ECO- FRIENDLY MANAGEMENT OF *Fusarium* ROT IN CARDAMOM AND ITS IMPACT ON SOIL HEALTH AND PLANT DIFENSE MECHANISM

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by

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THESIS

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2021

DECLARATION

I, hereby declare that this thesis entitled **"Eco-friendly management of *Fusarium* rot in cardamom and its impact on soil health and plant defense mechanism"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Eco-friendly management of *Fusarium* rot in cardamom and its impact on soil health and plant defense mechanism"** is a record of research work done independently by Ms. Veny Krishna K.C under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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VENY KRISHNA K. C.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

@	At the rate of
B	Boron
Ca	Calcium
^{0}C	Degree Celsius
CD	Critical difference
cfu	Colony forming units
cm	Centimetre
CRD	Completely Randomised Design
DI	Disease Incidence
et al.	And other co-workers
Fig.	Figure
g	Gram
g L^{-1}	Gram Per litre
h	Hour (s)
hpi	Hours post infection
ha	Hectare
ha^{-1}	Per hectare
i.e.	That is
K	Potassium
KAU	Kerala Agricultural University
kg	Kilogram
kg ha^{-1}	Kilogram per hectare

km	Kilometre
L	Litre
L^{-1}	Per litre
Mg	Magnesium
mg	Milli gram
mL^{-1}	Per millilitre
mm	Milli metre
µm	Micro meter
N	Nitrogen
NS	Non –significant
No.	Number
%	Per cent
P	Phosphorus
PDA	Potato Dextrose Agar
$Plant^{-1}$	Per Plant
S	Sulphur
SE	Standard error
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
viz.	Namely
v/v	Volume/ volume

Introduction

1. INTRODUCTION

Cardamom, commonly known as “queen of spices”, is the third most valued spice crop in the world. In India, the Cardamom Hill Reserves (CHR) of Kerala’s Western Ghats is the main production home of this crop. Sustainable cardamom production in CHR is challenged by several diseases that cause quantitative and qualitative crop loss. Fusarium rot caused by *Fusarium oxysporum* Schlecht is one of the most widespread and important fungal diseases of small cardamom (Thomas and Vijayan, 2002).

F. oxysporum disease may become destructive at all growth stages of the crop under favourable weather conditions and can cause severe infection in this changing climate scenario. Dhanya *et al.* (2018) reported 50 per cent yield loss from poorly managed plants due to the disease. The pathogen survives in soil and crop residues for many years making the management of the pathogen very difficult.

The *Fusarium* sp. initially attacks the tiller producing pale discoloured lesions leading to dry rotting. The infected tillers are weakened at the point of attack resulting in partial breakage. These tillers bend down and hang from the point of infection (Josephrajkumar *et al.,* 2007). Symptoms include burnt appearance of panicles and rotting of roots from the tip causing yellowing of the plant and thereby reduction in the yield (Murugan *et al.*, 2016).

In the scenario of growing concerns over environment pollution and health hazards the need for an integrated approach in the disease management strategy is of high importance. The integrated disease management using various biocontrol agents and bio inputs improved the vegetative growth and the yield of the plant in addition to disease management of Fusarium rot of cardamom (Dhanya *et al*., 2018). The farmers are also adopting integrated approaches by using combinations of bioagents and organic inputs for combating various diseases.

The present study entitled as “Ecofriendly management of *Fusarium* rot in cardamom and its impacts on soil health and plant defence mechanism” was undertaken with an objective to assess the Fusarium rot severity in Idukki district, develop an effective eco-friendly management practices for the disease and to study the impact of

these practices on the soil and plant health. The study emphasized on the following aspects:

1. Symptomatology of the disease
2. Isolation of the pathogen and pathogenic studies
3. Cultural, morphological and pathological studies of the pathogen
4. Management of Fusarium rot with biocontrol agents
5. Post treatment analysis of plant and soil for primary, secondary and micro nutrients
6. Pre and post treatment studies on biochemical defense mechanisms in experimental plants

Review of literature

2. REVIEW OF LITERATURE

Fusarium infection in cardamom has been reported earlier in the form of capsule rot by Wilson and co-workers in 1979, Siddaramaiah (1988) also reported seed rot and seedling wilt in nurseries due to this pathogen. Incidence of Fusarium started as stem rot and finally resulted in partial breakage and lodging of pseudostem at the point of infection (Dhanapal and Thomas, 1996).

Fusarium has a worldwide distribution and was responsible for severe vascular wilt or root rot in a wide range of plant families. Small cardamom is affected by a number of fungal diseases. Recently *Fusarium oxysporum*, the well known wilt fungus is becoming a major pathogen to small cardamom. The wide spread "foliar yellowing and plant decline" noticed in several cardamom plantations of Idukki District was reported to be caused by this fungal pathogen.

2.1 SURVEY AND COLLECTION OF INFECTED SAMPLES

Thomas and Vijayan (2002) conducted survey at 37 plantations in different parts of Idukki district during 2000-2002. They had found that symptom appearance was varied according to locality and the disease incidence was calculated individually for the each symptom. They have concluded that plants with 2-3 years were vulnerable to root rot and foliar yellowing. It was the first report of panicle wilt noticed on Njallani with disease incidence of 41%.

Vijayan *et al.* (2014) conducted survey in 93 plantations of Idukki district during 2009-2011. They had observed that root rot and foliar yellowing was varied upto 0-48% in 2009-2010 and it was increased to 68% in 2010-2011. Plants of 4-6 years are found to be most vulnerable to panicle wilt symptom and severe during April-July, and the symptoms appearance was varied to the location.

Disease severity or per cent disease index (PDI) was worked out to assess the extent of damage caused by the disease using a 0-5 scale by visual observation of symptoms on infected tillers and panicles (Dhanya *et al.*,2018).

2.1.1Symptomatology

The major symptoms caused by *Fusarium oxysporum* in small cardamom were root tip rot and leaf yellowing, pseudostem rot and panicle blight. These diseases were often severe and wide spread and lead to crop losses.

In several cardamom plantations in the Idukki district, root tip rot and leaf yellowing were widespread. After the monsoon rains, the disease made its appearance and became extreme during the summer months. The manifestations are the yellowing of the foliage that resulted in the drying of the leaves. The symptoms usually started with the older basal leaves and exceeded the middle portion of the tillers. The younger leaves are greener in colour. The earlier affected basal leaves become fully yellow and soon dried off. The root tip of affected plant showed symptoms of decay, proceeding slowly towards the plant base. Such roots shriveled and gave an off white to grey colour to the root tips. If sufficient moisture was present in the soil, the affected portions showed rotting. During dry weather, these portions became shriveled (Vijayan *et al.*, 2009).

Pseudostem rot was a characteristic symptom of the fungus seen on the pseudostem (tillers) of small cardamom. The infection proceeded in the form of dark brownish, round or oval necrotic patches on the pseudostems and these portions elongated and the infection spread towards the leaf sheaths. Later the pseudostem at the lesion portion splits off or tears resulted in the breaking and falling of tillers. The infection could be seen usually on any portion of the matured tillers (Vijayan *et al.*, 2009). If the infection occurs in the lower part of the tillers, it will fall off and give lodged appearance (Murugan *et al*. 2016).

In several cardamom plantations in the Idukki district over the past few years, Blight disease with drying of flower buds, young capsules and panicle tips has been noted. After the southwestern monsoon season or even directly after the monsoon rains, the disease was more observed. The matured capsules have dried off in extreme cases. The signs are so distinctive that the drying begins from the tip of the panicle and progresses toward the base of the panicle. Sometimes the dried parts showed a faint pinkish to purple hue (Vijayan *et al.*, 2009).

2.1.2 Isolation of pathogen

The pathogen was isolated from infected root, panicles, rhizomes and pseudostem and identified as *F. oxysporum* Schlecht (Thomas and Vijayan (2002), Vijayan *et al.* 2013, 2014).

2.1.3Screening for virulence

Thomas and Vijayan (2002) proved pathogenicity of root isolate through pin pricks on the base of healthy pseudostem using macerated 10 day old fungal cultures and spore suspensions in distilled water (5×10^5 cfu mL^{-1}) whereas Vijayan *et al.* (2014) used spore suspension of 10^7 cfu mL^{-1}. Detached panicles from healthy plants were used for inoculation of panicle isolates using pin prick method. Root isolates developed brownish coloration of the base in 6-10 days and foliar yellowing occurred 20 days after inoculation. The reisolation had proved the pathogenicity of *F. oxysporum* Schlecht.

Vijayan *et al.* (2014) used crude extracts of metabolites (toxins) of the pathogen for inoculation on the seedlings maintained in the green house. They had also proved pathogenicity and cross infectivity of the pathogens and *F. oxysporum* was re-isolated from the diseased plant parts such as roots, panicles, rhizomes and pseudostem of small cardamom.

The pathogenicity of *F. oxysporum* in large cardamom was also proved by placing a 5 mm mycelial plug in an opened flower and covering the spike with polythene bag. The typical disease symptoms appeared five days after inoculation. For stem isolate, an injury was made near the collar region with the help of sterilized needle on the pseudostem near ground level and inoculated with mycelia plug and it was secured with polythene bag to maintain humidity. The symptoms appeared after three days of inoculation as blackish lesion (Gopi *et al.*, 2016)

2.2 PATHOGEN VARIABILITY STUDIES BY MORPHOLOGICAL, CULTURAL AND PATHOLOGICAL CHARACTERS

2.2.1 Morphological variation of isolates of *Fusarium* sp. causing pseudostem rot in cardamom

Thomas and Vijayan (2002) was reported that fungal isolates from infected root and panicle showed white colonies with purple tinge with delicate mycelium, and the microscopic study revealed the production of macro and micro conidia by the fungus. Macro conidia was long, 3-5 segmented with 23.6×3.48 µm in size and slightly curved, while micro conidia are small, straight with 9.03×2.58 µm size. Gopi *et al.* (2016) characterized macro conidia as multi-septate, pointed and curved towards end with 26.91- 57.64 to 2.01- 2.59 µm size with one or two celled microconidia of size 5.62 - 8.44 to 1.86- 2.71µm was reported in large cardamom.

2.2.2 Cultural variability of *Fusarium* sp. isolates causing pseudostem rot in cardamom on PDA medium

Thomas and Vijayan (2002) was reported the Fusarium infection on the cardamom with distinct symptoms and identified as *Fusarium oxysporum* Schlecht. They observed the Fusarium isolates are white in appearance with purple tinge.

Vijayan *et al.* (2013) reported five morphologically varied types of *Fusarium oxysporum* Schlecht isolated from infected roots, rhizomes, pseudostem and panicles in different parts of cardamom plantations in Idukki district of Kerala based on colony characters.

Gopi *et al.* (2016) first reported Fusarium infection on large cardamom. The aerial floccose mycelium of the pathogen appeared as white initially and turned to purple later.

2.3 MANAGEMENT OF FUSARIUM ROT USING BIOCONTROL AGENTS

2.3.1 AMF

Mycorrhizal fungi colonized the roots of over 90 per cent of plant species, to the mutual benefit of both the plant host and fungus. These symbiotic fungi found in most terrestrial ecosystems and played a major role in both the growth of plants and

important ecosystem processes. Although there are several different types, the most common one is arbuscular mycorrhiza, which is formed by most plant species, including the majority of commercially important crop and horticultural plants. The hyphae of the symbiotic fungi penetrated the roots of susceptible plants forming specialized structures known as arbuscules, and also sometimes vesicles (Hooker *et al.,* 1994).

Thomas *et al.* (1994) studied the possibility of using the vesicular arbuscular mycorrhizal (VAM) fungus, *Glomus fasciculatum* in biological control of damping-off of cardamom caused by *Fusarium moniliforme, Pythium vexans* and *Rhizoctonia* sp. All treatments with *G. fasciculatum* either alone or in combination with pathogens showed more phosphorus in shoots than treatments with the three pathogens alone. They also observed that introduction of the mycorrhizal fungus together with *F. moniliforme* reduced the severity of disease caused by the pathogen less stunted appearance to the plants compared to *F. moniliforme* inoculated plants.

Fracchia *et al*. (2000) reported the effect of the saprophytic fungus *F. oxysporum* on arbuscular mycorrhizal (AM) colonization and plant dry matter status under greenhouse studies and field conditions. Application of the saprophytic fungus increased the number of propagules of AM fungi in field plots where pea was grown, but this increased was not sufficient to increase AM colonization of sorghum after the pea crop.

A study conducted by Akkopru and Demir (2005) on Fusarium wilt of tomato using AMF and some rhizobacteria, showed that single and dual inoculation of plant reduced disease severity and enhanced dry root weight effectively with better colonization.

Al-Askar and Rashad (2010) reported the effectiveness of AMF against Fusarium rot disease (*Fusarium solani* f. sp. *phaseoli*) of common bean. Mycorrhizal colonization led to a significant increase of the phenolic content and defense related enzymes resulted in significant reduction of disease severity and incidence.

Brito *et al.* (2018) conducted a study on protection against biotic stress in tomato challenged with Fusarium wilt fungus, using a two- phase pot experiment under

green house condition. It was observed that when an intact extra radical mycelium (ERM) of AMF was present in the soil where tomato planted, growth was not significantly affected by the pathogen inoculum.

Vijayan *et al.* (2018) identified exotic strains of VAM (*Glomus microcarpum, G. mosseae and G. fasciculatum*) are suitable for seedling inoculation in cardamom. They also standardized the protocol of AMF inoculation for cardamom seedling in nursery.

Math *et al.* (2019) observed that AMF (*Glomus fasciculatum*) could activate reactive oxygen species (ROS) mediated immune response in tomato roots (Pusa ruby) challenged with *F. oxysporum* f. sp. *lycopersici* microconidia. As per the study there was an increasing trend of ROS in roots up to 72 hr after pathogen inoculation. Antioxidant enzymes such as superoxide dismutase, catalase and peroxidase were also analysed by them and was found significantly higher level in AMF inoculated plants compared to control plants.

Ahammed *et al.* (2020) discovered that external application of melatonin promoted the root colonization of AMF in Fusarium infected cucumber roots with significant reduction in disease index, and improved physiological characters (photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration) in cucumber.

2.3.2 *Trichoderma viride*

According to Sivan and Chet (1989) sesame seeds treated with three isolates of *T. viride* reduced the pre and post emergence damping off caused by
R. solani and *F. oxysporum* f. sp. *sesami* under pot culture and field conditions.

John *et al*. (2010) proved *Trichoderma* as an effective biocontrol agent against two fungal pathogens (*Fusarium oxysporum* f. sp. *adzuki* and *Pythium arrhenomanes*) infecting soybean. During an *in vitro* study, *Trichoderma* showed mycoparasitism and destructive control against the tested fungal pathogens.

Perveen and Bokhari (2012) studied the effect of volatile metabolites of different species of Trichoderma on the growth of *Fusarium oxysporum* results showed that all *T. viride* significantly inhibited the mycelial growth of the pathogen. The results

of dual culture showed that all antagonists significantly inhibited the mycelial growth of the pathogen.

Sundaramoorthy and Balabaskar (2013) studied the effectiveness of *Trichoderma* sp. in control of soil/seed borne fungal diseases in several crop plants including cardamom. Fifteen native isolates of *Trichoderma* spp. were screened for their *in vitro* antagonistic property against the *F. oxysporum* f. sp. *lycopersici* by dual culture technique. The results indicated that *Trichoderma* sp. inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* to an extent of 38.12%, The application of native strains of *Trichoderma* through seedling dip and soil application was found as an effective strategy in suppressing the wilt incidence (by 15.33-25.50%).

Infection of seedlings by *Fusarium oxysporum*, the common root rot pathogen of cardamom seedlings was found to be greatly reduced in nursery beds pre-treated with *T. harzanium* and *T. viride. Trichoderma* are efficient biocontrol agents that are commercially produced to prevent development of several soil pathogenic fungi. Several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants (Vijayan *et al.*, 2015).

Moosa *et al.* (2016) conducted an experiment on combined application of locally available manures (cow, horse, chicken or sheep) and antagonistic isolates (*T. viride* and *T. harzianum*) as *in vivo* and *in vitro* against Fusarium wilt pathogen of tomato (*F. oxysporum* f. sp. *lycopersici*). They had observed that both Trichoderma isolates combined with cow dung resulted in minimum disease incidence after 21st and 45th day of *in vivo* studies, result of *in vitro* studies showed that *T. viride* along with cow dung showed least disease incidence on 45th day compared to *T. harzianum.*

Mohapatra and Mittra (2017) observed that wheat seedlings pre inoculated *T. viride* were less vulnerable to the Fusarium wilt when co –stressed with *F. oxysporum* Schlecht. The seedlings showed improved physiological characters (relative water content, total soluble protein, total chlorophyll content, carotenoid and H_2O_2) and growth parameters (root and shoot length) .

Hewedy *et al.* (2019) isolated fifteen *Trichoderma* isolates from various locations of Egypt and based on the internal transcribed spacer sequencing, they categorized the isolates into four different species (*T. harzianum, T. asperellum, T.*

longibrachiatum and *T. viride*) with BLAST identify per cent range of 99-100. Efficacies of the above isolates were examined with *F. oxysporum* f. sp. *capsici* of pepper by dual culture technique and *T. viride* showed maximum (81.51 per cent) inhibition.

Meena and Roy (2020) isolated and characterized *F. oxysporum* (*isbfu*-1) from infected isabgol (*Plantago ovate* Forsk.). Sequence analysis showed that the pathogen formed a separate cluster in *F. oxysporum* with more similarities to *F. oxysporum* f. sp. *dianthi* followed by *F. oxysporum* f. sp. *vasinfectum* and resulted in huge economical loss for the crop. They had formulated a field experiment for managing the disease and found that furrow application of *T. viride* enriched neem cake before sowing and seed treatment with carbendazim showed least disease incidence continuously for two years (2017 and 2018).

2.3.4 *Pseudomonas fluorescens*

Ramamoorthy *et al.* (2002) found that phenolics were accumulated in bacterized tomato root tissues challenged with *F. oxysporum* f. sp. *lycopersici* one day after pathogen inoculation. Isoform analysis revealed that a unique PPO1(polyphenol oxidase) isoform were induced and PO1(peroxidase) and PPO2 isoforms were expressed at higher levels in bacterized tomato root tissues challenge inoculated with the pathogen. Similarly, β-1, 3-glucanase, chitinase and thaumatin-like proteins (TLP) were induced and accumulated at higher levels within 3-5 days after challenge inoculation in roots.

Saravanan *et al.* (2004) reported that vascular discolouration in banana associated with Fusarium wilt disease is reduced by the accumulation of enzymatic resistance induced by *P. fluorescens*. A green house study conducted by them with Rasthali cultivar of banana showed lesser discoloration index and no wilting symptoms or death. The biocontrol agent induced defence enzymes such as peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in roots. They also separated peroxidase and polyphenol oxidase isoforms by native poly acrylamide gel electrophoresis from infected banana treated with *P. fluorescens.*

Vijayan *et al.* (2013) reported that fungal diseases such as Colletotrichum blight (*C. gloeosporioides*), Phoma leaf spot (*Phoma* sp.) and Leaf streak (*Pestalotiopsis royenae*) of large cardamom in Sikkim can be effectively controlled by *P. fluorescens* in the field condition.

Manikandan and Raguchander (2014) observed suppression of *F. oxysporum* f. sp. *lycopersici* in tomato plants while using liquid formulation of *P. fluorescens.* Isoform analysis revealed that five PO and six PPO isoforms were observed respectively in the tomato plants pretreated with Pf1 liquid formulation challenge inoculated with this pathogen, The expression pattern of catalase isoforms in tomato roots in responded to *F. oxysporum f. sp. lycopersici* inoculation revealed the induction of catalase isoforms, *viz*CAT1, CAT2, CAT3, CAT4 and CAT5.The isoform pattern of β-1, 3 glucanase showed maximum number of isoforms, viz., Glu1, Glu2, Glu3 and Glu4.Whereas no isoform was observed in the control. The defence related proteins, viz. phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), catalase, β-1, 3-glucanase and superoxide dismutase, were induced tomato plants by Pf1 liquid formulation upon challenged with pathogen.

Boukerma *et al.* (2017) evaluated two Pseudomonas species (*P. fluorescens* PF15 and *P. putida* PP27) against *F. oxysporum* f. sp. *lycopersici* by antagonism in three different media. *P. fluorescens* showed good mycelial inhibition in PDA, Kings B and mixed media than *P. putida* and also reduced the symptom development considerably in tomato plants.

Vijayan *et al.* (2018) opined that *P. fluorescens* spray @ 3-5 kg per 100 L or drench could reduce the incidence of *F. oxysporum* and other disease like *Colletotrichum* blight of large cardamom at field condition during pre monsoon period.

Alzandi and Naguib (2019) developed a bio priming method using *P. fluorescens* metabolite against Fusarium wilt of tomato (*F. oxysporum* f. sp. *lycopersici*). Bio primed seeds which were planted in Fusarium infected soil induced systemic resistance through increased level of different biochemical enzymes (peroxidase, poly phenol oxidase, super oxide dismutase, chitinase, β 1,3-glucanase and lipoxygenase) and signaling molecules such as jasmonic acid and H_2O_2.

Rathore *et al.* (2020) isolated and evaluated ten *P. fluorescens* strains (Pf 1-10) against cumin wilt pathogen (*F. oxysporum* f. sp. *cumini*). Based on the dual culture technique Pf-5 showed maximum inhibition (82.51%) with high level of cellulase and pathogen related enzymes (chitinase, β 1,3- glucanase and protease enzyme) in the culture medium. These results confirmed the lytic activity of the Pf-5, which could be used as an effective biocontrol agent.

2.3.5 Plant growth promoting rhizobacteria

Leeman *et al.* (1995) performed studies on of Fusarium wilt management in radish using PGPR as a commercial greenhouse trial. They observed that the crude extract containing lipid A induce systemic resistance in radish which is moderately resistant to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *raphani.*

Liu *et al.* (1995) conducted an experiment on induction of resistance by PGPR in cucumber against Fusarium wilt. Straight 8 the susceptible cultivar was used for the study and roots of two week seedling were uprooted split into equal halves which was inoculated with pathogen and PGPR, then transplanted by joining halves. Reduction in disease development appeared to be related to delayed movement of the pathogen within PGPR treated plants.

Pal *et al.* (2001) reported that plant growth promoting bacteria isolated from maize rhizosphere were found effective against *Fusariumm oniliform* and *F. graminearium* causing foot rot and wilting. *In vitro* antagonism strongly inhibited the fungi, *F. moniliforme* according to them the fluorescent *Pseudomonas* sp. EM85 was found to produce antifungal antibiotics (Afa^+) like siderophore (Sid^+), cyanide (HCN^+), and fluorescent pigments (Flu^+)where as *Bacillus* sp. MR-11(2) exhibited the production of antifungal antibiotics (Afa^+) like siderophore (Sid^+) and antifungal volatiles (Afv^+).

Recep *et al.* (2009) conducted a study on inhibition of potato dry rot caused by *Fusarium oxysporum*, *F. sambucinum* and *F. culmorum*by PGPR strains both under *in vivo* and *in vitro.* Agria and Granola cultivars of potato were used in the study and showed inhibitory effects.

Sundaramoorthy *et al.* (2012) studied the combined effect of strains of endophytic bacteria and PGPR against wilt disease of chilli (*Fusarium solani*).Thus isolated two endophytic bacterial strains of *Bacillus subtilis* EPCO16 and EPC5 were isolated from coconut and cocoa respectively and found induction of defense enzymes and pathogenesis-related (PR) protein in plants in responded to it.

Audipudi *et al.* (2016) isolated thirteen morphologically different bacterial colonies (AVP 1-13) from chilli rhizosphere and categorized them into five (*Pseudomonas*, *Bacillus*, Achromobacter, *Klebsiella* and *Stenostrophomonas*) based on the morphological, biochemical and 16S rRNA sequence. They had identified that *Pseudomonas* sp. (AVP-3) showed high salt tolerance and phosphate solubilisation properly than other isolates. Co-application of AVP3 and AVP7 (*Bacillus* sp.) induced systemic resistance by maximum production of peroxidase, phenylalanine ammonia lyase and growth promotion (highest of root and shoot length) in chilli.

Khan *et al.* (2018) conducted an experiment on four *Bacillus subtilis* strains for their plant growth promotion and biocontrol abilities against *Fusarium* spp. (*F. oxysporum* f. sp. *conglutinans*, *F. oxysporum* f. sp. *matthioli*, and *F. solani*). *In vitro* antagonism of *B. subtillis* 30VD-1 on *Fusarium spp.* effectively prevented the growth by hyphal thinning. The examination showed with phase contrast microscope that there was an abnormal swelling on the pathogen hyphae. They also reported, there was an escalated production of chitinase enzyme and volatiles during antagonism over *Fusarium* spp. Seed bacterization of pea with same strain also showed improved growth characters. Based on the study results Khan and coworkers concluded that *B. subtillis* 30VD-1 had multivariate mode of action against *F. oxysporum.*

Xu *et al*. (2020) isolated six bacterial strains from rhizosphere of healthy watermelon identified in Fusarium wilt infected field and among them, strain WB showed best antagonistic capabilities against *F. oxysporum* f. sp. *niveum*. The bacterial strain was characterized using 16S rRNA, *gyr* A and *gyr*B genes revealed that the new strain WB could not be identified as *Bacillus amyloliquefaciens* WB or *B. subtillis* WB so the strain was renamed as *Bacillus* sp. WB. Detailed mechanism of inhibition showed that cell free supernatant of *Bacillus* sp. WB affect the pathogen by restricted

growth (82 per cent at 72hr) and abnormalities on conidia (aggregation of organelles and heterogeneity of electron density of the cytoplasm)

2.3.6 Chemical control (carbendazim)

Ebben (1977) reported that carbendazim drenching at the time of planting is an effective management strategy of Fusarium wilt in carnation caused by *Fusarium oxysporum* Schlecht f. sp. *dianthi*. Minuto *et al.* (1995) also observed that application of carbendazim can reduce or delay the appearance of Fusarium wilt symptoms in Cyclamen (*Cyclamen persicum* Mill.) with split applications at transplanting time and one month later. Podder *et al.* (2004) made same observation while managing chickpea wilt caused by *F. oxysporum* f. sp. *ciceri*, by inhibition zone method..

Rajput *et al.* (2006) found that carbendazim was an excellent fungicide against cotton wilt caused by *F. oxysporum* f. sp. *vasinfectum.* An increase in the root and shoot length of plants along with reduction of root colonization by the pathogen were also recorded in the above study. The study results made by Guo *et al.* (1993) on cotton is in agreement with this..

Badia and Rai (2007) observed that mycelial growth of cumin wilt pathogen *F. oxysporum* f. sp. *cumini* was maximum inhibited by carbendazim at the lowest concentration *in vitro*. Field evaluation of the carbendazim done by seed treatment with a rate of 2g kg^{-1} cumin seed and noticed significant reduction in disease incidence. From the study they also concluded that combined application of carbendazim and biocontrol agent *Trichoderma harzianum* are compatible and significantly reduced the disease incidence at field level.

A study conducted by Amini and Sudovich (2010) showed that glasshouse tests revealed efficacy of all concentration of fungicide in reducing disease infestation in tomato *in vitro* and *in vivo.* Pathogenicity tests were conducted using root-dip inoculation with differential tomato cultivars: Beliy naliv-241 (susceptible), Blagovest and Benito. Their roots were dipped into a conidial suspension (10^6 cfu mL^{-1}) of tested isolate for 10 min.The mycelial growth of *F. oxysporum* f. sp. *lycopersici* was highly inhibited and the EC_{50} were calculated to be 0.008.

Animisha *et al.* (2012) observed that in poisoned food technique carbendazim was highly inhibiting the growth of *F. oxysporum* f. sp. *ciceris* at all the concentrations of 0.1, 0.2 and 0.3 per cent. Carbendazim were used as seed treatment at the rate of 2g kg^{-1} seed in field condition also. The treatment not only reduced disease severity but also highest mean yield of chickpea.

Mailto *et al.* (2014) evaluated fourteen fungicides against *F. oxysporum* f. sp. *ciceris* pathogen *in vitro* with five different concentrations ranging from 1-10000 ppm. Among them carbendazim and Thiophanate-methyl was found most effective at all used concentrations. The pathogen was failed to grow at low concentration and negligible growth at 1ppm. In greenhouse studies carbendazim application at 10 ppm is found to be reduced plant mortality, less phytotoxic, less root infection and also higher shoot length and weight. In field condition the minimum pathogen infection recorded in plants treated with carbendazim (7%) with remarkably enhanced growth compared to the untreated plants. In terms of root length carbendazim treated plants records highest (15cm) also the grain yield increased.

Deo (2013) in his study evaluated the efficacy of carbendazim fungicide (50% SC) at four rates (0.1, 0.3, 1 and 3 mg mL^{-1}) on *in vitro* radial growth of mycelia of two different pathogens *Fusarium oxysporum* f. sp. *lycopersici* "strain F20" isolated from tomato (*Lycopersicon esculentum*) and *Colletotrichum capsici* from chilli (*Capsicum annum* L.). There was mycelial growth was observed at 0 and 0.1 mg mL^{-1} concentration both fungus but in addition to that *C. capsici* showed significant growth in 1 and 3 mg mL^{-1} also. Maximum inhibitory effect found at higher concentration of 1 and 3 mg mL^{-1} in case of *F. oxysporum* f. sp. *lycopersici*. This concentration was most toxic to the Fusarium pathogen.

Mahmood *et al.* (2015) found out that *in vitro* assay of carbendazim was proved to be best for checking the mycelia growth of *F. oxysporum* f. sp. *ciceris* with mean of 83.7 % growth inhibition compare to control. The green house study revealed that increased in concentration of each treatment were directly proportion to its efficacy, and increased percent disease reduction of wilted plants over control plants. Carbendazim of dose 200 ppm and 500 ppm drenching was proved to be best.

Yadav and Ansari (2017) reported that carbendazim is completely inhibited the mycelial growth of *F. oxysporum S*chlecht of soya bean *in vitro* using poisoned food technique at concentration of 200 ppm, 500 ppm and 1000 ppm they had also found that, fungicide were lethal and killed spores of pathogen. So carbendazim can be used for effective control of Fusarium blight of soybean.

Mbasa *et al*. (2020) conducted an experiment on chemical management over cashew Fusarium wilt disease. They found that 10 g/L of carbendazim enhanced 49.4 per cent of cashew recovery from 50.0 per cent severity after 120 days and was found effective as that of asoxystrobin fungicides.

2.3.7 Influence of biocontrol agents on plant nutrients

Balliu *et al*. (2015) conducted an experiment on nutrient uptake and acquisition by AMF inoculated tomato plants under salt- stress condition. The study showed that N, Ca, Mg, P, Na, and Al content was significantly higher in leaves of salt stressed transplanted tomato plants after 30^{th} day of AMF inoculation than control. AMF inoculated tomato seedlings treated with fresh water irrigation also showed relatively higher uptake in the rate of N, P, Mg, Ca, and other micronutrients. They also concluded that salt stress reduced the K content in leaves.

Colla *et al.* (2015) made experiments on winter wheat seeds are coated with coveron (coating product consisting of *Glomus intraradices* BEG72, *G. mosseae* and *T. atroviride* MUCL 45632) and its effect on yield and quality. The experiment was conducted in growth chamber and field condition. They found that inoculated seedling had having better seed emergence and growth characters (root and shoot biomass and number of leaves). Nutrient analysis conducted at the end of the experiment showed that, seed coated experimental plants had higher nutrients concentration in harvested grain (P, K, Fe and Zn) and leaves (N, P, K, Ca, Mg, Fe and Zn) than uncoated.

Radhapriya *et al.* (2015) conducted experiments to study the effect of indigenous PGPR on growth of Indian beech (*Pongamia pinnata*) in degraded soil. 160 isolates were obtained from which *Pseudomonas aeruginosa* RRALC3 taken as best and it was subjected field experiment. The best treatment (*P. aeruginosa* RRALC3 and

inorganic fertilizer) enhanced N, P, K, Mg, S and Ca content in shoots than control plants.

Shinde *et al*. (2017) observed the reaction of trembling aspen (*Populus tremuloides*) seedling when soaked with three strains of *P. fluorescens* (pf-5, pf0-1 and WH6) and allowed to grow under nutrient stress condition. Co culture with all three strains elevated the P content than un- inoculated control plants.

Wahid *et al*. (2020) used phosphate solubilizing bacteria (*Bacillus* sp. P1S7) and consortia of AMF in calcareous soil where P availability was reduced due to its fixation by calcium in the soil. The pot culture experiment was conducted with two crops (maize and wheat). In this experiment combined application of AMF and *Bacillus* sp. along with rock phosphate showed highest grain yield and maximum plant P uptake.

Metwally (2020) studied the effect of combined application of AMF and *Trichoderma viride* on the biochemical, protein pattern and mineral content of the onion plants under green house condition. Increased nutrient contents of N, P, K, Ca and Mg were observed in treatments compared to the control plants. Root colonization with AMF was positively influenced by the application of *T. viride*. Dual inoculation record about 112 per cent P content was in the onion shoot than their individual application.

2.3.7.1 Influence of biocontrol agents soil nutrients

Lehmann *et al*. (2012) reported that the hyphal network of AMF helped the annual crop roots to absorb unavailable P from the soil, thereby increased the biomass production.

Radhapriya *et al.* (2015) conducted experiments on indigenous PGPR (*P. aeruginosa* RRALC3) combination with different fertilizers in degraded soil and their effect on growth and establishment of Indian beech (*Pongamia pinnata*). According to them the combined application of *P. aeruginosa* RRALC3 and inorganic fertilizer enhanced not only the growth but also soil nutrients such as N, P, K, Mg, Zn, Cu and Fe.

Singh *et al.* (2019) opined that *Trichoderma* spp. could enhance the acquisition of N from the soil to the roots of crops. Some *Trichoderma species* were capable of uptake some nitrogen containing compounds and induce nitric acid production in the

plants, these NO were acts as signaling molecules and had role in disease resistance and plant growth promotion.

Soumya *et al*. (2020) found that combined application of *Pseudomonas* spp. in the presence of NPK fertilizer promote growth (shoot and root length, fresh weight and leaf area) and productivity of *Amaranthus tricolor*. This field study revealed that the combined application had reduced the application of fertilizers by 50 per cent.

Wahid *et al*. (2020) conducted a pot culture experiment in calcareous soil for maize cultivation with the application of phosphate solubilizing bacteria (*Bacillus* sp. P1S7) and AMF consortia. Combined application above bioagents along with rock phosphate resulted in highest grain yield and soil P concentration.

2.4 INDUCTION OF DEFENCE MECHANISM IN CARDAMOM PLANTS IN RESPONSE TO BIO CONTROL AGENTS

2.4.1 Phenol

Phenolic compounds are secondary metabolites that constituted one of the most common and widespread groups of substances in plants. Term phenolic" or "polyphenol" can be defined as a chemical substance, which possesses an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents, including functional derivatives (esters, methyl ethers, glycosides, etc.)(Farkas and Kiraly, 1962).

Carrasco *et al*. (1978) observed stimulation of phenols in susceptible tomato variety Marmande with quinic acid treatment. Treated plants showed resistance against *Fusarium oxysporum* f. sp. *lycopersici*, from Procida (France). Eventhough quinic acid had no fungitoxic effect by itself, induced phenolic level of the plant in response to its application and contributed the resistance.

Niemann and Bayyen (1988) observed fungistatic property of phenols in association with *F. oxysporum* f. sp. *dianthi* inoculated carnations. Novada (highly resistat), Pallas (moderately resistant) and Lena (susceptible) were selected for the study, and Novada accumulated more phenols in stem than other cultivars. The phenols present in the stem extract showed germination-inhibiting properties for conidia of the

fungus. The accumulation of several phenolics and the fungistatic activity were roughly correlated to the degree of resistance of the three cultivars.

De Vecchi and Matta (1989) investigated the presence of phenolic compounds in the middle lamellae and intercellular spaces near infected vessels of resistant tomato varieties, which contributed resistance to *F. oxysporum* f. sp. *lycopersici.*

Curir *et al*. (2003) also found the fungitoxic effect of phenols present in carnations namely protocatechuicacid, vanillic acid, flavonol, glycoside and peltatoside as part of *in vivo* studies and flavones datiscetin obtained from *in vitro* tissues. The carnation cultivars used for experiment which showed highly resistant and partially resistant reaction towards *F. oxysporum* f. sp. *dianthi* pathotype 2 strain P 75, was appreciable amount of protocatechuic acid and vanillic acid were found in cultivar Gloriana, in accordance with fungal inoculation.

de Ascensao and Dubery (2003) reported soluble and wall-bound phenolic polymers in root tissues of banana cultivar Gold finger and was found in conjugated form usually in plants. A rapid synthesis was occurred by the elicitor fraction derived from the cell wall of *F. oxysporum* f. sp. *cubense* race four.

Mandal *et al*. (2009) found that cell wall bound phenolic compounds associated with roots of the tomato variety Arka saurabh susceptible to *F. oxysporum* f. sp. *lycopersici*. 4-hydroxybenzoic acid, vanillic acid, 4-hydroxybenzaldehyde, vanillin, 4-coumaric acid and ferulic acid where the pheolic compounds detected in this study by HPLC *in vitro* antifungal compound against pathogen analysis of infected roots. Media amendment method was used to find out their antifungal properties, and observed that ferulic acid, the concentration of which was highest in tomato roots and are the most effective.

Shukla *et al*. (2013) investigates the possibility of phenolic compounds as the management strategy against pathogens. Their study revealed that, phenol at concentration of 0.15% control and inhibits growth of *Fusarium udum* and *F. oxysporum* f. sp. *ciceri* by 100% in an *in vitro* study by poisoned food technique.

Hanifei *et al.* (2013) observed peak production of phenols in the roots of melons when challenge inoculated with the pathogen *F. oxysporum* f. sp. *melonis* race1.2under green house condition. Experiments include sensitive and semi- resistant genotypes. The study revealed that accumulation of phenolic compounds at the cell wall acted as one of the defence mechanisms.

Rajeswary (2014) reported a hike in the production of phenolic compounds in the leaves of 75 days old groundnut (*Arachis hypogea*) treated with *T. viride, T. harzianum* and *P. fluorescens.* Groundnut of JLR- variety was used for the study and leaves were inoculated with *Fusarium oxysporum.* According to them, the application of bio control agents elevated the plant defence enzymes and there by its resistance against the pathogen.

Oriyomi *et al*. (2019) evaluated phenol production in five different varieties of maize plants artificially inoculated with *F. verticillioides,* one of the important fungi causing root rot, stalk rot and ears rot. The experiment revealed that each variety reacted to the pathogen inoculation with varying levels of disease severity and more phenol production observed in seedling inoculation than soil inoculation method.

Dukare and Paul (2020) studied the biocontrol potential and growth promotion in pigeon pea over Fusarium wilt by the application of *Pseudomonas sp*. NS 1 and *Bacillus* sp. NS 22. Both biocontrol agents inhibited the fungal growth by the production of extracellular metabolites, diffusible metabolites and volatile metabolites. The study revealed that they were capable of producing fungal cell wall degrading enzymes and other hydrolytic enzymes (endochitinase, exochitinase, chitobiase chitinase, cellulase, pectinase, amylase, lipase, and protease) while compete with the pathogen. The phenol content of *Bacillus* sp. NS-22 and *Pseudomonas* sp. NS-1 treated plants were highest than the control plants and chemical check. The disease severity expressed by the plants treated with biocontrol agents was on par with the effective chemical control also.

2.4.2 Ortho-dihydroxy phenol

Mandal *et al.* (2009) observed an increased production of OD phenol in resistant tomato plants and showed resistance to *Fusarium* inoculation. Fusarium wilt generally occurred when plants are about 60 days old and especially when flowering initiated. Antimicrobial activity regarding defence system of tomato plants revealed that OD phenol is a key enzyme, which produced on the 20^{th} day of infection.

Rajeswari (2014) reported the importance of OD phenol as a defence enzyme in groundnut produced by the applications of bioagents like *T. viride*, *T. harzianum* and *P. fluorescens* against the *Fusarium oxysporum* infection. The analysis on the leaves showed two folds activity in bioagents treated plants when compared to the control plants.

Shinde (2017) screened thirty genotypes of pigeon pea for their resistance against Fusarium wilt, and concluded that in resistant cultivars there was a raise in the OD phenol content of leaf sample. She also found that the cultivars possess a specific amplicon for the resistance compared to the control plants.

Rajeswari (2019) conducted an experiment with foliar application of different combination biocontrol agents on the peanut for the management of Fusarium wilt pathogen. The experiment showed that *T. viride* and *P. fluorescens* combination are the best treatment with greater suppression of *F. oxysporum* with higher levels of OD phenol and proline content in the leaves

Raja and Rajikumar (2020) found that amending composted poultry manure could reduce charcoal rot of sunflower (*Macrophomina phaseolina*) with increased level of phenol and OD phenol concentration in plants.

2.4.3 Peroxidase

Maraite (1973) reported that PO activity was detected in an autoclaved muskmelon tissue colonized *in vitro* by *F. oxysporum* f. sp. *melonis* and four isoenzymes of peroxidase were separated by poly- acrylamide disk electrophoresis.

Significant increase occurred during early pathogenesis in all plant tissues and a sudden hike in concentration occurred with symptom appearance.

Luhova *et al.* (2006) investigated susceptibility leaves of *Pisum sativum* to *Fusarium oxysporum* and *F. solani* among the two genotypes (cv. Smaragd and line DP1059) and evaluated higher POX activity found in infected plants was confirmed by histochemical methods. These enzymes were mostly observed in both roots and shoots during pathogenesis.

Rai *et al.* (2011) also observed the peroxidase enzyme activities in tomato cultivars (*Lycopersicon esculantum* Mill.), tolerant and susceptible to Fusarium wilt disease. The tolerant cultivars of tomato viz., FEB-2, FEB-4, Flora Dade and NF-31 had significantly higher peroxidase activities than the susceptible ones (Sel-7, Sel-18 and Punjab Chhuhara). The maximum peroxidase activity was recorded in the resistant cultivar, Flora Dade (02.073unit mL^{-1}) and minimum in the susceptible cultivar, Sel-18 (0.241unit mL^{-1}). The hierarchical cluster analysis was performed using NTSYS-pc (V.1.8) software. The dendrogram using the average linkage between the groups, showed proximity of resistant cultivars viz., FEB-4, FEB-2, Flora Dade and NF-31 to the wild species with respect to similarity of banding patterns.

Fallahpori *et al.* (2013) was assayed the activity of peroxidase enzyme as a resistance mechanism sesame damping off resistant Asfij local germplasm (from Bahabad, Yazd province) and susceptible Kahnoj local germplasm (from Kerman province). Evaluation of the resistance in tolerant and susceptible germplasms in 2, 4, 6, 8, 10 and 12 days after inoculation with *F. oxysporum* f. sp. *sesami* showed that it increased in resistant germplasm with highest level on 4 days after inoculation. In susceptible germplasm, enzyme activity was increased slightly. Based on this study they concluded that peroxidase activity played a probable role in induction of plant was resistance against the disease.

Xue *et al.* (2014) discovered a new peroxidase gene in common bean roots using cDNA-AFLP libraries with *F.oxysporum* f. sp. *phaseoli* infection. Real-time PCR analyses indicated that expression of the PvPOX1 gene was up regulated by Fusarium infection and peroxidase activity, as measured by hydrogen peroxide (H_2O_2)

accumulation was enhanced during infection. This study results helped in understanding the molecular mechanism controlling resistance to Fusarium wilt pathogens and provide a diagnostic marker for selection of resistance to this disease based on peroxidase expression in common bean.

Moghbeli *et al*. (2017) screened ten different cucumber varieties with *F. oxysporum* f. sp. *radicis- cucumerinum* based on their reaction and production of different biochemical defense enzymes after pathogen inoculation. The susceptible varieties showed very less production of peroxidase activity than resistant variety, and the peak production of peroxidase was observed on 4^{th} day of pathogen inoculation and the concentration was gradually reduced by 10^{th} day.

Jalaluldeen *et al*. (2020) reported active enzymatic changes in chilli pepper roots in response with AMF (*Glomus mossaeae*) colonization against *F. oxysporum*. In the peroxidase production of was enhanced with reduced mortality of roots when combined application of AMF and actinomycetes was given under stressed condition.

2.4.4 Polyphenol oxidase

PPO is a nuclear encoded, plastid copper-containing enzyme, which catalyzes the oxygen dependent oxidation of phenols to quinones. Because of conspicuous reaction of products and induction by wounding and pathogen attack, PPO has frequently been suggested to participate in plant defence against pathogens.

Retig (1974) observed an increased activity of polyphenol oxidase in roots of the resistant varieties of tomato shortly after inoculation of *F.oxysporum* f.sp.*lycopersici* and there was no significant increase in case of susceptible. By the application of ethephon to plants the enzyme level even in susceptible plants escalated.

Mohammadi and Kazemi (2002) reported induced resistance of wheat cultivar, when inoculated with *Fusarium graminearum*. Green house experiments were conducted using resistant (Wangshui- bai and Sumai 3) and susceptible (Falat a Golestan) wheat plants. They were inoculated with eight fungal isolates and the PPO specific activity in extracts of wheat head from resistant (Wang-shui-bai and Sumai) and susceptible (Falat and Golestan) wheat cultivars at different growth stages were

extracted. Inoculation of *F. graminearum* conidia resulted in a significant increase in PPO activity in resistant as well as susceptible wheat heads at all growth stages except at the ripening stage in susceptible heads.

Rai *et al.* (2011) studied the PPO enzyme activities in different cultivars of tomatoes (*Lycopersicon esculantum* Mill.) which were tolerant and susceptible to Fusarium wilt disease. Polyphenol oxidase activity assayed from the leaf samples of the resistant as well as susceptible cultivars revealed that higher enzyme activity was recorded in the resistant cultivars viz., FEB-2, FEB-4, Flora Dade and NF-31 as compared to the susceptible (Sel-7, Sel-18 and Punjab Chhuhara**).**

Shafique *et al*. (2017) evaluated six chilli varieties of against *F. oxysporum* Schlecht. The Dandicut variety showed less disease index with higher levels of biochemical compounds (riboflavin, flavonoides, phenols, saponins, tannins and coumarins) and defence related enzymes (PPO, peroxidase and PAL). The molecular analysis confirmed that there was enhanced expression of genes like PR2a, acidic glucanase, chitinase 3, osmotin like PR5 and metallothionein in chilli during Fusarium infection.

Naguib (2018) studied the effect of exogenous application of natural defensin in Fusarium wilt management of wheat as seed priming. Based on experiment it was found that wheat leaves showed significantly increased concentration of PPO and other enzymes such as superoxide dismutase and peroxidase.

Jalaluldeen *et al*. (2020) studied biochemical enzymatic changes in chilli pepper roots when inoculated with AMF (*Glomus mossaeae*) against wilt pathogen *F. oxysporum*. Activity of PPO was highest at 10^{th} day of pathogen inoculation when co-application of AMF and actinomycetes was made.

2.4.5 Phenylalanine ammonia lyase (PAL)

PAL is the first enzyme of the phenylpropanoid pathway and flavanoid pathway, increased in both compatible and incompatible in plant–pathogen interactions and play an important role in the biosynthesis of phenolics that were effective chemical barriers against pathogen infection.

Corsini and Pavek (1980) reported that of potato tuber variety pioneer showed high levels of PAL in the tissues infected with *F. roseum* var. *sambucinum* collected from Idaho.

Buiatti *et al.* (1984) investigated the relation between PAL activity and defence reaction of carnation callus against *F. oxysporum* f. sp. *dianthi*. Duca,Mei-Ling, Niki and Pulcino are partially resistant to *F. oxysporum.* f. sp. *dianthi* race 2 and their callus extracts had produced more accumulation of PAL compared to susceptible variety like Corridamose and Georgia.

Stevens *et al*. (1999) found that production of PAL in sweet potato when exposed to UV treatment to prevent Fusarium infection. Sweet potato variety Jewel is selected for the study and dose of 3.6 KJ m^{-2} UV radiations was provided through a low pressure mercury vapor discharge lamp.

Anguilar *et al.* (2000) observed that *F. oxysporum* f. sp. *cubense* inoculation would increase the activity of PAL in the roots of banana Goldfinger cultivar. Goldfinger,William, Michel and sugar are the two resistant and susceptible cultivars respectively used for the green house experiment. After pathogen inoculation plants showed varying amount of enzyme accumulation. Resistant cultivar goldfinger recorded highest production of PAL enzyme (about 20 fold times than that of control plants).

Morkunas *et al.* (2005) opined that peak quantity of PAL in the embryo axes of lupin (*Lupinusluteus* L. cv. *Polo*) was observed after inoculation with *F. oxysporum Schlecht* f. sp. *lupine.* Eventhough the sugar added to the media also could induced the PAL enzyme markedly enhanced production was achieved by inoculation with pathogen.

Modafar *et al.* (2006) analysed Fusarium infected roots of date palm seedling and found enhanced PAL activity. Bousthami Noir and Jihel were the cultivars resistant, susceptible respectively, used for the study. They also observed that inoculation of the seedling with hyphal wall elicitor (HWE) of the pathogen induced PAL activity in cultivars but it was not influenced by the fungal culture filtrate.

Arfaoui *et al.* (2007) reported that Rhizobium pre treated chickpea seedling could produce PAL enzyme when challenge inoculated with *F. oxysporum* f. sp. *ciceris*. Accumulation patterns of PAL transcripts responded over pathogen inoculation was detected by Northern gel blots.

Christopher *et al.* (2010) found that *T. viride* treated tomato plants (especially seedlings) shows increased activity of PAL enzyme on 9th day of pathogen inoculation with *F .oxysporum* f. sp. *lycoperici.* \

Chang *et al.* (2008, 2015) also observed that PAL gene was expressed at the shoot base of watermelon. Resistant JSB line when examined for comparison of *F. oxysporum*-watermelon interactions with the susceptible Grand Baby (GB) cultivar, they found aprogressive colonization of pathogen in the plant system of JSB cultivar but its growth was limited to below shoot level. This peculiar of the growth and enzyme analysis confirmed that high activity of PAL imparted resistance against Fusarium.

Datta and Lal (2018) discovered temporal and spatial variation in the PAL activity of Fusarium infected pigeon pea. As per the study there was a significant increase in PAL activity at pre- initiation, disease initiation and advanced disease stages of inoculated plant.

Duba *et al.* (2019) studied the selected characters (leaf morphology, phenylalanine ammonia lyase, Fusarium resistance and reactive oxygen species) influencing the Fusarium infection in wheat species. Resistant variety Tas-581 showed highest production of PAL 24hr after inoculation with *F. culmoram* and gradually reduced the concentration by 48th and 168th hr. Tas-581 also showed higher ROS activity than susceptible varieties.

Cacique *et al*. (2020) evaluated moderately resistant (BRC 1035) and susceptible (30F35Y) maize varieties to their reactive mechanism (transpiration rate, stomatal conductance, internal CO_2 concentration and enzyme activities) during Fusarium stalk rot caused by *F. verticillioides*, showed internal necrosis of internodes and nodes less intense than the susceptible variety. Higher PAL activity was observed during advanced stages of fungal infection (10th and 30th DAI) in resistant varieties.

2.4.6 β- 1, 3-glucanase

Plants responded to the attack of potentially pathogenic organisms through an increased synthesis of hydrolases such as chitinase and β-1,3-glucanase.

Netzer and Kritzman (1979) made an attempt to find relation of β -1,3-glucanase activity and the growth of *F. oxysporum* f .sp. *melonis* in near isogenic lines of resistant and susceptible muskmelon. The enzyme activity gradually increased with the artificial inoculation of the pathogen, especially on hypocotyls and in roots in resistant cultivars. From this study it was concluded that, wilt inducing pathogen was highly inhibited by the activity of host β -1,3-glucanase.

Mauch *et al*. (1988) reported that chitinase and β-1,3-glucanase purified from pea pods acted synergistically in the degradation of fungal cell walls. Combinations of purified chitinase and β-1, 3-glucanase inhibited all fungi tested as effectively, even though the pea pathogen, *F. solani* f. sp. *pisi*, was inhibited by B-1,3-glucanase alone and the inhibition of fungal growth was caused by lysis of fungal tips.

Chang *et al*. (1992) described the molecular characterization of β-1, 3-glucanase from pea, which is induced by *F. solani*. This enzyme is the most prominent protein in endocarp tissue during fungal infection. They had cloned and sequenced the cDNA and genomic southern analysis revealed that only one β-1,3-glucanase gene which was present in pea corresponding to the probe that is used in their study.

Sela-Buurlage *et al.* (1993) discovered that only class I vacuolar chitinase and β-1, 3-glucanase isoforms exhibited antifungal properties against *F. solani* in tobacco. But class II chitinase and β-1,3-glucanase showed no inhibitory activity in sole or combination.

Jongedijk *et al*. (1995) also observed enhanced synergistic activity of chitinase and β-1, 3-glucanase in transgenic tomato plants having expression of tobacco class I gene. The plants were highly protected from *F. oxysporum* f. sp. *lycopersici* by these enzymes.

Li *et al*. (2001) isolated two specific β-1, 3-glucanase clones using cDNA from wheat spikelets inoculated with conidia of *F. graminnearum*. Northern blot

hybridization showed that the expression of these genes is induced upon infection with *F. graminearum.* The accumulation of transcripts for these PR-proteins was more rapid in the resistant variety Sumai-3 than in its susceptible mutant during the first 24 hr.

Ying-Zang *et al*. (2003) observed that the resistance response of cotton (*Gossypium hirsutum* L.) cultivars to crude toxin of *Verticillium dahliae* (VD) was correlated with the activities of β-1, 3-glucanase in their callus cells. The activities of β-1, 3-glucanase in the callus cells treated with the pathogen toxin were increased to the higher level at earlier time point in resistant cultivars than these in the susceptible cultivars. Polyclonal antibody against β-1, 3-glucanase identified a 28 kD protein when was induced by VD-toxin by western blotting technique.

de Marco and Felix (2007) also observed that production of β-1,3-glucanase from the *Trichoderma harzianum*. Two proteins showing β -1,3-glucanase activity were purified.

Jin *et al.* (2007) discovered a full length of β-1, 3-glucanase cDNA MpGlu from plantain (*Musa paradisica*) leaves, peel and pulp by rapid amplification of cDNA ends (RACE) technique, which inhibited the growth of *Fusarium oxysporum* f. sp. *cubense*. In Northern blot analysis it was revealed that the expression of the gene was up-regulated in two days after inoculating with Fusarium.

Quarantin *et al*. (2016) studied the role of *Fusarium graminearum* cerato-platanin proteins (FgPP1 and FgCPP2) localized in the fungal cell wall. FgPP1 gene was expressed 24 hpi on wheat spikelet**.** β 1, 3- glucanase released -3.5 µg glucose equivalent from mycelia from gcpp1,2 mutants and this revealed that protective function of FgCPPs against cell wall degrading enzymes.

Taif *et al*. (2019) identified a β-1, 3-glucanase gene from Chinese ginseng (*Panax notoginseng*) encoding 379 potential peptides. The gene was named as pnGlu1 and produced transgenic tobacco lines, they were also showed a significant increase in the JA and other PR protein synthesis.

Reyes- Zambrano *et al.* (2020) observed *F. oxysporum* infected Agave (*Agave Americana*) roots for 0^{th}, 7^{th}, 15^{th} and 30 days under scanning electron microscopy

(SEM) and quantified chitinase and β-1,3-glucanase activity from the sample. They concluded that chitinase activity was maximum in the roots 15 days after pathogen infection.

Materials and Methods

3. MATERIALS AND METHODS

The location of current study entitled 'Éco-friendly management of *Fusarium* rot in cardamom and its impacts on soil health and plant defence mechanism' was Department of Plant Pathology, College of Agriculture, Vellayani and Cardamom Research Station, Pampadumpara, Idukki during the period of 2018-2020.

3.1. SURVEY AND COLLECTION OF INFECTED SAMPLES, ISOLATION OF PATHOGEN AND SCREENING FOR VIRULENCE

Since the pathogen (*Fusarium oxysporum*) infection in cardamom plantation is prevalent during the summer season, survey was carried out in February- May 2019 in two major cardamom cultivated blocks of Idukki districts *viz*., Kattappana and Nedumkandam. One Panchayat each of above blocks were surveyed. Three plantations of size 1ha each were selected from each panchayat and the details are given below. Each plantation was divided into four plots each with 250 plants. Ten plants were selected from each plot and five tillers randomly selected from each plant were scored. The following score chart developed by Dhanya *et al.* (2018) used for the study.

0= No disease

1= 1-10 % of tillers or panicles had fungal lesions

2= 11-25 %of tillers or panicle had fungal lesions

3=26-50 % of tillers had fungal lesions with drying of panicles from tip

4= 51-75 % of tiller stake hold of fungal lesions with partial panicle blight

Based on the data, disease severity (PDI) was calculated using the formula

$$PDI = \frac{\text{Sum of score}}{\text{Total number of tillers observed} \times \text{maximum score given}} \times 100$$

Percent Disease Index was calculated using the formula of Singh (2002).

$$\text{Disease Incidence} = \frac{\text{No. of infected plants}}{\text{Total number of plants observed}} \times 100$$

Five year old well established plantations with yielding plants of most popular local variety Njallani, which is highly susceptible to the disease was chosen for the survey.

3.1.1. Isolation of pathogen

Infected samples (pseudostem, root and panicles) were collected from each surveyed plots for isolation. The infected samples were washed under running water and cut into small bits containing diseased portion along with a small healthy portion. These bits were surface sterilized with 0.1% mercuric chloride for 1 min followed by three washings in sterile distilled water. The excess moisture was removed by placing the surface sterilized bits over a sterile filter paper. The bits were then transferred to Petri dishes containing potato dextrose agar (PDA) medium under aseptic conditions. The Petri dishes were incubated at room temperature (27 ± 3ºC) for 24 – 48 hr. The fungal growth developed on the Petri dishes was transferred to PDA slants (Thomas and Vijayan, 2002).

3.1.2. Screening for virulence

Pathogenicity studies were carried out to find out the virulence of the isolates obtained in 3.1.1. Inoculation was carried out on the live pseudostem of one year old Njallani cardamom plants raised in pots under green house condition. The pseudostem were initially washed with running tap water and artificial inoculation of the pathogen was done by making gentle aberrations on the tillers by pin pricks and placed 5 mm mycelial discs from actively growing seven day old culture using a cork borer. These fungal cultures were kept in position by wrapping it with a thin layer of wet absorbent cotton. The inoculated portion was covered with perforated polythene covers sprinkled with sterile water to provide ideal condition for disease development. The control plants were also maintained in the same way without pathogen inoculation.

For root isolates (Fr_1-Fr_6) the following inoculation procedure was repeated. In addition to that the spore suspension of the pathogen (10^6 cfu mL^{-1}) per plant was

drenched to the base of the plant. The pathogen was reisolated from the infected tissue and was compared to the original isolated to prove the pathogenicity.

3.2. PATHOGEN VARIABILITY STUDIES BY MORPHOLOGICAL, CULTURAL AND PATHOLOGICAL CHARACTERS

3.2.1. Morphological variation of isolates of *Fusarium* sp. causing pseudostem rot in cardamom

The morphological characters of virulent isolates *viz*., the mycelial characters, sporulation and size and shape of conidia were studied by preparing slides stained with cotton blue and observed under 400X and 1000X magnification with Leica DM 750 (Nisha, 2018).

3.2.2. Cultural variability of *Fusarium* sp. isolates causing pseudostem rot in cardamom on PDA medium

The cultural characteristics of the isolates were studied by growing the virulent isolates on PDA medium. The sterilized PDA medium was poured in Petri dishes and allowed to solidify under aseptic conditions. Mycelial disc of 5 mm size from five day old culture was placed at the centre of the Petri dish. The inoculated dishes were incubated at room temperature (27 ± 3°C). Five replications were maintained for each treatment. Observations were made on the radial growth, rate of growth of isolates, growth pattern, colony colour and number of days taken to cover Petri dishes. The growth of the mycelium and sporulation of the isolates were studied by single spore isolation (Booth, 1971).

3.2.3. Sporulation and hyphal characters

Hyphal and sporulation characters of pseudostem isolate Fp_1 and root isolate Fr_1 was studied by slide culture method. Aseptically with a forceps placed a sheet of sterile filter paper in a Petridish. Two flame sterilized glass rod was placed over the filer paper and wet the paper by pouring 4ml of sterile water and place sterile slide on the glass rod. 2 % agar block of 7 mm×7 mm was cut from previously poured in to culture dish to the depth of 3mm. The block was transferred on to the glass slide kept on the glass rods. With inoculation loop the four corners of the agar block was inoculated with cultures and cooled flame sterilized cover slip was placed. The Petri dish was sealed

and kept for incubation for 1-2 days. After incubation period the cover slip was removed and placed it on microscope slide containing drop of lacto phenol cotton blue and observed hyphae and spores (Rijal, 2019).

3.3. MANAGEMENT OF Fusarium ROT USING BIO-AGENTS

3.3.1. Preparation of inoculum of pathogen (mass multiplication)

Sick soil inoculation (Nikam *et al.,* 2011) was followed in one year old cardamom suckers raised in grow bags under green house condition. The pure culture of Fusarium isolate was multiplied on sand maize flour medium prepared in the ratio (9:1). 15 g of maize flour was mixed in 85 g of sand and filled in the polypropelene cover of size 15×20 (150 g per pp cover) and sterilized in an autoclave at 15 Lbs for 30 min. Then, the pure culture of Fusarium isolate (Fp_1 and Fr_1) was inoculated separately into this media and under asceptic condition incubated at room temperature for 15 days. After 15 days, the inoculum rich media (10^7cfu g^{-1}) was taken out from the cover and drenched to the base of cardamom plants raised in polythene bags at a rate of 150 g per plant.

3.3.2. Preparation of *Trichoderma* sp. enriched organic manure

4.5kg dry cowdung was mixed with 0.5 kg thoroughly powdered neem cake. The mixture was moistened by sprinkling water on it. After that 120g talc based preparation of *Trichoderma viride* (KAU strain, 10^8 cfu g^{-1}) was added to it. After thorough mixing *Trichoderma* inoculated cow dung –neem cake mixture was covered with a jute bag and kept in shade for one week. Intermittent mixing and maintained with sprinkling water was done for better growth (KAU, 2016).

3.3.3. Treatments used to manage the Fusarium rot of cardamom

Different treatments of biocontrol agents and fungicide were scheduled into nine treatments as basal applications and foliar spraying. For pot culture experiment fumigated soil was used. The endophytic biocontrol agent AMF was incorporated into the pot as T1 at the time of planting at the rate 20g per 10 kg potting mixture. In the treatment T2, *Trichoderma* enriched organic mixture was prepared and applied at the base of the plants. The bacterial bio agent *Pseudomonas fluorescens* strain PN026 (2 per cent) prepared by mixing 20 g bioagents in one litre of cowdung slurry served as the

treatment T3. The treatments T4, T5 and T6 comprised of combined application of above bio agents (Table 1). In these treatments the spraying was given at monthly interval for three times. T7 comprised of basal application as well as spraying of treatment plants with 2 per cent plant growth promoting rhizobacteria mix II (KAU strain). The fungicide carbendazim @ 2 per cent as spray and drench was used as chemical check (T8) and T9 served as control plants without any management practices. Pathogen was multiplied in sand maize media and was inoculated into the base of treatment plants.

Table 1.Biocontrol agents and fungicide used to manage Fusarium rot of cardamom

Treatments	Basal application (single)at the time of planting	Spray(3 times)Monthly
T1	AMF (KAU strain)-@ 20g pot^{-1} (2%)	-
T2	*Trichoderma viride* (KAU strain) in neem cake FYM mixture @)1kg pot^{-1}	-
T3	*Pseudomonas fluorescens* (KAU strain) (2% in cowdung slurry @1L pot^{-1})	-
T4	AMF (20 g pot^{-1})	*P. fluorescens* (2% in cowdung slurry) @ 0.5 $plant^{-1}$
T5	AMF(20g)+ *T. viride* (FYM mixture @1 kg pot^{-1}) + *P. f*	*P. fluorescens* (2%) @ 0.5 L $plant^{-1}$
T6	AMF(20 g) + *P. fluorescens* (2% @ 1Lpot^{-1}) + *P. f*	*P. fluorescens*(2%) @ 0.5L $plant^{-1}$
T7	PGPR mix II (2% drench) @1L pot^{-1}	PGPR mix II(2%) @ 0.5 L $plant^{-1}$
T8	Carbendazim (2 g L^{-1} drench) 1L pot^{-1}	Carbendazim (2 gL^{-1} spray) 0.5 L $plant^{-1}$
T9	Control (untreated plants)	

3.3.4. Disease incidence and severity of cardamom inoculated with *Fusarium* sp. in response to treatments

To evaluate the disease severity of experiment plants during pot culture study 0-4 scale score chart was used. The severity and incidence was calculates as per the equation mentioned in 3.1.

0- No disease

1- Lesion on 1-10% of tiller

2- Lesion on 11-25% of tiller

3- Lesion on 26-50% of tiller

4- Lesion >50% of tiller

3.3.5. Population dynamics of *Fusarium* sp. and bio control agents in the soil

After inoculation of the pathogen the soil of treated plants were analyzed periodically (monthly) by serial dilution method to assess the load of pathogen and antagonists.10g soil was transferred to 90 mL of sterilized water in a 250 mL conical flask and shook well for 10 min in a shaker to get a solution of 10^{-1} dilution this by transferring and again diluted 1ml from this solution to another 9 ml of sterile water. Serial dilution was continued until a dilution of 10^{-6} was obtained. 10^{-3} dilution was used for enumeration of *Fusarium* sp.,10^{-4} is taken for *Trichoderma* sp. and 10^{-6} gave colonies *P. fluorescens* and *Bacillus* sp. with spread plate method on MRBA (Martin rose Bengal agar), *Trichoderma* specific media (TSM), Kings B media and nutrient agar respectively. One mL from10^{-3},10^{-4} and 10^{-6}dilution was poured into sterile Petri plates contain molted and cooled media is spread over uniformly. The Petri dishes were closed, sealed and kept at room temperature and on the next day onwards the observations on colony count were recorded up to five days (Waksman, 1922).

3.3.6. Root staining for Arbuscular Mycorrhizal Fungi

At the end of the experiment to study the mycorrhizal colonization pattern of AMF inoculated the plant roots were collected, and washed with water. 10 % KOH (W/V) was added to container and made root samples were submerged but the fluid did not fill more than the half of the container. The tissues were soaked overnight and replaced with fresh KOH before the heat treatment. The solution turned into brown

colour by overnight that indicated the tannins were left from tissues. Heat treatment was given by boiling the processed roots in a water bath. The sample solution was removed and rinsed with tap water for three times. After that 5% HCl was added and allowed to retain for about a minute. After that HCl was poured out and added trypan blue stain and examined the stained roots under a microscope (Philip and Haymann, 1970).

$$\text{Per cent root colonization} = \frac{\text{No. of root bits showing colonization}}{\text{Total number of root bits observed}} \times 100$$

3.3.7. Nutrient analysis of plant and soil

The major nutrients (N, P, and K), secondary and micronutrients (Ca, Mg, S and B), are used for evaluating the soil and plant growth status in relation to the effect of biocontrol agents treated plants under Fusarium infected condition. Soils collected three months after treatment application from plants were used for nutrient studies were assessed. Fully expanded 3rd leaf was taken for the nutrient analysis of leaves. The shade dried leaves were further dried in hot air oven at about 60±2^{0}C for three to four hour for complete drying and powdered with help of grinder. The powdered samples were passed through 2mm stainless steel sieves for chemical assay.

3.3.7.1. Determination of total nitrogen in plant

0.5 g of ground plant sample was taken in digestion tube, 10 mL of concentrated sulphuric acid and 5g of catalyst mixture was added to it. The digestion tubes were loaded in to the digester, heated the digestion block and switched the digestion unit. The block temperature was raised to 400^0 C. The sample turned light green colour at the end. The digested sample was distilled in a distillation unit. The delivery end was dipped in a mixture of 20 mL of 4 %boric acid with mixed indicator kept in 250 mL conical flask.40 mL NaOH (40 %) was also added to the distillation unit. The digested sample was heated by passing steam and the liberated ammonia was absorbed in 20 mL of 4% boric acid containing mixed indicator**.** The pinkish coloration of solution had turned to green due to the absorption of ammonia. This distillate was titrated along with blank using 0.02N sulphuric acid and the colour was changed to pink (AOAC, 1995).

$$\text{Nitrogen content in plant(\%)} = \frac{\text{R (sample titer-blank titer)} \times \text{Normality of acid} \times \text{Atomic weight of Nitrogen} \times 100}{\text{sample weight (g)} \times 1000}$$

3.3.7.2. Determination of total Phosphorous in plant

0.5 g of ground plant sample was weighed and collected in a conical flask and 5 mL of conc.HNO_3, 2 mL mixture of equal volume of H_2SO_4 and $HCLO_4$ were added and mixed. Keep the mixture in hot plate and heated till the solution become colorless. The mixture was cooled, filtered and washed the residue from filter paper several times and made up the volume to 100 mL using distilled water. 10 mL of aliquots were transferred to 50 mL volumetric flask and added 10 mL vanadate –molybdate solution and diluted to 50 mL volume with water. 10 minutes after thorough mixing, read absorbance at 440 nm with blank. Took 0, 1, 2.0, 3.0, 4.0 and 5mL of standard P solution separately in 50 ml volumetric flask and 10 mL of vanadomolybdate reagent was added and made up the volume with distilled water. Standard curve was prepared by plotting P concentrations on X axis and reading on Y axis. (Jackson, 1973)

$$\text{Total (\%) P} = \frac{X}{10{,}00{,}000} \times 50 \times \frac{\text{Vol. of extract made}}{\text{Vol. of extract taken}} \times \frac{100}{\text{Wt. of plant sample}}$$

3.3.7.3. Determination of Potassium in plant

Grounded plant sample (1g) was taken in 1000 mL digestion flask and added 20-25 mL of diacid mixture into it. The mixture was heated on a heat plate until the completion of digestion till the solution became colorless. After cooling, 20-25 mL of distilled water was added. The solution was filtered with Whatman No.40 filter paper, filtrate was collected into 100 mL volume flask and made up the volume with distilled water. The aliquots were taken from the solution and the reading was recorded using flame photometer (Black, 1965).

$$\text{K (\%) in plants sample} = X \times 4 \times 10^{-3}$$

3.3.7.4. Determination of Calcium and Magnesium in plants

For determining Ca, 10 mL of plant extract was pipetted out into a conical flask and to which 2 mL of 10 per cent potassium hydroxide solution and 2 mL of murexide indicator solution was added and mixed thoroughly. The solution was titrated with standardized EDTA solution until colour changes from pink to purple. Mg in the plant extract was determined by, to the 10 mL of the plant extract 5 mL of buffer solution and four drops of eriochrome black T solution was added. The solution was titrated against EDTA till a blue colour appeared (Padhye, 1957).

3.3.7.5. Determination of Sulphur in plant

One gram of powdered leaf sample taken in digestion flask, to which 10 mL of Diacid mixture was added and the whole were placed over hot plate. After digestion was completed, it was filtered through filter paper and washed by hot water. Filtrate was collected in 100mL conical flask. Made up the volume using distilled water (Dwivedi, 2012).

Preparation of standard curve:

0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15 mL of working standard S solution was placed into different volumetric flasks of 25 mL.10 mL of aliquot was added into the each flasks and 1 g of $BaCl_2$ crystals was added and mixed till the crystals are dissolved. 1 mL of 0. 25 % gum aracia solution was added to the solution and made up the volume with distilled water. A white colour turbid was developed and read the absorbance at 440 nm by spectrophotometer along with the blanks. The readings were plotted on a graph against sulphur concentration.

$$\text{Amount of Sulphur } (\text{kg ha}^{-1}) = \text{conc.}(\mu\text{g mL}^{-1}) \times 12.5 \times 2.24$$

3.3.7.6. Determination of Boron in plant

0.5 g of sample was taken in porcelain dish and 0.5g of $Ca(OH)_2$ was added. It was ignited in muffle furnace at 550^0C for four hours to get white grey ash. The dishes were cooled and the ash was moistened by adding little amount of distilled water. 5 mL

of 0.1N HCl was added to it and transferred the content in to 25 mL volumetric flask and make up the volume using distilled water. 1 mL of aliquot of blank and diluted boron standard or sample solution in to a polypropelene tubes and 2mL of buffer was added and mixed thoroughly. To the mixture, 2 mL of azomethine-H reagent was mixed and kept for 30 minute and read the absorbance at 420 nm. A standard curve was plotted by B concentration on X axis and absorbance on Y axis (Zenki *et al.,* 1989)

3.3.7.7. Determination of nitrogen in soil (Alkaline Permanganate method)

20 g of soil sample were transferred to digestion tube along with 2-3 beads to prevent bumping and 1mL of paraffin wax were added to prevent frothing. 100mL of potassium permanganate (0.32%) and 100mL of sodium hydroxide solution were added to the distillation flask. A hose was immersed into a conical flask containing 20 mL of boric acid solution with mixed indicator. On heating ammonia gas was liberated and collected in to the boric acid and turned to green colour from wine red. The distillate was collected and titrated against 0.02N, sulfuric acid till the end point as pink colour (Subbiah and Asija,1956)

$$\text{Nitrogen content in soil(\%)} = \frac{\text{R (sample titer blank titer)} \times \text{normality of acid} \times \text{Atomic weight of Nitrogen} \times 100}{\text{Sample weight (g)} \times 1000}$$

3.3.7.8. Determination of Phosphorous in soil (Bray extraction and colorimetric estimation)

5 g of soil sample taken in100 mL conical flask along with 50 mL of Bray No. 1 reagent were mixed by shaking for five minutes. The mixture was filtered through Whatman No. 1 filter paper. 5 mL of the fitrate was pipetted out to 25 mL conical flask and made up the volume using distilled water. Read the density of colour after 10 minutes at 660 nm

Preparation of standard curve:

Different concentration of P (1, 2, 3, 4, 5, and 10 mL of 2µg mL^{-1} P solution are taken in a 25 mL volumetric flask and 5 mL of the extracting reagent was added to it. A blue colour was appeared when reagent B was added. By plotting concentration and absorbance curve on graph paper calculations was done (Watanabe and Olsen, 1965).

$$\text{Available P(kg ha}^{-1}\text{)} = \frac{\text{Absorbance for sample} \times 50 \times 2.24}{\text{Slope of the std. curve}}$$

3.3.7.9. Determination of Potassium in soil

5 g of soil sample was taken in 100 mL conical flask along with 25 mL of 1N ammonium acetate solution and shook for 5 minutes. This mixture was filtered through whatman No.1 filter paper and was read in flame photometer along with blank sample.

Preparation of standard curve:

From the stock solution dilute measured aliquots were taken in 100 mL volumetric flask with ammonium acetate solution to give 10 to 100ppm of Potassium. The flame photometer was adjusted at zero for the blank at zero ppm and 100 for 100ppm of Potassium. A standard curve was constructed by plotting reading against different concentrations of aliquots (Jackson, 1973).

$$\text{Available K(Kg ha}^{-1}\text{)} = R \times \frac{\text{Volume of the extract} \times 2.24 \times 10^6}{\text{Weight of soil taken} \times 10^6}$$

R=ppm of K in the extract (obtained from Std. curve)

3.3.7.10. Determination of Calcium and Magnesium in soil (Versanate method)

5 g of soil sample was shook with 25 mL of neutral normal ammonium acetate for five minutes. The mixture was immediately filtered using Whatman No.42 filter paper. The first few filtrate was discarded and remaining part was used for assay. For determination of Ca in solution, 5 mL of the extract solution was taken in a conical flask along with 5 mL of distilled water, 10 drops each of KCN, hydroxyl amine hydrochloride, tri ethanol amine were added and mixed thoroughly. 25 mL of NaOH was the mixed to the solution followed by 1 mL of calcon solution. The solution was titrated against EDTA to get blue colour. For determining both Ca+ Mg in solution, 5 mL of the soil extract were added with equal amount of water, 15 mL of buffer solution and mixed the solution well. Ten drops each of KCN, hydroxyl amine hydrochloride, tri ethanol amine and potassium hexacyano ferrate were added the solution and mixed.

Finally ten drops of erochrome black T solution was added to the solution mixed and titrated against EDTA. The titration was continued up to a blue end point had reached. Mg is calculated from the difference between Ca+ Mg and Ca determination (Hesse, 1971).

$$\text{Per cent Ca in sample} = \frac{T_{Va} \times N \times 0.02 \times 100 \times 100}{5 \times w}$$

$$\text{Per cent Mg in sample} = \frac{(T_{Vb} - T_{Va}) \times N \times 0.02 \times 100 \times 100}{5 \times w}$$

T_{Vb} = (Ca+Mg)
T_{Va} = (Ca)

3.3.7.11. Determination of Sulphur in soil ($CaCl_2$ extraction method)

Ten gram of air dried soil taken in 150 mL conical flask and 50 mL of 0.15% $CaCl_2$ was added and mixed for 30 minute in mechanical shaker. After thoroughly mixing, the solution was filtered through Whatman No.42 filter paper.

Preparation of standard curve:

0.25, 0.50, 1, 2.5 and 5 mL of standard S solution was taken in separate 25 mL volumetric flask and 10 mL of the extracting solution was added to each flask, then 1 g of $BaCl_2$ crystals were dissolved in the solution by swirling. To the each flask 1 mL of 0.25% gum aracia solution were added and made up volume with distilled water. After 5-30 minutes a white precipitate was formed and the turbidity was read by spectrophotometer at 340 nm. A curve was prepared by drawing S concentration on X axis and absorbance at Y axis (Massouni and cornfield, 1963).

$$\text{Available S in soil (mg kg}^{-1}) = R \times \frac{50}{10} \times \frac{1}{10}$$

3.3.7.12. Determination of boron in soil (Hot water soluble Boron method)

20 g air dried soil was taken in 250 mL conical flask and mixed with 40 mL water. 0.5 g of activated charcoal was added to the mixture and boiled for 5 minute in the hot plate. It was cooled and filtered through Whatman No.42 filter paper. 1 mL of

aliquot of blank diluted boron standard or sample solution was taken in to a polypropelene tubes and 2 mL of buffer was added and mixed thoroughly. 2 mL of azomethine-H reagent was then mixed and kept for 30 minute. Absorbance was read at 420 nm. A standard curve was plotted by B concentration on X axis and absorbance on Yaxis (Gupta, 1967)

$$\text{Amount of B in soil (mg Kg}^{-1}\text{)} = \frac{\text{Absorbance reading}}{\text{Slope from the curve}} \times \frac{40}{20}$$

3.4.INDUCTION OF DEFENCE MECHANISM IN CARDAMOM PLANTS IN RESPONSE TO BIO CONTROL AGENTS

To study the changes in biochemical defence mechanisms in treatment plants in response to bio agent application, post treatment analysis was carried out for following parameters.

3.4.1. Phenol

Fully expanded 3rd younger leaf of cardamom was used for the study, 1g sample was taken for phenol analysis. It was ground in a mortar and pestle with 10 mL of 80% ethanol and the extract was taken centrifuged at 10,000 rpm for 20 min at 4^0C. After collecting the supernatant residue ground with 80% ethanol(1:5) again centrifuged. The supernatant collected in Petri plates allowed to evaporate in evaporation chamber for overnight. The residue was dissolved in 5 mL of distilled water. 0.5mL of Foline-ciocalteu reagent was added to it and after three minutes also added2 mL of 20% saturated solution of sodium carbonate (Na_2CO_3)to that and the mixture was kept in boiling water for 1 minute. A blue layer was developed on the upper layer and a reagent blank was taken for measuring absorbance using spectrophotometer at 650 nm using a blank and regent only (Bray and Thrope, 1954).

3.4.2. OD phenol

The Ortho Di-hydroxy phenol content was estimated according to the method proposed by Johnson and Sachaal (1952).One gram of leaf sample was ground with 80 per cent ethanol to get a fine filtrate and centrifuged at 10,000rpm for 15 min. 1 mL of alcoholic extract, 1 mL of 0.5 N HCl and 1 mL of Arnone reagent, 2 mL of 1 N NaOH and 10 mL of distilled water were added to the supernatant. A pink colour appeared

immediately on addition of NaOH. The colour intensity was reduced by diluting it with 25 mL distilled water and the absorbance read at 515 nm. The concentration of OD was calculated based on a standard curve prepared with catechol.

3.4.3. Peroxidase

Peroxidase enzyme activity was analyzed by grinding 1g leaf sample with 5 mL of sodium phosphate buffer (pH 6.5). Homogenate was filtered using muslin cloth (cotton). It was centrifuged at 5000 rpm for 15 min at 4^0C and collected the supernatant was collected in labeled tube as enzyme extract. Reaction mixture was prepared with 1.5 mL of 0.05 M pyrogallol, 0.5 mL of enzyme extract and 0.5 mL of 1% H_2O_2. The mixture was precipitated at room temperature and took in a cuvette size of 0.5 mL. Spectrophotometer set with a wavelength of 420 nm. In the initial phase, 1 mL of 1% H2O2 was added in cuvette which had contains a mixture of sample and read for 5 minutes. The enzyme activity was expressed in U/mg/ min. One unit of activity was the rate of change of optical density (OD) at 420 nm per minutes (Srivastava, 1987).

3.4.4. Polyphenol oxidase

Pinch of PVP was added to the extract prepared by homogenizing 1g of leaf sample in 5mL of 0.1M sodium phosphate buffer (pH 6.5). It was centrifuged at 5000 rpm for 15 min. The supernatant was collected and the reaction mixture was prepared by adding 3 mL of 0.05 M pyrogallol, 1 mL of enzyme preparation and one ml of 1 % $H_2O_2$and incubated at 28 ± 1 °C. At the start of the enzyme reaction, the absorbance of the mixture was adjusted to zero at 495 nm and changes in the absorbance were recorded at 30 seconds intervals. Standard reaction mixtures consisted of 3 mL of 0.1 M phosphate buffer (pH 6.5), 2 mL of enzyme preparation and 1 mL of 0.01 M catechol. The above mixture was incubated at 28 ± 1°C and absorbance was set to zero at 495 nm at the commencement of the reaction. The PPO activity was expressed as changes in the OD of the reaction mixture at 495 nm in the specified time of reaction (Mayer and Harel 1979).

3.4.5. Phenylalanine ammonia lyase (PAL)

1g leaf sample was homogenized in 5 mL of 0.1 M sodium borate buffer of pH 8.8. After the addition of pinch of a PVP, the extract was centrifuged at 10,000 rpm for

10 min at 4^0C. The reaction mixture comprised of 3mL of 0.1M sodium borate buffer, 0.2 mL of enzyme extract and 0.1mL of12 µL phenylalanine kept for incubation at 40^0C for 30 min. OD value was recorded at 290nmin spectrophotometer.

Standard curve of cinnamic acid with different concentration was prepared with 10g cinnamic acid dissolved in 10ml sodium borate buffer. 1 mL aliquot of this solution made up to 10mLusing distilled water. From this stock solution different volume of solution was taken out (0,0.2, 0.4 …, 1ml) made up to 1ml and read absorbance at 290 nm (Dickerson *et al.,* 1984).

3.4.6. β-1,3-Glucanase

Leaf sample of 0.5g were extracted with 5 mL of 0.1M sodium acetate buffer(pH 5.0) at 4^0C. The extract was centrifuged to rpm of 10,000 for 15 min at 4^0C and the supernatant was collected. To the 0.075mL enzyme extract 0.075 mL of 4 per cent laminarin was added and incubated at 40^0C for 10 minute. The reaction was stopped by adding alkaline copper reagent and the mixture was boiled for 10 minute in water bath. After cooling the β-1,3- glucanase was read by spectrophotometer by 620nm and compared against heat inactivated controls.(Pan *et al.,*1991)

3.5.STATISTICAL ANALYSIS

The data obtained from *in vitro* and in *vivo* studies were subjected to analysis of variance (ANOVA) after appropriate transformation. When the effects were found to be significant, critical difference value was calculated for each observation using 't' values at 5 per cent level of significance. Significant treatments were compared with CD value. All the data were analyzed using WASP 2.0 software and OP STAT.

Results

4. RESULTS

The present study on "Eco-friendly management of *Fusarium* rot in cardamom and its impact on soil health and plant defence mechanism'' was conducted during the period 2018-2020 at Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram and Cardamom Research Station, Pampadumpara, Idukki, Kerala. The main objective of the study was to evolve an effective management strategy for Fusarium rot of cardamom through the individual and combined application of biocontrol agents and its comparison with the fungicide carbendazim as chemical check. The results from the study are detailed here.

4.1. SURVEY, COLLECTION OF INFECTED SAMPLES, ISOLATION OF PATHOGEN AND SCREENING FOR VIRULENCE

4.1.1. Survey and Collection of samples

The incidence of Fusarium rot is at its peak during the summer season Survey was carried out during February -May of 2019 in the plantations of Idukki district. Three plantations each from Pampadumpara panchayat and Erattayar panchayats of Nedumkandam and Kattappana block respectively were identified as hotspots for the disease, panchayats located in Nedumkandam block and Kattappana block respectively were selected for the survey and sample collection. In surveyed locations, the per cent disease index or severity ranged from 50.40-84.8 per cent. The maximum disease index was noticed in farmer's field -2 (84.8%) located at Pampadumpara panchayat followed by fields of Cardamom research station (field-1) and farmer's field-6 (77.4%) of Erattayar panchayat of Kattappana. Least disease incidence was recorded in the farmer's field-4 (plate.7) of Erattayar panchayat and farmer's field -2 of Pampadumpara panchayat, both with 60 % infection (Table 2). The prominent symptoms observed during the survey were eye shaped brown lesions on the pseudostem, lodging of tillers and partial or complete drying of panicle (Plate.1).

Table. 2. Incidence and severity of Fusarium rot of cardamom in different blocks of Idukki district

Sl no.	Location of collection	Block	Panchayats	PDI (%)	DI (%)
1.	Cardamom research station (field-1)	Nedumkandam	Pampadumpara	77.40	100.00
2.	Farmers field-2		Pampadumpara	84.80	66.00
3.	Farmer’s field-3		Pampadumpara	64.00	92.00
4.	Farmer’s field-4	Kattappana	Erattayar	50.40	60.00
5.	Farmer’s field-5		Erattayar	55.00	73.07
6.	Farmer’s field-6		Erattayar	77.40	80.00

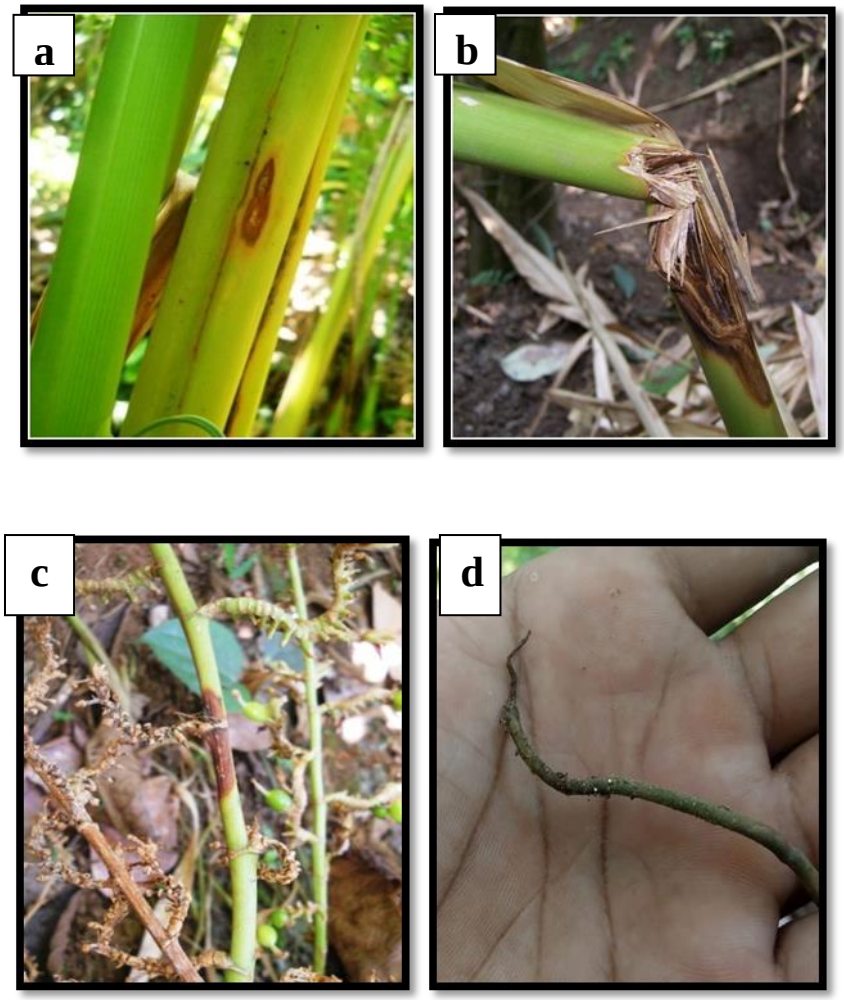


Plate 1. Fusarium rot symptom on pseudostem, panicle and root (a) Intial stage(b)Advenced stage(c) burnt appearance on panicle (d) root rotting

Plate 2.Fusarium infection on pseudostem in cardamom fields of CRS,Pampadumpara (field -1)
(a)Eye shaped lesion on the tiller (b)stem lodging(c) severe stem lodging

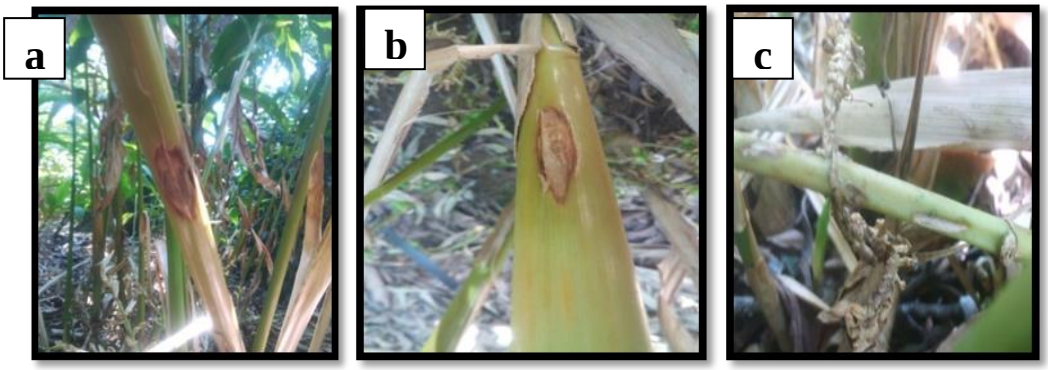


Plate 3.Fusarium infection on pseudostem and panicle of cardamomom in the farmers field of Pampadumpara (field -2)
(a) and (b) Eye shaped lesion on pseudostem (c) lesion on panicle

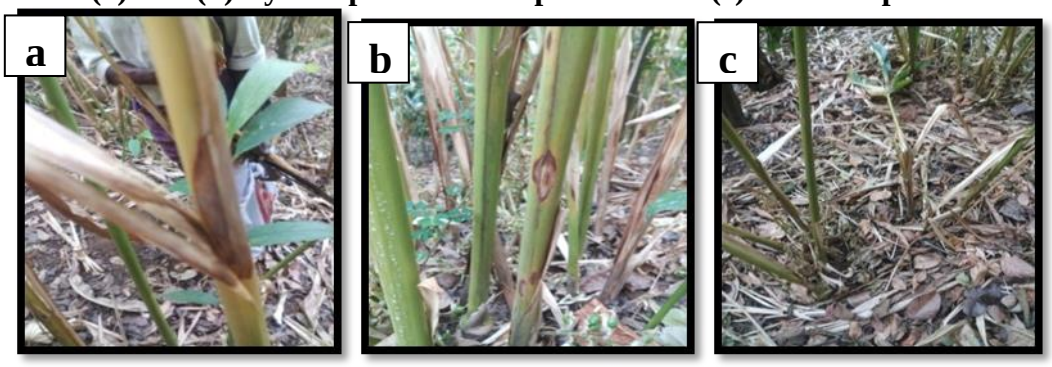


Plate 4. Fusarium infection on pseudostem of cardamom in farmers field of Pampadumpara (field-3)
(a) and (b) Eye shaped lesion on pseudostem(c) Stem lodging

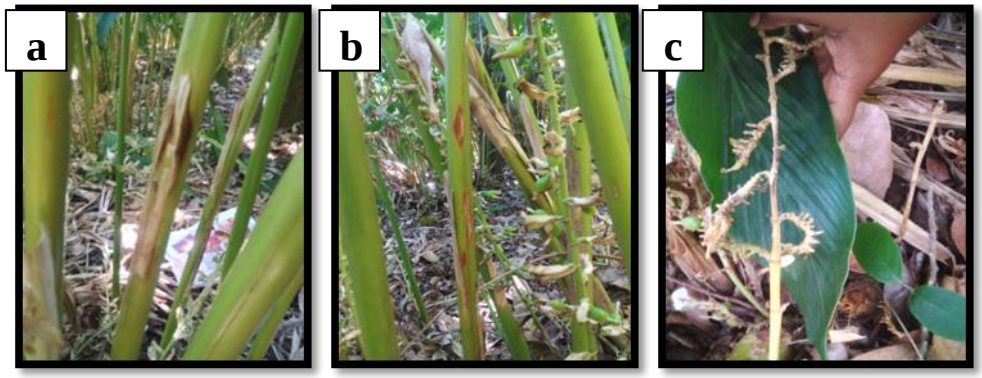


Plate 5. Fusarium infection on pseudostem and panicle of cardamom in farmers field of Erattayar (field-4) (a) and (b) Eye shaped lesion on pseudostem (c) Burned appearance of panicle

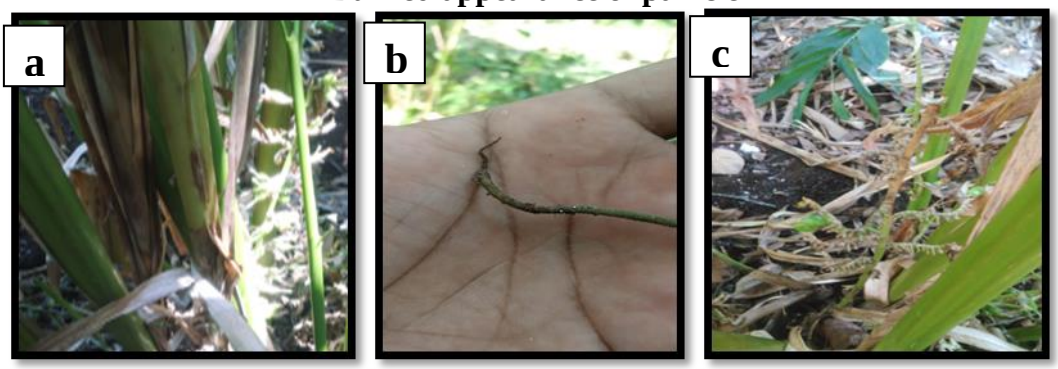


Plate 6. Fusarium infection on pseudostem, panicle and root of cardamomin farmers field of Erattayar (field-5) (a) Eye shaped lesion on pseudostem (b) Root tip rotting (c) Burned appearance on panicle

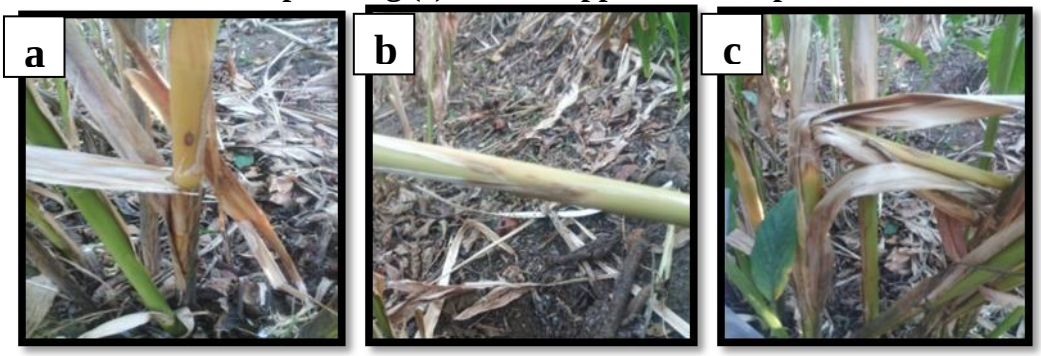
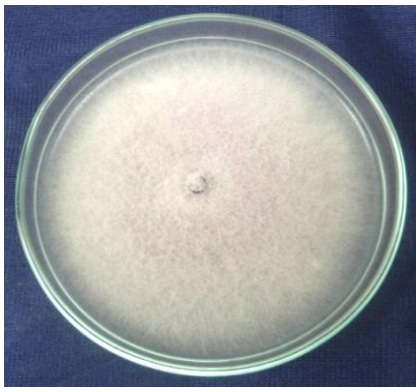
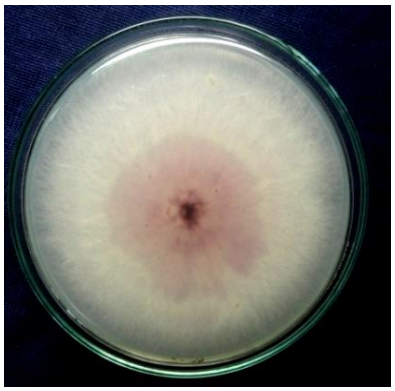


Plate7.Fusarium infection on pseudostem of cardamomo in farmers field of Erattayar (a) and (b) Eye shaped lesion on pseudostem (c) Stem lodging

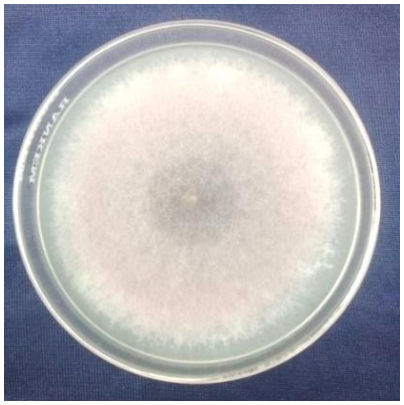
Front view **Rear view**



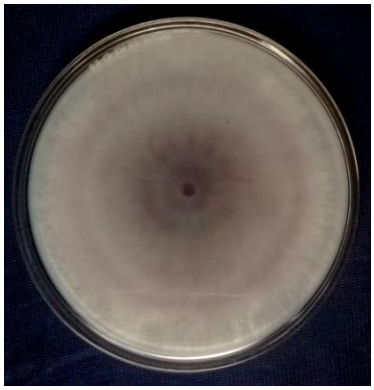
(a)



(a)



(b)



(b)

Plate 8.*Fusarium* sp. cultures isolated from pseudostem and roots of cardamom and grown on PDA medium-7th day (a-pseudostem isolates Fp$_1$, b-root isolates Fr$_1$)

4.1.1. Symptomatology of the disease

Characteristic symptoms of the disease were dry rotting on the pseudostem mainly in the summer season. Eye shaped lesions were developed on various parts of the pseudostem. In the advanced stages the affected portion of the pseudostem broke at the point of infection. The roots also showed root rotting from the tip (Plate1.) resulted in reduced water and nutrient uptake. Leaves of the severely infected plants turned yellow. Symptoms on panicles started as drying of the tip and subsequently spread to the whole panicle resulted in a burnt appearance.

4.1.2. Isolation of the pathogen

The fungus was isolated from infected pseudostem and roots having typical symptoms (eye shaped lesion and rotting respectively) collected from all six surveyed fields of Pampadumpara and Erattayar panchayats. The pathogen was isolated as per the standard procedure described in 3.1.2. Based on the morphological characters (colour and hyphal growth pattern) and microscopical studies (hyphal characteristics and nature of spore) the fungus was identified as *Fusarium* sp. The isolate obtained from tiller is labeled as Fp (Fp_1-Fp_6) and root as Fr (Fr_1-Fr_6). The cultures were maintained on sterile PDA medium.

4.1.3. Screening for virulence

The pathogenicity studies of the collected isolates of *Fusarium* sp. were conducted by artificial inoculation of the pathogen on the pseudostem of one year old cardamom plants by pin prick method. The isolate Fp_1produced symptoms on pseudostem as necrotic spots with brown border within one month after inoculation. The isolate produced typical eye shaped lesion (1.5×0.35 cm) as Plate 9.

Koch‘s postulates

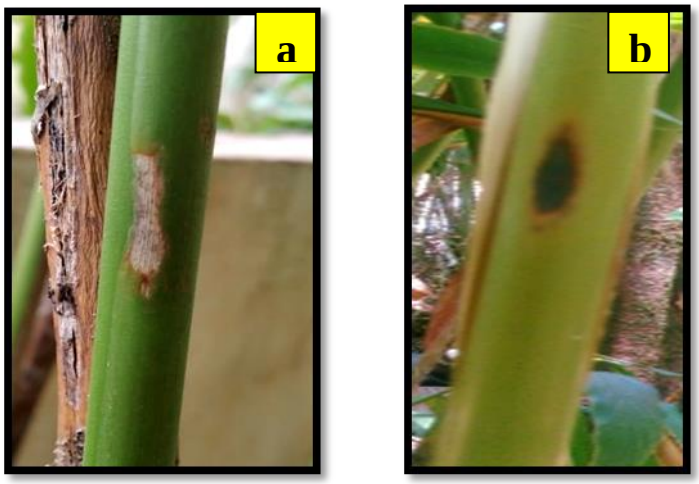


Plate 9. Symptom development on pseudostem of cardamom by *Fusarium* sp. on artificial inoculation by pin prick method (a) Fp_1 (b) Fr_1

Table 3. Characteristics of lesions produced on pseudostem of cardamom by isolates of *Fusarium* sp.

Isolates	Locations	Mean lesion size after inoculation		Rate of development of lesion (cm day^{-1})	Days taken for symptom development
Fp_1	Pampadumpara	length	1.50±0.25	0.08	4 weeks
		Width	0.35±0.05		
Fr_1	Erattayar	Length	0.90±0.10	0.03	6 weeks
		Width	0.45±0.05		

(Values are mean of 6replication±SD)

Fr_1 inoculated plants showed typical root tip rotting symptom about two months after inoculation (Plate 9). The lesion developed by this root isolate on pseudostem was of size 1×0.4cm. Symptom development was delayed for other isolates and was not so prominent as that of Fp_1 and Fr_1 (Table 3). There for Fp_1 and Fr_1 were identified as virulent isolates. The pathogen was re-isolated from the artificially inoculated plants. The re-isolated cultures were identical to the original culture and hence the Koch's postulates were proved.

4.2. PATHOGEN VARIABILITY STUDIES BY MORPHOLOGICAL, CULTURAL AND PATHOLOGICAL CHARACTERS

4.2.1. Morphological characters

The morphological characters of the virulent isolates were studied using the technique mentioned in 3.2.1.and the results are given in table.3 and Plate 8

The virulent isolates (Fp_1 and Fr_1) were cultured in the PDA medium at room temperature for the detailed study of growth pattern and sporulation. Both cultures showed white fluffy growth, irregular marginal and pinkish pigmentation at the rear side of the Petri plate.

Table 4. Morphological characters of different isolates of *Fusarium* sp. causing pseudostem rot in cardamom on PDA medium

Isolates	Growth pattern	Characteristics of the mycelium on PDA after 7 days of growth	
		Frond view	Rear view
Fp_1(pseudostem)	Fluffy	White growth	Pinkish tinge at the base
Fr_1 (root)	Fluffy	White growth	Pinkish tinge at the base

4.2.2. Cultural characters

4.2.2.1. **Rate of growth of mycelium**

Isolate Fp_1 showed a faster growth (1.55cm per day) and covered the Petri dish (9 cm) within six days whereas the culture Fr_1 completed its growth by seven days (1.25 cm per day) under room temperature (Table 6). The initial growth of culture Fp_1 was comparatively slow but after five days, it gained more rate of establishment.

Table 5. Cultural variability of *Fusarium* sp. isolates causing pseudostem rot in cardamom on PDA medium

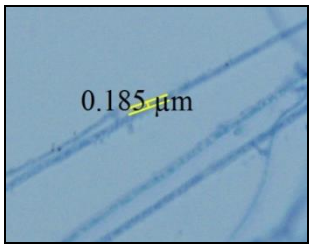
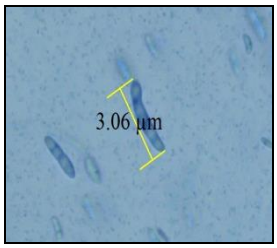
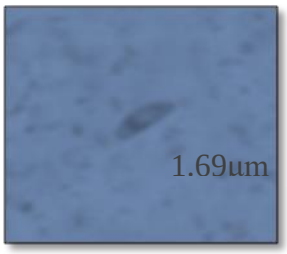
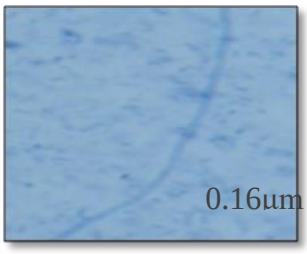
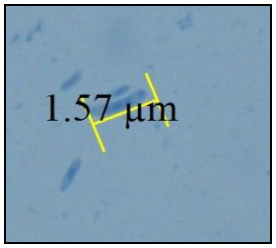
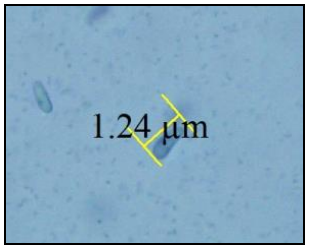
Isolates	Growth in Petri dishes			Rate of growth (cmday-1)	DTCP
	3rd day	5 th day	7 th day		
Fp1	3.30±0.70	5.80±0.10	9.00±0.15	1.55	6
Fr1	3.40±0.26	6.44±0.47	8.70±0.10	1.25	7
SE(m)	0.19	0.34	0.12	0.04	0.50

(DTCP: Days taken to cover 9cm Petri dish)

4.2.2.2. Sporulation and hyphal characters

Microscopical studies of Fusarium isolates confirmed production of macro and micro conidia (Table 6). Macro conidia were hyaline in nature, two to several celled and sickle shaped, whereas microconidia were 1-2 celled, hyaline and ovoid in shape.

Table 6. Morphological variation of isolates of *Fusarium* sp. causing pseudostem rot in cardamom

Isolates	Width of the mycelia(m)	Conidial characters	
		Macro conidia	Micro conidia
Fp1			
Fr1			

Both pseudostem and root isolates showed considerable variation in the size of conidia. The isolate Fr_1 produced macroconidia of size 1.57x0.43 µm in length and microconidia of average size of 1.34x0.34µm. While culture Fp_1 produced microconidia and microconidia of size 3.00 x0.22µm and 1.69 x 0.13 µm respectively (Table 6 and 8). Mycelia produced by the isolate Fp_1 were hyaline in nature with a width of 0.185µm. Eventhough Fr_1 also produced hyaline hyphae but its width was slightly less (0.165µ) compared to Fp_1 (Table 7)

Table7.Mycelial characters of Fusarium isolates causing Fusarium rot in cardamom

Isolates	Mycelial width (µm)	Nature of mycelium (µm)	Colour
Fp_1	0.185	Septate	Hyaline
Fr_1	0.165	Septate	Hyaline

Observations were made from seven days old culture

Table 8. Conidial characters of *Fusarium* isolates causing Fusarium rot in cardamom

Isolates	Conidial characters	
	Macro conidia(µm)	Micro conidia (µm)
Fp1	3.00×0.22	1.69×0.13
Fr1	1.57× 0.43	1.24×0.34

Plate 10. Mass multiplied of *Fusarium* sp. in sand maize media after 15 days

Plate 11. *Trichoderma* sp.mass multiplication in cowdung neem cake mixtures

Plate 12. Pot culture study on management of *Fusarium* sp. at Cardamom Research Station, Pampadumpara

4.2.3. Pathological characters

The isolate Fp_1 produced typical symptoms on pseudostem in about 29 days. Root isolate (Fr_1) also produced symptoms on pseudostem as well as the root. But it took more days (40) to reproduce the symptom on pseudostem compared to Fp_1.

Table 9. Pathological variability study of isolates of *Fusarium* sp. causing pseudostem rot in cardamom

Isolates	Symptoms on cardamom plants	No. of days taken for symptom development
Fp_1(pseudostem)	Typical eye shaped lesion on tiller	29
Fr_1 (root)	Eye shaped lesion on tiller and root rotting	40

4.3. MANAGEMENT OF FUSARIUM ROT USING BIO-AGENTS

Pathogen was multiplied as per the method mentioned in 3.3.1.and was inoculated into the base of treatment plants. The basal application of different treatment was done as described in 3.3.3. The spraying was given at monthly interval for three times. The pot culture unit was kept under shady area in CRS Pampadumpara.

4.3.1 Disease incidence and severity of cardamom inoculated with *Fusarium* sp. in response to treatments

Three months after the experiment, the impact of treatments on Fusarium disease management was recorded. Disease incidence and disease severity were highest for the untreated control plants (100% and 87.50 % respectively). Minimum disease incidence and severity was recorded for treatment T6 where combinations of AMF and *P. fluorescens* were given as basal application along with 2 per cent *P. fluorescens* as foliar spray (35.33% and 17.00% respectively). The effect of this treatment was on par with treatment T5 where combined application of AMF and *T. viride* was given as basal application with *P. fluorescens* spray (41.00% and 17.50%). This was followed by the chemical check carbendazim, effectively managed the Fusarium rot infection. Basal application of AMF along with 2 per cent *P. fluorescens* spray was found be the next best treatment recording 27.50% disease severity and it was on par with sole application of *T. viride* (as basal application) (30.00%). The effect of individual application of

AMF (as basal application) and PGPR mix II were also on par, but poorly managed the disease compared to all other treatments, but was significantly superior to untreated control.

Table 10. Disease incidence and severity of cardamom plants inoculated with *Fusarium* sp .in response to treatments

	Treatments	Disease incidence	Disease severity
T1	AMF(BA)	88.83(100.00)^{e}	39.23(40.00)^{cd}
T2	*Trichoderma viride* (BA)	46.43(52.50)^{c}	32.89(30.00)^{bc}
T3	*Pseudomonas fluorescens*(BA)	55.25(67.66)^{d}	37.75(37.50)^{bcd}
T4	AMF(BA)+*P.f* (spray)	43.56(47.50)^{c}	31.60(27.50)^{bc}
T5	AMF+ *T.viride*(BA)+*P.f*(spray)	39.81(41.00)^{b}	24.67(17.50)^{a}
T6	AMF+ *P. f*(BA)+*P.f*(spray)	36.27(35.33)^{a}	24.26(17.00)^{a}
T7	PGPR mix II	54.63(66.50)^{d}	39.23(40.00)^{cd}
T8	Carbendazim	40.10(41.50)^{b}	29.88(25.00)^{b}
T9	Control (untreated plants)	88.34(100.00)^{e}	69.38(87.50)^{d}
	CD (0.05)	3.08	8.89
	C.V	2.48	28.57

(Values in parenthesis are Arc sin transformed)

Plate 13. Reduction in symptom development of small cardamom plants inoculated with *Fusarium* sp. in response to the application of best treatments (T5 and T6)

4.3.2. Population dynamics of *Fusarium* sp. and biocontrol agents in soil

The mode of action of various bioagents against *Fusarium* sp. was studied based on the pathogen antagonist ratio in soil. Pathogen inoculum was added to the soil in month of August and the pathogen antagonist ratio was recorded from October onwards at periodical interval for three months (Table 11). Distinguishable, flat white cottony colonies of *Fusarium* sp. were got during enumeration from MRBA medium. From growth pattern of initial white spreading mycelial growth and later green spores was identification of *Trichoderma* sp. from TS media

One month after pathogen inoculation minimum pathogen population was observed for combined application of AMF with *T. viride* (T5) and *P. fluorescens* (T6) followed by combined application of AMF with *P. fluorescens* (as basal application and spray).

Two months after pathogen inoculation AMF with *P. fluorescens* (T6) application reduced the population of pathogen in the soil considerably followed by treatments of AMF with *T. viride* (T5) and *P. fluorescens* (as basal application and spray).

Data after three months revealed that pathogen population is still high in control pots. At this stage both the treatment ie, combined application of AMF either with *P. fluorescens*(T6) or *T.viride* (T5) as basal application and *P. fluorescens* spray as well the application of fungicide like carbendazim were the best in managing the pathogen population in soil and were on par.

It was also found that when bioagents like AMF was given as combination either with *T. viride* or *P. fluorescens* the interaction effect promoted the growth of bioagents in soil (Table 12).

Table 11. Population dynamics of *Fusarium* sp. and biocontrol agents in soil

Treatments	October				November				December			
	Fusarium sp. (10^3)	*P.fl* (10^6)	*Trichoderma*(10^4)	*Bacillus*sp. (10^6)	*Fusarium* sp. (10^3)	*P.fl* (10^6)	*Trichoderma*(10^4)	*Bacillus*sp.(10^6)	*Fusarium* sp. (10^3)	*P.fl* (10^6)	*Trichoderma*(10^4)	*Bacillus*sp. (10^6)
AMF	16.50^{bc}	-	-	-	19.50^{a}	-	-	-	8.50^{b}			
T.viride	16.50^{bc}	-	8	-	15.50^{c}	-	2.5	-	4.00^{de}	-		-
P. fluorescens	15.00^{cd}	5	-	-	17.00^{b}	2	-	-	5.00c	1	-	-
AMF +*P.f*	16.00^{bc}	7	-	-	12.00^{d}	1.5	-	-	3.50^{ef}	1	-	-
AMF+*T.viride*	12.75^{d}	3	8	-	11.00^{de}	1	5.5	-	2.00^{f}	1	6	-
AMF+ *P. f* + *P. f*	15.00^{cd}	3	8	-	10.50^{c}	1	5.5	-	2.00^{f}	6	-	-
PGPR II	17.50^{ab}	3.5	-	6	17.75^{b}	1	-	1	4.00^{cd}	1	-	6.5
Carbendazim	10.50^{e}	-	-	-	12.50^{d}	-	-	-	2.00^{f}	-	-	-
Control	19.00^{a}	-	-	-	20.50^{a}	-	-	-	22.00^{a}	-	-	-
CD(0.05)	1.67				2.73				1.16			

Table 12. Population dynamics of *T. viride* and *P. fluorescens* in soil three month after imposing treatments.

Treatments (basal application	*T. viride* (10^{-4}) cfu g^{-1}	*P. fluorescens* (10^6) cfu g^{-1}
T2- *T. viride*	2×10^4	-
T3- *P. fluorescens*	-	1×10^6
T5-AMF+*T. viride*	6×10^4	-
T6-A MF+*P. fluorescens*	-	6×10^6

4.3.3. Root staining for Arbuscular mycorrhizal fungi

At the end of the experiment roots in cardamom plants inoculated with AMF was stained as per the method in experimental cardamom plants as per the method described in 3.3.5.

T1 (application of AMF), T5 (AMF+ *T. viride* as basal dose) and T6 (combined application of AMF and 2 per cent *P. fluorescens* as basal dose) and control plants were selected for staining. All the treatment plants except control showed good colonization three months after inoculation (Plate 13).

Colonization per cent recorded was highest for cardamom plants treated with AMF and *P. fluorescens* as basal application (T6) followed by AMF with *T. viride* as basal application (T5) than their individual application (Table 13). Interaction effect of these biocontrol agents enhanced the colonization of AMF in cardamom roots challenged inoculated with *Fusarium* sp.

Table 13. Colonization per cent of AMF in cardamom roots when applied alone and in combination with *T. viride* and *P. fluorescens*

Treatments		Colonization per cent
T1	AMF	50.55
T5	AMF+*T. viride*	68.76
T6	AMF+*P. fluorescens*	80.00
T9	Control	0.00

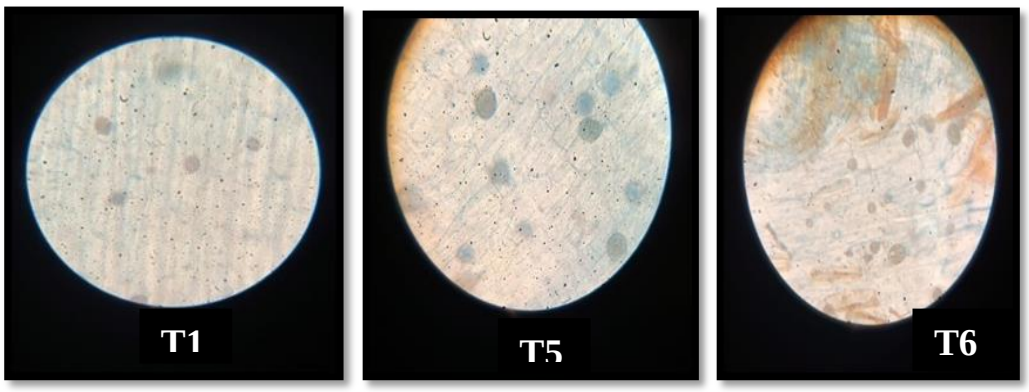


Plate 14. Colonization of AMF in roots of small cardamom plants in response to *P. fluorescens* and *T. viride* application

(T1) AMF, (T5) AMF+ *T. viride* and (T6) AMF +*P. fluorescens*

4.3.4. Effect of biocontrol agents on biometric characters of cardamom plants inoculated with *Fusarium* sp.

Biometric characters such as tiller number, tiller length, number of leaves, leaf length and width, of treated plants were recorded at the end of the experiment and given in the Table 14. The treatment plants responded in a significantly variable manner for the vegetative characters.

Basal application of (20 g) AMF with 2 per cent *P. fluorescens* as basal application and spray (T6) resulted in maximum plant height followed by basal application of *P. fluorescens* in cowdung slurry (T3). Combination treatment comprised of AMF as basal application and *P. fluorescens* as spray (T4) were the next best treatment. Untreated control plant had minimum plant height among the treatments.

The various treatments distinctively influenced the vegetative character like the total number of leaves in experiment plants. Sole application of *P. fluorescens* (T3) to the soil resulted in maximum number of leaves followed by combined application of AMF and *P. fluorescens* as basal dose along with *P. fluorescens* spray (T6). Individual application of AMF (T1) and its combination with *P. fluorescens* (T4) and *T. viride* (T5) also produced more number of leaves and their effect on par with that of individual application of PGPR mix II (T7). It was also found that bio agent application (individually and in combination) promoted the leaf production in plants compared to fungicide application.

Considerable variation was observed in leaf length of plants exposed to various treatments. Highest leaf length was recorded for basal application of AMF+ *P .fluorescens* as basal application and spray (T6), followed by basal application of AMF with *P. fluorescens* (T4). AMF and *T. viride* (T5) as combined application recorded as the next best treatment in leaf width whereas untreated control plants recorded least leaf width.

In the study there was no significant difference was observed in the case of spray, number of tillers and leaf width between the treatments.

As a conclusion drawn from the data on vegetative characters revealed that application of AMF and *P. fluorescens* as single basal application and in combination

with *P. fluorescens* spray (T6) showed maximum plant height and leaf length and number of leaves. Sole application of *P. fluorescens* also promoted plant height and number of leaves. There for the treatments comprised of *P. fluorescens* and AMF individually and in combination resulted in good vegetative growth of treatment plants.

Table 14.Effect of biocontrol agents on biometric characters of small cardamom plants inoculated with *Fusarium* sp.

Treatments	Plant height (cm)	No.of Tillers	No.of Leaves	Leaf length (cm)	Leaf width (cm)
AMF	53.15^{cd}	3.00	14.50 ^{abc}	38.50 ^{cd}	10.00
T. viride	66.90^{bcd}	3.00	10.45^{bcd}	37.75^{d}	9.50
P. fluorescens	92.00^{ab}	3.50	17.25^{a}	43.60 ^{bcd}	11.66
AMF+*P.f*	75.70 ^{abc}	4.00	14.75^{abc}	59.50^{ab}	11.25
AMF+ *T.viride* +*P.f*	69.30^{abcd}	3.50	14.00^{abc}	46.30 ^{abcd}	10.50
AMF+ *P. f* +*P.f*	96.50 ^{a}	4.50	15.75^{ab}	62.50^{a}	12.83
PGPR mix II	60.45 ^{cd}	4.50	14.25^{abc}	56.10 ^{abc}	11.80
Carbendazim	60.30 ^{cd}	2.50	10.25^{cd}	41.90^{bcd}	9.00
Control	41.00 ^{d}	2.00	7.50^{d}	30.00^{d}	8.00
CD(0.05)	29.34	NS	5.43	30.46	NS
C.V	18.86	NS	18.26	17.47	NS

4.3.5 Nutrient analysis of plant and soil

Nutrient analysis of soil and plant samples were carried out as per the method described in 3.3.7. From Table 15, it is evident that the treatment imposed significant effect on the available nutrient status viz. primary, secondary and micro nutrients. The mean values ranged between 210.78- 241.39 kg ha^{-1} (available N), 110.62-154.79 kg ha^{-1} (available P), 262.16-319.00 kg ha^{-1} (available K), 313.99-544.92 ppm (available Ca), 69.75-101.81 ppm (available Mg), 14.99-39.07 ppm (available S) and 0.16-0.33 ppm (available B).

In the case of available N, the highest level of N (241.39 kg ha^{-1}) was noticed in soil in combination of AMF and *T. viride* (T5) was given as followed by application of PGPR mix II (233.04 kg ha^{-1}) was on par with chemical treatment (232.37 kg ha^{-1}). From the Table 15 it was observed that the application of AMF alone enhanced the P content of the soil. The highest value was noticed in treatment T4 with the application of AMF and 2 per cent *P. fluorescens* in cowdung slurry (154.09 kg ha^{-1}), which was on par with combined basal application of AMF and 2 per cent *P. fluorescens* (152.82 kg ha^{-1}). The lowest value for available P was noticed in control plants (T9).

Significant effect of treatments was noticed with respect to available K status (Table 15). Basal application of AMF (303.81kg ha^{-1}) increased availability of K in soil than control (262.16 kg ha^{-1}). It was higher when AMF in combined applications (T4, T5, T6) and recorded highest in T6 (319.00 kg ha^{-1}) with 2 per cent *P. fluorescens* and was followed by sole application of *T. viride* (305.55 kg ha^{-1}).

Combined application of AMF and *P. fluorescens* (T6) resulted in the highest level of Ca (544.92 ppm) in soil followed by T2 with the sole application of *T. viride* (461.88 ppm). T1 with the application of AMF resulted in the highest B content (0.33 ppm) followed by combined application of AMF and *T. viride* (0.31 ppm).

Application of PGPR mix II (T7) enhanced the Mg and S content in the soil significantly (101.81 ppm and 39.07 ppm respectively). The lowest values with respect to available Mg and S was noticed in control plants (69.75 ppm and 14.9 ppm)

From Table 16, the plant nutrient analysis of small cardamom showed, significantly varied nutrients contents in accordance with different of treatment applications. The mean values ranged between 0.91 to 2.29% (available N), 0.01 to 0.20 % (available P), 0.64 to 2.62% (available K), 0.37 to 0.59 ppm (available Ca), 0.30 to 0.59 ppm (available Mg), 0.08 to 0.25 ppm (available S), 3.50 to 11.00 ppm (available B).

Available N was increased highest in the leaves of T2 plants with the sole application of *T. viride* (2.29 %) followed by its combination with AMF in T5 (1.84 %). Lowest N was found in PGPR mix II (T7), chemical control (T8) and control plants (T9) with 0.96%, 0.96%, and 0.91 % respectively were on par.

No significant difference was noticed in treated plants in availability of P. Available P was found to be highest in combined application of AMF with *T. viride*

(T5) of 0.20 % and it was followed by T3 (solely application of *P. fluorescens*) recorded with 0.19 % and the least content 0.01 % from control plants (T9).

It was observed that (Table 16) available K was significantly found higher in AMF combinations (T4 and T5) with either *P. fluorescens* (2.91 %) or *T. viride* (2.62 %) and was on par. The next best treatment was T2 with application of *T. viride* alone (2.05%). The lowest K content 0.64 % was recorded from control plants (T9).

Ca level was increased highest in individual basal application of bio control agents by enriched *T. viride* (T2) of 0.59 ppm and followed by *P. fluorescens* (T3) of 0.50 ppm. Least concentration was found in control plants (T9) with 0.37 ppm.

Level of Mg was elevated significantly in the leaves of combination treatments of AMF (T4, T5 and T6). It was highest (0.68 ppm) for treatment application of AMF with 2 per cent *P. fluorescens* in cowdung slurry (T4) followed by (0.67 ppm) combined application of AMF either with 2 per cent *P. fluorescens* (T6) or *T. viride* (0.64 ppm) in T5. 0.30 ppm (T9) was the lowest concentration recorded among the treatments.

S content in plants was higher with application of AMF (T1) and its combination treatments (T4, T5 and T6). Sole basal application of AMF (T1) and its combination with *P. fluorescens* (T6) had the similar effect on S concentration in plants by highest concentration of 0.25 ppm, followed by 0.24 ppm in AMF with enriched *T. viride* (T5) and 0.22 ppm in AMF with 2 per cent *P. fluorescens* in cowdung slurry (T4) were on par. T9 showed lower concentration of 0.08 ppm.

It was evident (Table 16) that plant B content was highest for AMF with 2 per cent *P. fluorescens* (T6) of 11.00 ppm followed by B concentration of 8.75 ppm in T5 (AMF with enriched *T. viride*) and 8.50 ppm in T4 (AMF with 2 per cent *P. fluorescens* in cowdung slurry), and was on par. Concentration was found to be lower in leaves of control plants.

From the study it was concluded that AMF with 2 per cent *P. fluorescens* as basal application along with three spays of *P. fluorescens* (T6) was the best treatment by improved the soil nutrient availability of N (225.25 kg ha^{-1}), P(152.82 kg ha^{-1}), K (319.00 kg ha^{-1}), Ca (544.92 ppm), Mg (95.75 ppm), B (0.28 ppm) and enhanced plant nutrient status of available N (1.33 %), P(0.15 %), K(1.93 %) ,Ca (0.46 ppm), Mg (0.67 ppm), S (0.25 ppm) and B(11.00 ppm) over control.

Table.15. Primary, secondary and micro nutrient content of soil after imposing various treatments

Trmnts	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Ca (ppm)	Mg (ppm)	S (ppm)	B (ppm)
T1	229.26 ^{c}	145.69 ^{b}	303.81^{b}	388.55^{d}	79.48^{e}	24.27^{f}	0.33^{a}
T2	216.10^{g}	119.48^{g}	305.55^{b}	461.88^{b}	76.90^{f}	36.68^{b}	0.21^{e}
T3	219.65^{e}	139.25^{c}	297.60^{c}	349.33^{f}	85.50^{d}	33.91^{c}	0.23^{de}
T4	226.02^{d}	154.09 ^{a}	316.51^{a}	450.96^{c}	71.63^{g}	30.71^{d}	0.25^{d}
T5	241.39^{a}	134.03^{d}	318.55^{a}	361.55^{e}	91.13^{c}	26.04^{f}	0.31^{b}
T6	225.25^{d}	152.82^{a}	319.00^{a}	544.92^{a}	95.75^{b}	28.28^{e}	0.28^{c}
T7	233.04^{b}	130.42^{e}	285.88^{d}	332.00^{g}	101.81^{a}	39.07^{a}	0.21^{e}
T8	232.37^{b}	125.78^{f}	263.75^{e}	325.96^{h}	70.05^{g}	18.68^{g}	0.18^{f}
T9	210.78^{g}	110.62^{h}	262.16^{e}	313.99^{i}	69.75^{g}	14.99^{h}	0.16^{f}
CD (0.05)	2.31	3.33	2.71	2.26	2.56	2.02	0.028
C.V	0.45	1.09	0.16	2.25	1.37	3.19	5.05

Trmnts-Treatments

Table .16. Primary, secondary and micro nutrient content of plants after imposing various treatments

Trtmt	N (%)	P (%)	K (%)	Ca (ppm)	Mg (ppm)	S (ppm)	B (ppm)
T1	1.21 ^{d}	0.16	1.83^{b}	0.39^{de}	0.38^{de}	0.25 ^{a}	5.75^{cd}
T2	2.29^{a}	0.15	2.05^{b}	0.59^{a}	0.48^{bc}	0.19 ^{bc}	4.50^{de}
T3	1.50^{c}	0.19	1.22^{d}	0.50^{b}	0.50^{b}	0.15^{de}	6.50^{c}
T4	1.17^{d}	0.13	2.91^{a}	0.40^{cde}	0.68^{a}	0.22^{a}	8.50^{b}
T5	1.84^{b}	0.20	2.62^{a}	0.46^{bc}	0.64^{a}	0.24^{a}	8.75^{b}
T6	1.33^{d}	0.15	1.93^{b}	0.46^{bc}	0.67^{a}	0.25 ^{a}	11.00^{a}
T7	0.96^{e}	0.06	1.59^{c}	0.44^{bcd}	0.47^{bcd}	0.17^{e}	6.50^{c}
T8	0.96^{e}	0.07	1.55^{c}	0.45^{bcd}	0.39^{cde}	0.17^{e}	4.00^{e}
T9	0.91^{e}	0.01	0.64^{e}	0.37^{e}	0.30 ^{e}	0.08^{f}	3.50^{e}
CD (0.05)	0.16	NS	0.30	0.06	0.09	0.03	1.64
CV	6.71	NS	8.26	8.58	11.32	10.60	11.08

4.4 INDUCTION OF DEFENSE MECHANISMS IN CARDAMOM PLANTS IN RESPONSE TO BIO CONTROL AGENTS

Application of biocontrol agents induced defence enzymes in treated plants challenge inoculated with *Fusarium* sp. Analysis of biochemical defence enzyme were carried out as per 3.4 from fully expanded top second or third leaf of cardamom before treatment application and three month after treatment application.

From the pre- treatment study there are very small concentration of bio chemical enzymes were produced in the cardamom leaves (Table 17). Three months after the pathogen inoculation and various treatment applications showed significantly higher levels of these enzymes was increased in the leaves (Table 18).

Highest level of phenol was observed in the leaves of cardamom plants treated with AMF (T1) in the soil. OD phenol value was highest for PGPR mix II applied plants, whereas combination of AMF and *T. viride* (T5) resulted in significantly higher level of peroxidase. Combination of AMF and *P. fluorescens* (T6) is the next best treatment for all the above parameters. Individual application of *T. viride* (T2) and AMF (T1) resulted in highest concentration of PAL in treatment plants. β 1,3 glucanase induction was highest in PGPR mix II treated plants followed by *P. fluorescens* treated plants (individually and in combination with AMF ie.,T3,T4 and T6).

Study results also confirmed the induction of defence related enzyme in biocontrol agents treated plants challenge inoculated with *Fusarium* sp., compared to control plots. The best treatment combined application of AMF with *P. fluorescens* (basal dose) along with 2 per cent *P. fluorescens* foliar spray elevated the production of phenol, OD phenol, PO, PPO and PAL in cardamom plants compared to control and treatment effectively managed the disease.

Table.17. Pre- treatment studies on biochemical defence enzymes in cardamom

Sl .No.	Treatments	Phenol (μgl^{-1})	OD-phenol (mg g^{-1})	PO ($\mu g\ min^{-1}\ g^{-1}$)	PPO ($\mu g\ min^{-1}\ g^{-1}$)	PAL ($\mu gmin^{-1}\ g^{-1}$)	β 1,3 glucanase (Umg^{-1})
T1	AMF	0.55	1.97	0.69	0.23	0.06	0.21
T2	*T.viride*	0.39	4.67	0.51	0.27	0.03	0.18
T3	*P.fluorescens*	0.50	4.10	0.60	0.18	0.04	0.20
T4	AMF+ *P.f*	0.06	3.81	0.46	0.22	0.03	0.18
T5	AMF+ *T.viride* +*P.f*	0.45	5.85	0.53	0.28	0.02	0.25
T6	AMF+ *P.f*	0.08	3.22	0.62	0.26	0.02	0.13
T7	PGPR mix II	0.45	3.23	0.41	0.16	0.02	0.12
T8	Carbendazim	0.42	4.58	0.57	0.15	0.03	0.25
T9	control	0.42	3.57	0.58	0.19	0.03	0.21
CD (0.05)		NS	NS	NS	NS	NS	NS
CV		44.88	25.91	15.93	25.68	79.52	18.76

Table.18. Effect of biocontrol agents on defence mechanism in cardamom

Sl .No.	Treatments	Phenol (μgl^{-1})	OD-phenol (μgl^{-1})	PO ($\mu g\ min^{-1}\ g^{-1}$)	PPO ($\mu g\ min^{-1}\ g^{-1}$)	PAL ($\mu gmin^{-1}\ g^{-1}$)	β 1,3 glucanase (μgl^{-1})
T1	AMF	10.10^{a}	38.05^{e}	12.85^{c}	0.49^{d}	0.19^{ab}	32.20^{g}
T2	*T.viride*	4.70^{e}	42.45^{c}	11.00^{d}	0.60^{cd}	0.21^{a}	267.50^{cd}
T3	*P.fluorescens*	4.25^{e}	31.83^{g}	16.95^{f}	0.92^{bc}	0.16^{bc}	345.00^{bc}
T4	AMF+ *P.f*	6.60^{cd}	40.56^{d}	20.14^{h}	2.39^{a}	0.14^{c}	365.00^{bc}
T5	AMF+ *T.viride* +*P.f*	7.05^{c}	36.03^{f}	30.82^{a}	1.17^{b}	0.04^{d}	137.50^{df}
T6	AMF+ *P.f*	7.95^{b}	45.61^{b}	23.09^{b}	2.03^{a}	0.05^{d}	385.00^{b}
T7	PGPR mix II	3.35^{f}	60.71^{a}	9.09^{e}	0.29^{d}	0.03^{d}	2228.00^{a}
T8	Carbendazim	5.87^{d}	38.43^{e}	1.10^{h}	0.37^{d}	0.15^{c}	187.50^{ef}
T9	control	2.90^{f}	20.85^{h}	3.06^{g}	0.26^{e}	0.006^{d}	21.00^{g}
CD (0.05)		0.79	1.68	1.65	0.39	0.03	79.31
CV		17.12	16.08	6.70	23.03	19.71	23.03

Discussion

5. Discussion

Cardamom of commerce, popularly known as 'Queen of Spices',is the herbaceous perennial *Elettaria cardamomum* Maton, belonging to the Zingiberaceae family. It is a shade-loving plant cultivated at an altitude of 600–1200 m above mean sea level (MSL) with an annual rainfall of 1500–4000 mm and a temperature range of 10–35 °C (Parthasarathy *et al.,* 2012). Cardamom is native to Western Ghats and widely cultivated in Cardamom hill reserves of Kerala. It is also used in processed food, perfumes, oleoresins and many other applications. But its production was highly fluctuated by fungal and viral diseases. Fusarium rot is one of its kinds, caused by the fungus *Fusarium oxysporum* Schlecht. The pathogen infects panicle, tillers and roots of cardamom plants of all growth stages (Thomas and Vijayan, 2002). Plantations with poor management practices are highly prone to the attack of Fusarium rot (Dhanya *et al.,* 2018).

The present study is framed to develop an eco-friendly management approach against the disease with biocontrol agents and its comparison with the effect of chemical fungicide. According to Murugan *et al.* (2016) higher yield acquired by cardamom planters in recent years was attributed to continuous and injudicious application of high dose of chemical pesticides to ward off pests and diseases in cardamom hill reserves (CHR). In this context, the present study helped the planters to develop an eco-friendly management approach with prophylactic application of various biocontrol agents individually and in combinations. The treatments include endophytes like AMF, Plant growth promoting rhizobacteria mix II, *Pseudomonas fluorescens* and *Trichoderma viride.*

A survey was conducted during February- May 2019 to study the incidence and severity of Fusarium rot disease in selected blocks of Idukki district. One panchayat was selected from each block. Three plantations located in Pampadumpara and Erattayar panchayats of Nedumkandam and Kattappana blocks respectively were identified as the hotspot areas for the disease. In surveyed locations, the disease severity ranged from 50.00- 84.80 per cent and per cent disease incidence ranged from 60-

100%. Highest disease severity (84.80 % and 77.40%) recorded from farmer's fields of Pampadumpara panchayat and one farmer's field from Erattayar panchayat (77.40%). Disease severity was calculated based on a 0-5 scale developed by Dhanya *et al.* (2018) by visual observation. Thomas and Vijayan (2002) conducted a survey in thirty seven plantation of Idukki district during 2000-2002 and observed severe root rot, yellowing and panicle wilt. The disease incidence ranged from 3-27.5 per cent. Vijayan *et al.* (2014) noticed the disease incidence was increased to 68 percent during 2010-2011 and was higher in Kallupalam, Pulyanmala and Puttadi of Nedukamkandam block.

Symptomatology of the disease was also recorded during survey. The disease was prominent mainly during summer season. Similar finding was made by Thomas and Vijayan (2002). The characteristic symptoms of the disease observed were eye shaped lesions of variable sizes on the pseudostem. The affected portion became weak at the point of infection and broke when the infection is in the advanced stage. Severely infected plants had leaf yellowing symptom due to root rotting and restricted nutrient and water uptake. Panicle initiation was also initiated during the summer season. Therefore Fusarium infection on the panicle resulted in a burnt appearance with drastic reduction in productivity. All the above symptoms were very conspicuous in all surveyed plots. Similar symptoms of root rot, rhizome rot, panicle wilt and pseudostem rot were reported by Vijayan *et al*. (2013, 2014) based on a survey conducted in Fusarium infected cardamom plantations of Idukki district.

The pathogen associated with Fusarium rot disease was isolated from infected samples (root tip, pseudostem, and panicle) collected from surveyed plots by tissue isolation method described by Booth (1971).

The virulence studies with the pseudostem isolate (Fp_1-Fp_6) and root isolate (Fr_1 - Fr_2) of *Fusarium* sp. were carried out using pin prick method on one year old cardamom plants under greenhouse condition. The isolate Fp_1 produced typical symptoms on tiller about four weeks after pathogen inoculation. Fr_1also produced lesion on pseudostem but it took more days for development (6 weeks).Thomas and Vijayan (2002) noticed that root isolates of *Fusarium* sp. produced characteristic symptoms of root rotting and yellowing on inoculated cardamom seedlings within 15-30 days after inoculation. But in the present study the root isolate took more days to develop the typical symptom.

This might be due to the strain variation of the pathogen evaluated in the present study. The spore suspension of the root isolate (10^6 cfu mL^{-1}) produced root-rotting symptoms when drenched to the base of the plant. Thomas and Vijayan (2002) also followed the same method for the virulent studies of Fusarium isolates from cardamom. Vijayan *et al*. (2013) observed that the different isolates of the fungus obtained from infected tiller, panicle and root could cross-infect the other plant parts of cardamom. In accordance with this, in the present study the root isolate (Fr_1) produced typical symptoms on cardamom roots as well as pseudostem. The pseudostem isolate Fp_1 produced large lesion with a size of 1.50×0.35 cm on the pseudostem. Whereas the lesion produced by the root isolates (Fr_1) was comparatively smaller (0.90×0.45 cm) and concluded that Fp_1 is virulent than Fr_1. Kumar *et al* (2011) conducted studies on resistance of Fusarium head blight by evaluating the lesion produced on the detached leaves of barley. They had found that susceptible cultivars produced larger lesions than resistant. Hawthorne *et al.* (1994) analyzed the pathogenicity variation among different strains of *Fusarium solani* on *cucurbita* sp. using healthy and wounded hypocotyls and mesocarp of cucurbits.

In the present investigation virulent isolates obtained from pseudostem and root did not vary much in their morphological characters. Colour of both isolates was white on the front view and pinkish on the rear view. Colonies were pink in appearance and produced fluffy mycelium with an irregular margin. The mycelial growth of the fungus covered the Petri plate within a week. The above results were in confirmation with the findings of Thomas and Vijayan (2002). Contradictory to the present observation Vijayan *et al*. (2013) categorized *F. oxysporum* isolates obtained from the infected cardamom samples into five morphological types, based on their colour of their colony. Gopi *et al.* (2016) recorded Fusarium rot in large cardamom (*Amomum subulatum* Roxb), where the isolated pathogen showed aerial floccose mycelium appeared white in the early growth stage and later changed to purple. Study conducted by Albores *et al.* (2014) on different *Fusarium* sp. isolated from gladiolus revealed that most of the *F. oxysporum* cultures had white mycelia with violet or cream colour on the rear view. All the above published results are in confirmation with the results we have arrived at in this study.

The pseudostem isolate Fp_1produced macro conidia of size 3.00×0.22 µm and micro conidia of 1.69× 0.13 µm, whereas the root isolate Fr_1 produced macro conidia and micro conidia of size1.57 ×0.43 µm and 1.24×0.34 µm respectively. Thomas and Vijayan (2002) described the macroconidia and microconidia of *Fusarium oxysporum* Schlecht in small cardamom for the first time from Idukki. According to them, macro conidia produced by the fungal pathogen is 3-5 septated with a size of 23.6×3.48µm, whereas that of microconidia, the size ranged from 9.03×2.58µm. Ploetz (2006) studied the conidial characters of *F. oxysporum* f. sp. *cubense.* As per that the macroconidia produced by the fungus ranged from 27to 55 × 3.3 to 5.5 µm, four- to eight-celled, and sickle-shaped, where as its micro conidia was of 5 to 16 × 2.4 to 3.5 µm size, one- or two-celled with oval- to kidney-shape. Albores *et al.* (2014) also recorded the size of microconidia as 50.50-67.49 µm for *F. oxysporum* isolated from gladiolus. Based on an *in vitro* study conducted by Gopi *et al.*(2016),it was reported that *F. oxysporum* infecting large cardamom had macro and micro conidia with a size of 26.91- 57.64 to 2.01- 2.59µm and of 5.62-8.44 to 1.86- 2.71µm respectively. The small sized conidia observed in the present study revealed that the strains of *F. oxysporum* infecting small cardamom were varying from location to location and were also quite different from that of large cardamom.

Nowadays the use of highly toxic chemical pesticides resulted in export rejection of small cardamom due to the presence of escalated residues in the cured products. Therefore the use of biocontrol agents against plant pathogens as consortia is a recent trend in cardamom disease management strategies.

The results of the pot culture trial carried out with various biocontrol agents (individually and in combination) and chemical fungicide (carbendazim) against Fusarium rot of cardamom revealed that combined application of bio agents as basal and foliar application resulted in good management of the disease compared to their individual application.

Disease severity was found to be minimum for plants treated with combination of AMF and *T. viride* or AMF and *P. fluorescens* as basal application along with 2 per cent *P. fluorescens* spray. Yusran *et al.* (2009) noticed that disease severity of *F. oxysporum* Schlecht f. sp *radicis- lycopersi* in tomato was reduced significantly by the combined application of AMF and *P. fluorescens.* In the present study basal application

of AMF with either *T. viride* or *P. fluorescens* reduced the disease severity by 69.50 and 70.00 per cent respectively. Similar to this, Srivastava *et al.* (2010) observed that combined application of AMF, *Trichoderma* sp. and *P. fluorescens* were effective in reducing the Fusarium wilt of tomato (*F. oxysporum* f. sp. *lycopersici*) severity by 63.00 per cent. Dehariya *et al.* (2004) also found that the disease severity of Fusarium wilt (*F. udum*) of pigeon pea was very much reduced when the bioagents like *Trichoderma* sp. and AMF were used in combination. Tayal *et al*. (2011), Mbuthia *et al.* (2019) also derived the same result when these bioagents were used in combination against *F.oxysporum* f. sp. *lycopercisi* infection in tomato. Field study conducted by Dhanya *et al.* (2018) also confirmed the effective role of bio agents like *Trichoderma* sp., *P. fluorescens* and AMF as combination against the Fusarium rot of cardamom. While conducting the compatibility studies between *Trichoderma* sp. and AMF, synergistic nature of these bioagents were confirmed by Camprubi *et al.* (1995). Filion *et al.* (1999) observed that in the presence of AM fungal extract the conidial germination of *Trichoderma harzianum* was enhanced. Likewise germination per cent and further mycelial development of *G. mosseae* was accelerated in the presence of *Trichoderma* spp (Calvet *et al.,* 1992). Al-Asbahi (2012) found that volatile bio molecules released by *T. harzianum* Rifai KRL-AG2 indirectly increased the association of AM fungi with wheat plant roots (cv. Avocet S) and the wheat protein homologous to arbuscular mycorrhizal protein was over-expressed, which could play a role in the interaction between AM and plant. Sharma *et al.* (2016) reported that combination of *G. mossae* and *T. viride* induced more phosphatase activity in the roots of black gram variety UH-1 than their individual application resulted in enhanced P availability from the soil. Synergistic effect between AMF and *P. fluorescens* could be due to the production of antifungal compounds like diacetylphloroglucinol (DAPG) which had positive influence on mycorrhizal colonization (Dwivedi *et al.,* 2009) and high DAPG production peaks in the presence of AMF in wheat roots infected with *Gaeumannomyces graminis* var. *tritic*i (Siasou *et al.,* 2009). Basu and Santhaguru (2009) also demonstrated that percent colonization by *G. fasciculatum* was increased in the presence of *P. fluorescens*. Pivato *et al.* (2009) observed that saprophytic growth of *P. fluorescens* C7R12 was enhanced by *G. mosseae* when *in vitro* conditions. Jäderlund *et al*. (2008) had shown that unique associations with *G. intraradices* and *P. fluorescens*

increased the plant dry weight in *Microdochium nivale*-infested winter wheat (*Triticum aestivum* cultivar Tarso).Mbuthia *et al.* (2019) suggested that, the complimentary effect of the above bioagents in combination might be due to their additive or synergistic nature.

The pathogen - antagonistic ratio was least in cardamom plants treated with combinations of AMF and *T. viride* as well as AMF and *P. fluorescens*. Khan *et al.* (2004) reported a drastic reduction in the population of soil wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* in *Trichoderma* sp. treated chickpea plants. From the present study it was also concluded that when the bioagents were used in combination, *(ie,* AMF with either *Trichoderma viride* or *P. fluorescens*) their interactive effect enhanced the population of both biocontrol agent in soil. In accordance with this study Deharia *et al.* (2014) reported that AMF and *Trichoderma* sp. in combination against *Fusarium udum* in pigeon pea, both AMF colonization in plant roots and *Trichoderma* sp. population in soil were triggered. Sangeetha *et al.* (2013) also reported high population of *P.fluorescens* in soil even after 75 days if applied along with AMF compared to its sole application. All these research findings strongly support the present study results.

Root staining was done at the end of experiment and observed good colonization in roots of cardamom plants treated with AMF. Colonization percentage and number of vesicles were comparatively higher when AMF was given in combination either with *T. viride* and *P. fluorescens*. Sarma *et al*. (2014) opined that *Trichoderma* sp. induced an enhanced colonization of AMF in vascular plants. Boer *et al.* (2005) also reported that frequency of mycorrhizal colonization in roots were enhanced from 50-60% when its co- inoculated with *P. fluorescens*. Kumar *et al.* (2012) confirmed the same result in sorghum. Gamalero *et al.* (2003) also found significant colonization of AMF in tomato plant inoculated with *P. fluorescens*. According to them good establishment of AMF on roots treated with *P. fluorescens* was by the synergistic interaction of the bioagents. Colonization percent obtained for combined application of AMF with *T. viride* was 68.76 per cent in the present study. An experiment conducted on groundnut by Yadav and Aggarwal (2015) also made almost the same results. In the study, soil application of AMF with *Trichoderma* sp. and *P. fluorescens* resulted in 77.8 per cent colonization in the roots. An evaluation study with different *P. fluorescens* strains and AMF strains in

sorghum as combined soil application resulted in 50.90 to72.80 per cent colonization (Kumar *et al*., 2012)

Biometric observations were taken from the experimental plants to study the additional role of biocontrol agents in plant growth promotion. Plant height and leaf length were significantly increased when AMF was given with *P. fluorescens* as basal application. Similar to observation was made by Mohandas *et al.* (2010) in a field experiment on *F. oxysporum.* f. sp. *cubense* in neypovan cultivar. In the study, combined application of AMF and *P. fluorescens* resulted in good plant height and girth. Neeraj and Singh (2011) also found an increase in yield potential of French bean by the synergistic effect of AMF and *P. fluorescens* used in the management of *Rhizoctonia solani*. In the present study, next best treatment for plant growth promotion was combination of AMF and *T. viride*. Seed pre inoculation of AMF and *T. viride* increased vegetative and yield parameters of groundnut inoculated with *Macrophomina phaseolina* (Doley and Jite, 2014). The biocontrol agents in combination enhanced the vegetative growth like tiller length, number of leaves, leaf length and width of small cardamom. Mwangi *et al.* (2011) also remarked that AMF and *Trichoderma* sp. when applied together could improve plant's biometric parameters such as plant height and growth of tomato plans. Dehariya *et al.* (2014) also found significant growth response in pigeon pea plants inoculated to *Fusarium udum* when treated with combinations of *Trichoderma* sp. and AMF. Yadav and Aggarwal (2015) also found a significant difference in the growth parameters (plant height, shoot biomass, and root biomass) and yield (number of pods, protein content and oil content) of ground nut plants when treated with combination of bioagents (AMF+*Trichoderma* sp.+ *P. fluorescens*) compared to their individual application. Duc *et al.* (2017) proved that triple inoculation of the plant with AMF, *Trichoderma* sp and *P. fluorescens* resulted in high yield of pepper plants.

Change in nutrient status of plant as well as soil in response to various treatments was assessed three months after treatment application. Considerable increase in the level of nitrogen, phosphorus, potassium, calcium, magnesium and boron was observed in bio agent treated soil of experiment plants. The highest level of nutrients was recorded when combination of bioagents ie., AMF with *Trichoderma viride* enriched cowdung (T5) or *P. fluorescens* (T6) as basal application. AMF application

along with *T. viride* recorded highest soil content of nitrogen (241.39 kg ha^{-1}). The activity of soil organisms ensure supply of nutrients to the plants and play a significant role in organic matter decomposition and release of plant nutrients like N, P, K, Mg (Egamberdieva and da Silva, 2011). Enhancement of nitrogen availability by accelerated decomposition of organic materials was observed by Hodge *et al.* (2001). Highest concentration of P was observed in soil of co-inoculation of AMF and *P. fluorescens* (154.09 kg ha^{-1}) in the present study. Krey *et al.* (2013) reported that phosphorous availability of the soil could increase with the soil application of *P. fluorescens*. Phosphorus is a relatively less mobile element compared to nitrogen and a large portion of applied P might be get fixed into the soil (Carpio *et al*. 2005) and reduced the leaching of P from soil (Cavagnaro *et al.,* 2015), AMF application improved the P mobility by 18 per cent (Bender *et al*, 2015) and delivered to the plants by 80 per cent (Marschner and Dell, 1994). Nurbaity *et al.* (2016) reported that when *Glomus* sp. was applied along with *Pseudomonas diminuta* the use of chemical fertilizers in potato plants was reduced by 50 per cent. Dual inoculation with AMF and *P. fluorescens* resulted in added uptake of phosphorus in maize and faba bean from rock phosphate (Najjar *et al.,* 2012). Marschner and Dell (1994) also found that AMF application enhanced the release of K by its external hyphae. In the present study also K availability was significantly recorded highest in treatment soils with AMF (T1: 303.81 kg ha^{-1}, T4: 316.51 kg ha^{-1}, T5: 318.55 kg ha^{-1}, T6: 319.00 kg ha^{-1}). *Pseudomona*s and *Bacillus* sp. had active role in nutrient solubilisation (Pindi and Satyanarayana, 2012). Yusran *et al*. (2009) observed an enhanced uptake of P by combined inoculation of *Pseudomonas* sp., *Bacillus amyloliquefaciens* FZB42 and AMF. Azarmi *et al*. (2011) reported that application of *Trichoderma* sp. to soil could enhance the ability of the insoluble compounds and micronutrients by the production of organic acids and subsequent reduction in soil pH. The combination of AMF with *T. viride* or *P. fluorescens* had improved soil nutrient availability from their sole application. Wu *et al*. (2014) opined that through the production of soil binding glomalin by mycorrhizal fungi indirectly increased the nutrient availability in the soil. Soil aggregate stability improved the glomalin resulted in good root growth and activity by increasing the organic carbon status of the soil. The higher organic matter content of soil also resulted in retention of cations like Ca^{2+} and Mg^{2+}, and improvement of nitrification processes

(Morrison *et al*., 2017). Ca and Mg content of AMF with *T. viride* (Ca: 361.55 ppm Mg: 91.13 ppm) and *P. fluorescens* (Ca: 544.92 ppm and Mg: 95.75 ppm) were also found to be higher than the untreated control plants from the pot culture study. Several *Bacillus* sp. (*Bacillus megaterium,B. circulans, B. subtilis, B. polymyxa, B. sircalmous*) had been described as effective phosphate solubilizers (Mohammadi and Sohrabi, 2012). But for PGPR mix II (P: 130.42 kg ha^{-1}), which contains *B. subtilis* as one of its components gave poor phosphorus availability in the present study. The low status of P, in the treatment involving might have been due to the fixation of P which was released by the action of P solubilising organism in PGPR. The contradictory result might be due to the variation of the strain used in the study.

Nutrient analysis of leaves of experimental plants also showed an increased concentration of primary, secondary and micronutrients but their level significantly varied with treatments. Tanwar *et al.* (2013) observed more nitrogen content in roots of broccoli plants when treated with consortium of bioagents. Based on their findings nitrogen and phosphorus accumulation in the shoots and the roots of tomato seedlings inoculated with *F. oxysporum* f. sp. *lycopersici*, were enhanced significantly in response to combined application of *Trichoderma* sp. and AMF compared to their individual application. In the present study also combination treatment of AMF with *T. viride* (T5) recorded high content of N (1.84 per cent) and with *P. fluorescens* (1.33 per cent) in the cardamom leaves. Yadav and Aggarwal (2015) also found high concentration of phosphorous in roots of plants treated with combination of AMF, *Trichoderma* sp. and *P. fluorescens.* There was no significant difference in the P content in the leaves but recorded highest in combined treatments of AMF with *T. viride* (T5:0.20 per cent) and *P. fluorescens* (T6: 0.15 per cent) in this study. These findings were in agreement with the present study results. Role of *Trichoderma* sp. in plant growth promotion and nutrient level of phosphorus were observed by Li *et al.* (2015) while conducting an experiment with tomato in hydroponics.

High potassium level was observed in the plants treated with combination of AMF with either *P. fluorescens* (1.93 per cent) and *T. viride* (2.62 per cent). The ability to dissolve insoluble phosphate forms and potassium fixed as silicates by

Trichoderma sp. was observed by Nahidan *et al.* (2019). Similar observations also made by Li *et al.* (2015) in tomato plants.

In the present study higher concentration of Sulphur was observed in AMF treated plants (T1: 0.25 ppm, T4: 0.22 ppm, T5: 0.24 ppm, and T6: 0.25 ppm) than control plants. Bisht *et al.* (2009) reported a hike in sulphur level in shoots as well as roots of *Dalbergia sissoo* trees treated with AMF. Poveda *et al.* (2020) also opined that AMF inoculation could increase the mobility of sulphur in soil.

The bioagents could also trigger the plant's defense mechanism against the pathogen. In the present study combined application of bioagents like AMF and *P. fluorescens* as basal application along with *P.fluorescens* spray induced significantly higher biochemical reaction in plants compared to untreated control plants.

Inoculation with AMF increased the phenol content and phenylalanine ammonia lyase activity in the experiment plants. Khatun and Chatterjee (2011) supported this challenge finding by describing the activation of added phenol and PAL in AMF treated coleus plants inoculated with *Fusarium oxysporum*. Grapes and Asgari grape cultivar accumulated significant amount of phenol in the leaf tissue than root tissues in accordance with AMF establishment (Eftekhari *et al.* 2012). Eke *et al.* (2016) also reported that the soil application of AMF increased the phenol and PAL contents of common bean (*Phaseolus vulgaris*) inoculated with *Fusarium solani* f. sp. *phaseoli*.

T. viride application also increased the PAL activity in cardamom plants inoculated with *Fusarium sp.* Quaas *et al.* (1993) observed that conidial suspension of *Trichoderma* sp. could induce increased PAL activity in citrus leaves. Jayalakshmi *et al*. (2009) also made same observation in chickpea plants in response to inoculation with *Trichoderma sp.* According to Christopher *et al.* (2010) PAL activity was significantly higher in tomato plants, treated with *Trichoderma* sp. as soil and seed application followed by inoculation with *Fusarium oxysporum f sp. lycopersici*. Surekha *et al.* (2014) found that *Trichoderma* sp. pretreated black gram plants when inoculated with *Fusarium oxysporum* showed higher PAL activity than control. Hima (2017) conducted a study on *Fusarium oxysporum* f. sp. *cubense* disease management of ginger using mutants of *Trichoderma* sp. and observed that mutant K80M13 induced high activity of PAL in inoculated plants.

Basal application of *P.fluorescens* showed high polyphenol oxidase and phenylalanine ammonia lyase activity in the present study. Paul and Sarma (2005) observed *P. fluorescens* mediated systemic resistance in black pepper through the synthesis of defence enzymes such as PPO and PAL against *Phytophthora capsici.* Sujatha and Ammani (2011) observed induction of defence related enzymes (PPO and PAL) in *Vigna mungo* (blackgram) by *P.fluorescens* strain Os25 against *Fusarium oxysporum*. Nandi *et al.* (2013) reported high PPO and PAL activity in cowpea seedlings when subjected to seed treatment with *P. fluorescens* against damping off disease (*Sclerotium rolfsi*). Rajeswari (2014) found comparable amount of PPO in response to *P. fluorescens* application in groundnut plants challenge inoculated with *Fusarium oxysporum.*

Basal application of bioagents in combination *ie,* AMF and *T. viride* along with *P. fluorescens* spray induced production of phenol, orthodihydroxy phenol, peroxidase,poly phenol oxidase and β-1,3-glucanase in experimental plants from the study. Allay and Chakraborty (2013) reported induction of defence enzymes like β-1,3-glucanase and peroxidase in mandarin against *Fusarium solani* by dual application of AMF and *Trichoderma asperellum.* Doley *et al.* (2014) also reported the role of *Glomus fasciculatum* and *Trichoderma viride* pre inoculated ground nut seeds in managing *Macrophomina phaseolina* through the induction of phenol, peroxidase and poly phenol oxidase. Duc *et al.* (2017) observed that, application of *Trichoderma* sp, AMF and *P. fluorescens* in combination accelerated the production of poly phenol oxidase in Kapria cultivar and peroxidase in Karpex cultivar of pepper plants

Basal application of AMF and *P. fluorescens* along with *P.fluorescens* spray enhanced orthodihydroxy phenol, peroxidase and β 1,3glucanase activities in the cardamom leaves and is the best management practice against Fusarium rot of cardamom through the induction of defense related enzymes. Mohamed *et al.* (2019) observed high level of PO in common bean against *Sclerotium rolfsi* when treated with *P.fluorescens* and AMF. High concentration of orthodihydroxy phenol (90.6µg) was observed in *Solanum viarum* seedling (Hemashenpagam and Selvaraj, 2011) when combined inoculation with AMF and *Trichoderma harzianum* under green house condition. Sangeetha *et al.* (2013) reported that treatment with AMF and *Pseudomonas*

sp. induced high level of phenol on 75th day of inoculation in maize. In accordance with this in the present study three months after biocontrol application *P.fluorescens* alone and in combination with AMF promoted induction of phenol, OD-phenol, PO, PPO and β 1,3 glucanase in cardamom plants challenge inoculated with *Fusarium oxysporum*.

PGPR mix II application also induced OD-phenol and β-1, 3-glucanase in *F. oxysporum* inoculated cardamom plants. Saravanakumar *et al.* (2007) found that induced systemic resistance induced by β-1,3-glucanase contributed by PGPR bioformulations enhanced the disease resistance in tea plants against blister. Shanmugam *et al.* (2011) found the role of *Bacillus* sp. on the induction of β-1,3-glucanase activity in root and shoots of tomato plants by 1.4-1.6 fold against *Fusarium oxysporum* f. sp. *lycopersici*. Senthilraja *et al.*(2013) also reported PGPR application enhanced the expression of defence enzyme like β-1, 3-glucanase in groundnut plants against collar rot pathogen and found that hundred per cent reduction over the disease. Similar results were observed in mulberry against *Macrophomina phaseolina* (Ganeshmoorthi *et al.,* 2008), in tomato against *Fusarium oxysporum* f. sp. *lycopersici* (Shanmugam *et al.,* (2011).

Summary

6. SUMMARY

Cardamom commonly referred as ‘Queen of Spices’ is the third costliest spice crop after saffron and vanilla. It has good medicinal values and widely used for various culinary purposes. Fusarium rot or pseudostem rot is one of the major fungal diseases in plantations, particularly during the summer months. Typical symptoms of the disease appeared on pseudostem as eye-shaped lesions, which later broke off from the point of infection. Panicle wilt caused by the fungus resulted in drastic yield reduction whereas rotting of root tips caused poor of water and nutrients uptake and subsequent yellowing of the plants. Frequent application of fungicides to manage the disease reduced the exporting value of the cured product.

The research work entitled "Eco- friendly management of *Fusarium* rot in cardamom and its impacts on soil health and plant defence mechanism” was undertaken at the Department of Plant pathology, College of Agriculture, Vellayani, Thiruvananthapuram and Cardamom Research Station, Pampadumpara, Idukki during 2018-2020, with the objective to assess Fusarium rot severity and pathogen variability in Idukki district, develop an effective ecofriendly management practices for the disease and study the impact of the practices on soil and plant health.

During summer months, survey, sample collection and pathogen isolation were performed from the cardamom plantations where the disease was pronounced. Kattappana and Nedumkandam blocks of the Idukki district, which are the two major cardamom-raising blocks of the district, were selected for the study. Three of one hectare size was selected from each panchayat. Pampadumpara and Erattayar panchayats were selected from the Nedumkandam and Kattappana blocks respectively. Based on the survey Pampadumpara panchayat showed maximum disease incidence and severity (100% and 84.80%) compared to Erattayar panchayat (60.00% and 50.40%). All the distinctive manifestations of Fusarium rot disease like eye shaped lesions on pseudostem, stem lodging, panicle wilt and root tip rotting were noted in the surveyed fields.

Six isolates of *Fusarium oxysporum* Schlecht were obtained during the study from different plantations (six each from infected pseudostem and roots) identified at Kattappana and Nedumkandam blocks. The pseudostem isolates were named as Fp_1-Fp_6

and root isolates as Fr_1–Fr_6. The morphological characters and microscopic observations like colour, colony characters, hyphal and conidial characters as well as rate of growth in Petri dish confirmed the fungus as *F. oxysporum*. Koch's postulates are proved and based on that Fp_1 and Fr_1 identified as most virulent isolates. The virulent isolates from pseudostem and root were appeared as white fluffy colony with pinkish tinge at the rear side of the 7 day old culture. Macro conidia of Fp_1 had a size of 3.00×0.22 µm and microconidia of size 1.69×0.13µm, while Fr_1 produced macro and micro conidia of size 1.57×0.43µm and 1.34×0.34µm respectively. Cultural characters such as rate of growth was slightly higher for Fp_1 (1.55 $cmday^{-1}$) than Fr_1 (1.25 $cmday^{-1}$) in Petri dishes under room temperature. The Pathological study carried out using pseudostem isolate Fp_1 re-produced typical eye shaped lesions on the pseudostem within 29 days after inoculation where as the root isolate Fr_1, which took a longer period about 40 days for symptom production. *In vitro* studies revealed that pseudostem and root isolates exhibit major differences in their morphological, cultural and pathological characteristics. Pseudostem isolate Fp_1 (obtained from Pampadumpara panchayath) was identified as the most virulent isolate based on the virulence studies (viz., days taken for symptom formation, lesion size, mycelial growth) and was used for further studies.

A pot culture study was designed with a view to evaluate the efficacy of selected bioagents administered individually and in combination against Fusarium rot disease of cardamom. The experiment was carried out with nine treatments and three replications under CRD design. The bio agents were given as both soil and foliar application. T1: AMF @ 20 g per 10 kg soil, T2: 2 per cent *Trichoderma viride* in FYM @ 1 kg per 10 kg soil, T3: 2 per cent *Pseudomonas fluorescens* @ 1 L per 10 kg soil, T4: basal application of AMF @ 20 g per 10 kg soil with two per cent *P. fluorescens* spray @ 0.5 L / plant, T5: AMF+2 per cent *T. viride in* FYM @ 1 kg per 10 kg soil with two per cent *P. fluorescens* spray @ 0.5 L / plant, T6: AMF + two per cent *P. fluorescens* @ 1 L per 10 kg soil with two per cent *P. fluorescens* spray @ 0.5 L / plant, T7: 2 per cent PGPR mix II @ 1 L per 10 kg pot and T8: 0.2% carbendazim as drench @ 1L per 10 kg soil and spray @ 0.5 l per plant was given at the time of planting, foliar spraying was given and repeated at monthly interval for three times. Pathogen inoculum (150 g / 10 kg soil) multiplied in sand-maize flour medium was used for the soil inoculation of experimental plants, 15 days after the basal application of respective biocontrol agents.

Symptoms of the disease developed on experimental plants and become severe on 45 days after pathogen inoculation and the severity varied according to different treatment applications. Among the nine treatments, soil application of AMF inoculum and *P. fluorescens* along with *P. fluorescens* spray (T6) resulted in successful disease management (disease incidence: 40 percent and disease severity: 24.26 percent) compared to pathogen inoculated control plants (disease incidence: 100 percent and disease severity: 69.38 per cent) followed by combined application of AMF and *T. viride* as basal application with *P. fluorescens* foliar spray (disease incidence: 39.81 % and disease severity: 24.67 %).

Population dynamics studies of pathogen and biocontrol agents in soil at periodic intervals of up to three months confirmed a substantial reduction in the pathogen antagonist ratio, especially in combination treatments, when AMF was given in combination either with *P. fluorescens* or *T. viride* as basal application. The population of the pathogen was drastically reduced. In addition to this it was found that the interaction effect of bioagents in the above treatments enhanced the population of *P. fluorescens* and *T. viride* in soil, three month after application. Root staining of cardamom plants inoculated with AMF was performed at the end of the experiment and it was found that the per cent colonization in the root was improved when AMF combined with either *P. fluorescens* (colonization per cent: 80.00) or *T. viride* (colonization per cent: 68.76)

Apart from the disease management strategy by the bio control agents,

their effect on vegetative attributes such as plant height, number of leaves, number of tillers, leaf length and width were measured at the end of the experiment. The best treatment *i.e.,* the co- inoculation of AMF with *P. fluorescens* contributed to highest biometric characteristics to the treated plants (plant height: 96.50 cm, leaf length: 62.50 cm, leaf width: 12.83 cm, tiller number: 4.5 and leaf number: 15.75) compared to untreated control (plant height: 41 cm, tiller number: 2, leaf number: 7.50, leaf length: 30 cm and leaf width: 8 cm).

The nutrient status of plants and soil in response to bioagents application was also carried out at the end of the experiment. The primary (N, P, K), secondary (Ca, Mg, S) and micro nutrient (B) status were analysed as per the standard protocol. Best treatment T6 (AMF + *P. fluorescens* as combined basal application along with foliar

spray of *P. fluorescens*) improved the availability of N (225.25 kg ha^{-1}), P(152.82 kg ha^{-1}), K (319.00 kg ha^{-1}), Ca (544.92 ppm), Mg (95.75 ppm) and B (0.28 ppm) in soil as well as in plants (N:1.33 %, P: 0.15 %, K: 1.93 % ,Ca: 0.46 ppm, Mg :0.67 ppm, S: 0.25 ppm and B: 11.00 ppm) over control.

The changes in concentration of Phenol, ortho-dihydroxy phenol, peroxidase, poly phenol oxidase, phenylalanine ammonia lyase and β- 1, 3- glucanase were analysed to study the variation induced in plants exposed to biotic stress by the bio agent application. It was done three month after treatment application using fully expanded 3rd leaf of experimental plants. The highest level of defense-related enzymes such as phenol (7.95µgL-1), ortho dihydroxy phenol (45.61 µgL-1), peroxidase (23.09 µgmin-1), polyphenol oxidase (2.03 µgmin-1) and β-1, 3-glucanase (385.00 µgL-1) was observed in response to the soil application of AMF and *P. fluorescens* along with foliar spray of *P. fluorescens* (T6). Through the induction of above defence related enzymes, this treatment managed the disease effectively in the pot culture study.

Result of the pot culture study, including disease incidence and severity, pathogen antagonistic ratio in soil, AMF colonization, effect on growth of plants parameters, soil and plant nutrient analysis and induction of various defence related enzymes, revealed that Fusarium rot of cardamom, could be effectively managed by soil application of vermiculite based AMF inoculum (20 g per pot) and 2 per cent *P. fluorescens* @ 1L per 10 kg soil at the time of planting along with 2 per cent *P. fluorescens* spray @ 0.5 L per plant for three times at monthly interval.

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Appendices

APPENDIX-1

Composition of stain used

1. Lactophenol cotton blue

Anhydrous lactophenol : 67.0mL

Distilled water :20.0 mL

Cotton blue :0.1 g

Glycerol :3mL

Anhydrous lactophenol prepared by dissolving 20 g phenol in 16 mL lactic acid in 3mL glycerol

2. Trypan blue (0.05%)

Lactophenol :10 mL

Glycerol : 10mL

Distilled water : 10mL

Trypan blue :0.0015g (0.05per cent of total solution)

APPENDIX-II

Preparation of media

1. Potato Dextrose Agar (PDA) medium

Potato : 200g
Dextrose : 20 g
Agar : 20 g
Distilled water : 1 L

2. Martin's Rose Bengal Agar Medium

Peptone :5 g
Monopotassium phosphate (KH_2PO_4) : 1 g
Magnesium sulphate ($MgSO_4.7H_20$) :0.5 g
Dextrose :10 g
Rose Bengal dye :33 mg
Agar :15 g
Distilled water :1 L
Streptomycin :1%

3. Trichoderma Specific Medium

Glucose :3 g
Magnesium sulphate (MgSO4) :0.2 g
Monopotassium phosphate (KH_2PO_4) :0.9 g
Rose Bengal dye : 0.5 g
Chloramphenicol : 0.25 g
Agar : 15 g
Ammonium nitrate : 1g
Potassium chloride : 0.15 g
Distilled water : 1L

4. Nutrient Agar

Peptone	:5 g
Beef extract	:3g
Sodium chloride	:5g
Agar	:15g
Distilled water	:1000 mL

5. King's medium

Proteous peptone	:20 g
Potassium hydrogen phosphate	:1.5 g
Magnesium sulphate heptahydrate	: 1.5g
Glycerol	:10 mL
Agar	: 15 mL
Distilled water	: 1000 mL

APPENDIX-III

Buffers for Enzyme Analysis

1. 0.1 M Sodium phosphate buffer (pH 6.5)

Stock solutions

A: 0.2 M solutions of monobasic sodium phosphate (27.8 g in 1litre)

B: 0.2M solutions of dibasic sodium phosphate (53.65g in 1 litre)

68.5 mL of A mixed with 31.5 mL of B diluted to a total of 200 mL

2. 0.1 M sodium borate buffer (pH 8.8)

Stock solutions

A: 0.2M solutions of Boric acid (12.4 g in 1000 mL)

B: 0.2 M solutions of Borax (19.05g in 1000 mL)

50 mL of A is mixed with 30 mL 0f B, diluted to a total of 200 mL

3. 0.1 M sodium acetate buffer (pH 5)

Distilled water: 800 mL

Sodium acetate : 5.772g

Acetic acid: 1.778g

Abstract

Eco- friendly management of *Fusarium* rot in cardamom and its impact on soil health and plant defense mechanism

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ABSTRACT

The study entitled 'Eco-friendly management of Fusarium rot in cardamom and its impact on soil health and plant defense mechanism' was conducted at College of Agriculture, Vellayani and Cardamom Research Station Pampadumpara during 2018-2020 with the objective to assess Fusarium rot severity and pathogen variability in Idukki district, develop an effective ecofriendly management practices for the disease and study the impact of the practices on the soil and plant health.

Six isolates of *Fusarium oxysporum* Schlecht were obtained, three each from infected pseudostem and roots collected from Kattapana and Nedumkandam blocks of Idukki district. The isolate, Fp_1 from pseudostem (Pampadumpara panchayath) was identified as most virulent based on virulence rating (*viz.,* days taken for symptom development, lesion length, mycelial growth), and was used for further studies. Inoculation of the root isolate (Fr_1) also produced typical lesion on pseudostem indicating that the, fungus can also attack pseudostem besides the roots. But the root isolate took more days (40 days) for symptom development compared to pseudostem isolate (29 days). The morphological characters including the colour, colony characters, hyphal and conidial characters as well as rate of growth in Petri dish confirmed the fungus as *F. oxysporum*. *In vitro* studies revealed that the isolates from pseudostem and roots showed significant variation in their morphological, cultural and pathological characters.

A pot culture experiment was conducted to assess the efficacy of selected bioagents (individually and in combination) for the management of the disease at CRS Pampadumpara in CRD using nine treatments with three replications. Pathogen inoculum (150 gm/10 kg soil) multiplied in sand-maize flour medium was standardised as the inoculum level enough for cent per infection on the pseudostem resulting in complete crop loss. Soil application of vermiculite based AMF inoculum (20 g) with @ 2% *Pseudomonas fluorescens* (1 L/10 kg soil) per 10 kg soil at the time of planting along with 2% *P fluorescens* spray @ 0.5 L/ plant at monthly interval for three times resulted in effective disease management (disease incidence: 40% and disease severity: 24.26%) compared to the inoculated control (disease incidence: 100% and disease severity: 69.38 %). Studies on the population dynamics of the pathogen and the

biocontrol agents at periodical interval upto three months revealed significant reduction in the pathogen antagonist ratio and enhancement in AMF colonization of the treatment plants. Among the best treatment the combination of AMF and *P. fluorescens* resulted in good biometric characters of the treatment plants (plant height: 96.50 cm ,leaf length: 62.50 cm and number of leaves: 15.75) compared to control (plant height: 41 cm ,leaf length: 30 cm and number of leaves: 7.50) through enhancement of soil nutrients (P, K, Ca and Mg) and plant nutrient status (K, Mg, S and B) compared to control plants. Laboratory studies also revealed the induction of defense related enzymes (phenol, ortho dihydroxy phenol, peroxidase, polyphenol oxidase, and β 1,3 glucanase) in high concentration as a response to the application of above treatments.

Thus, the present study revealed that Fusarium rot of cardamom a devastating disease can be effectively managed by soil application of vermiculate based AMF inoculum (20 g) with 2 per cent *P. fluorescens* (1L/10 kg soil) per 10 kg soil at the time of planting along with 2 per cent *P. fluorescens* spray at 0.5 L per plant at monthly interval for three times; this treatment also resulted in good vegetative growth of cardamom plants therefore this can be used as an ecofriendly management strategy for the production of good quality cardamom.