae Crops: Present and Future, [Singh, H.P., V.A. Parthasarathy, K. Kandiannan, K.S. Krishnamurthy (Eds.)] Westville Publishing House, New Delhi. pp. 288-331.

vi. Extension Bulletins

1. Ankegowda, S.J.,Senthil Kumar,R.,Prasath, D.,**Praveena, R.** and Biju, C.N. 2012, Technical Bulletin on Propagation techniques in Cardamom. p 1-13.

(c).Intellectual Property Generation(Patents: filed/obtained; Copyrights: filed/obtained; Designs: filed/obtained; Registration details of variety/germplasm/accession) :

(d) Presentation in Workshop/Seminars/Symposia/Conferences
(relevant to the project) :

1. **Praveena, R**. and C.N. Biju. 2012, "Diversity of rhizome and root rot pathogens in cardamom".*(Abstracts presented in "National symposium on Blending conventional and Modern Plant Pathology for Sustainable Agriculture", Organized by IIHR and Indian Phytopathological Society at IIHR, Bangalore on 4-6th December.)*
2. **Praveena, R.,**Biju. C.N. and Sujatha, A.M., 2013, "Sequential events in the colonization and proliferation of rhizome and root rot pathogens in small cardamom"(ORAL) "National Symposium on Pathogenomics for Diagnosis and Management of Plant Diseases", 24-25 October, 2013, Organized by CTCRI and Indian Phytopathological Society at CTCRI, Thiruvananthapuram.
3. **Praveena, R.,**Biju. C.N. and Sujatha, A.M., 2013,"Geospatial diversity and ecological dynamics of rhizome and root rot pathogens in small cardamom".35th Annual Conference and Symposium of Indian Society of Mycology and Plant Pathology on "Innovative and Ecofriendly Research Approaches for Plant Disease Management" held at Dr Panjabrao Deshmukh KrishiVidyapeeth, Akola during 8-10th January, 2014.
4. **Praveena, R.** Biju. C.N. and Sujatha, A.M. Evaluation of *Trichoderma* isolates against rhizome and root rot pathogens in small cardamom.(Paper presented in the International Symposium on Plantation Crops (PLACROSYM XXI) hosted by ICAR – Indian Institute of Spices Research, Kozhikode, Kerala and Indian Council of Agricultural Research, New Delhi at Kozhikode during 10th – 12th, December, 2014).

(e) Details of technology developed(Crop-based; Animal-based, including vaccines; Biological-biofertilizer, biopesticide, etc; IT based-database, software; Any other-please specify) :

(f)Trainings/demonstrations organized: Nil

(g) Trainings received:

Winter School for 21 days on Viral Genomics and Transgenic Development	8th to 28th September,2010	Division of Plant Pathology, IARI, New Delhi
Short course on Plant Disease Diagnostics: Theory and Practices	4-13th, July 2012	Central Potato Research Institute, Shimla
Training programme on "PEST SURVEILLANCE".	October 3-10th, 2013.	National Institute of Plant Health Management (NIPHM), Hyderabad.
Short term training on "Genomics and proteomics of plants and microbes towards translational research".	21st January - 10th February, 2015	ICAR- Indian Institute of Spices Research, Kozhikode.

(h) Any other relevant information:

13. (a) Extent of achievement of objectives and outputs earmarked as per RPP-I :

Objective wise	Activity	Envisaged output of monitorable target(s)	Output achieved	Extent of achievement (%)
1. Diversity analysis of pathogens.	Collection, isolation, maintenance and identification of rhizome and root rot pathogens from different geographical locations of cardamom growing areas.	Collection, maintenance and identification of pathogens.	Collected and maintained the isolates of *R. solani*, *P. vexans* and *Fusarium* spp.	90
	Characterization and diversity analysis of isolates based on cultural, morphological, biological and molecular characters.	Characterization and diversity analysis of isolates.	Characterised the isolates of *R. solani*, *P. vexans* and *Fusarium* spp.	80
	Pathogenicity of isolates on susceptible cultivar.	Pathogenicity of isolates.	Tested the Pathogenicity of all fungi isolated from diseased samples	100
	Sequential inoculation studies to identify the primary causal pathogen of rhizome/root rot diseases.	Identification of the primary causal pathogen.	Observations of sequential inoculation studies revealed that *P. vexans* and *R. solani* are primarily involved in rhizome rot infection.	100
2. Diversity analysis of antagonists.	Collection, isolation and characterization of antagonists from cardamom growing areas of Kerala, Karnataka and Tamil Nadu.	Collection, isolation and characterization of antagonists.	Collected, characterized and maintained the isolates of *Trichoderma.*	80
3. Identification of hotspots.	Identification of hotspots of the disease.	Identification of hotspots.	Meppadi panchayat of Kerala and Appangala and Kadagudalu areas of Karnataka	90

			were Identified as hotspots	
	Study the temporal variation in the occurrence of pathogens in hot spots.	Temporal variation in the occurrence of pathogens.	*P. vexans* and *R. solani* were present during the period August - September and June – October respectively whereas *F.oxysporum* showed continuous association with cardamom from May – January.	90
4.Identification of potential antagonists	Identification of potential isolates of antagonists by assessing their antagonistic potential under *in vitro* conditions.	Identification of potential isolates of antagonists.	Nine isolates of *Trichoderma* were effective against *P. vexans*, *R. solani* and *F. oxysporum*.	85
	Evaluation of potential antagonists under pot culture condition.	Evaluation of potential antagonists.	Among the shortlisted efficacious *Trichoderma* isolates, twoisolates ,KA-3 and KA-20 were effective under pot culture conditions	90

(b) Reasons of shortfall, if any: Nil

14. Efforts made for commercialization/technology transfer :

During training programmes on cardamom disease management, the farmers were

educated about the disease occurrence, symptomatology and the specific chemicals used for rhizome rot disease management.

15. (a) How the output is proposed to be utilized?

The sequential inoculation studies revealed that, *P. vexans*, *R. solani* and *F.oxysporum* differed significantly with respect to the events involved in the infection process and pathogenesis. The fungi *P. vexans* and *R. solani* were primarily involved in causing rhizome rot and *F.oxysporum* in root rot diseases in cardamom. Understanding the processes involved in the sequential progression of pathogenesis would help in screening of cardamom genotypes for disease resistance. Identification of the primary causal organism will also help to develop disease management schedule for rhizome and root rot diseases with specific fungicide molecules. The most effective *Trichoderma* isolates can also be used for managing rot disease under nursery and field conditions.

(b)How it will help in knowledge creation?

The project helped to identify the primary causal organism of rhizome rot disease and also the processes involved in the sequential progression of pathogenesis. Surveillance of the disease showed that, the pathogens occurred during different periods. *P. vexans* and *R. solani* were present during August - September and June – October, respectively whereas *F. oxysporum* showed continuous association with cardamom from May – January. Based on the temporal variation of pathogens an integrated disease management schedule for different periods can be developed for rhizome and root rot diseases.

16. Expected benefits and economic impact(if any) : -

Identification of the primary causal organism will help to develop disease management schedule to manage rhizome and root rot diseases with specific fungicide molecules. Based on the occurrence of pathogens during different months, an integrated disease management schedule can be developed.

17. Future line of research work/other identifiable problems :

1. Field trials to evaluate the efficacy of most effective *Trichoderma* isolates and the identified new fungicide molecule, Tebuconazole against rhizome and root rot pathogens.
2. Development of an integrated disease management schedule for rhizome and root rot diseases based on the temporal variation of pathogens.

18. Details of research data (registers and records) of the project deposited with the institute :

Register No.141 and field record book (Maintained in the Division of Crop Protection)

19. Signature of :

PICo-PI

20. Signature of Head of Division :

21. Observations of PME Cell based on Evaluation of Research Project after completion :

22. Signature (with comments if any along with rating of the project in the scale of 1 to 10 on the overall quality of the work) of Director :