CHAPTER - IV.

Field studies for the management of rot diseases of
*Elettaria cardamomum* and *Piper nigrum*

Introduction

India is known as the land of spices and among the spice grown in India, *E. cardamomum* and *P. nigrum* are important not only in terms of production and export but also in having centres of diversity in the evergreen forests of western Ghats. Both these spice crops are used for flavoring of various food preparations, confectionaries, beverages and liquor. These are also used for medicinal purpose, both in allopathy and ayurveda systems. Many rot diseases are affecting these crops and causing considerable crop loss. Capsule rot of *E. cardamomum* is a major disease. Similarly rhizome rot and stem rot are another set of rot diseases threatening the cultivation of cardamom results in 40 to 50 % of crop losses. Similarly in pepper also seventeen diseases have been reported, foot rot is the major disease occurring in all pepper growing tracts and losses upto 20 to 30 percent have been estimated due to this disease (Samraj and Jose, 1996 and Nambiar and Sarma, 1977).

The rot diseases of cardamom and pepper were managed through plant sanitation coupled with fungicidal application (Joseph Thomas, 1989). The excessive application of fungicides cause soil sealing, fertility diminishing and residual problem. The leftover chemicals seriously affect the quality of these spices and also caused environmental pollution. Some of the beneficial bacteria and fungi are capable of increasing the growth and vigor in several agriculturally important crops including pepper and cardamom (Berg et al., 200; Kleopper et al., 1980; Sarma et al., 2000). Plant growth promoting rhizobacteria, AM fungi and *Trichoderma* are of increasing important as inoculants for bio-
fertilization, biostimulation and biological control of plant pathogens in sustainable agriculture (Weller, 1988). Considering above fact the present work was undertaken with the following objectives.

1. To study the influence of biocontrol agents in combination with fungicide on disease control in cardamom and pepper;
2. To develop suitable package for the management of rot diseases of cardamom and pepper in the main field; and
3. To study the effect of bioagent in combination with fungicide to increase the productivity

**Materials and methods**

**Maintenance of the experimental plants**

The field trial was conducted in planters' field at Anamalai hills for cardamom and lower pulney hills for pepper.

**Detail of the field trial**


**Inoculum**

Inocula of *G. fasciculatum* and *P. fluorescens* were obtained from Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. *T. harzianum* was isolated from the rhizosphere of the *E. cardamomum* and maintained in a special medium (Elad and Chet, 1983) containing: g l⁻¹, MgSO₄-0.20, K₂HPO₄-0.90, NH₄NO₃-1.00, KCl-0.15, Glucose-3.00, Metalaxyl-0.30, PCNB-0.20, Rose Bengal-0.15, Chloromphenicol-0.25, Agar-15.00, Dis. H₂O-1000 ml. 400 ml of
suspension of *T. harzianum* incorporated in Talcum powder (1 kg) and shade dried. The talc based *T. harzianum* was used for mass multiplication.

**Mass multiplication of bioagents**

The bioagents such as *T. harzianum* and *P. fluorescens* were further multiplied in cow dung (well dried and powdered cow dung at 25% moisture level) before application to the plants. For this *T. harzianum* was mixed with cow dung in the ratio 1: 50 (1 kg *T. harzianum* with 50 kg of cow dung), sprinkled the water to get about 25% moisture and spreaded out to a thickness not exceeding 25 cm height in this shade for about 10 days. The multiplication of *Pseudomonas fluorescens* also carried out in the same procedure (Joseph Thomas et al., 2000) After multiplication of the bioagent, these were applied in the respective plot.

**Application of the bioagents in the field**

Before onset of the monsoon, plant sanitation work was completed. 2.5 to 3.0 kg of cow dung containing *T. harzianum* and *P. fluorescens* was applied on the plant base of cardamom plants. In the case of pepper 4.0 to 5.0 kg of cow dung containing *T. harzianum* and *P. fluorescens* was applied in the respective plots. 200 g of AM fungi (*Glomus fasciculatum*) was also applied at the base of the plants as per the treatment schedule and covered with thin layer of the top soil.

**Spraying of Copper oxy chloride**

After twenty days of the application of the bioagents, Copper oxychloride (0.20%) was sprayed in the respective plots of cardamom and pepper as per the treatment schedule.

**Quantitative estimation of biocontrol agents was undertaken**

(vide chapter-1)

**Enumeration and isolation of AM fungal spores**

Spore population in each soil sample was enumerated after isolation by a modified wet-sieving and (Muthukumar et al., 1996)
decanting technique and identified according to Schenck and Perez (1990) (vide chapter - 1)

Quantification of pathogen using baiting technique

To assess the population level of *Phytophthora meadii* in the soil for disease development, leaf baiting technique was followed. The soil samples were collected from both diseased and healthy plants. The collected soil sample was sieved to less than 2 mm and 25 g of each soil sample placed in petriplates. Fifty ml of glass-distilled water was added to make a soil water suspension, so that free water stood above the soil. Five replications of 20 leaflets of *Albizia falcatarea* and young cardamom leaf disc were separately floated with the adaxial surface in contact with water. Plates were incubated at 28°C for 72 h. Depending upon the density of inoculum, all or some of the baits turned black and mycelium emerged at the edges and sporulated. The infected baits were recorded and percent bait infection was calculated in order to assess the population levels of *P. meadii* (Tsao, 1960).

Recording observations on diseases

Cardamom

The incidence of rhizome rot and stem rot was recorded according to Joseph Thomas *et al.* (1996) (vide-chapter-II). In capsule rot, infection on capsule was recorded by counting total number of the capsules and infected capsules. The percentage of incidence was calculated.

Pepper

Foot rot incidence was recorded (vide-chapter-III)

Statistical analysis

The data were statistically analysed by Analysis of Variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test (DMRT). Pearson's correlation analysis was used to assess the relationships between seedling biomass, microbial population and plant tissue and soil nutrients (Zar, 1984) (Vide chapter - l).
Results

*Elettaria cardamomum*

**Rhizome rot**

The maximum disease incidence was in *E. cardamomum* recorded in T1 and minimum rhizome rot was in T16 followed by T15, T14, and T13 at 30 DAI. The disease reduction over control was 74.24%. At 60 DAI maximum rhizome rot incidence was recorded in T1 and minimum recorded in T15 and T16. The disease reduction over control was 87.77% and 87.43%, respectively. In general at 90 DAI maximum rhizome rot was recorded in T1 and T12 minimum incidence occurred in T16 plants. The disease reduction was 72.7% over control plants (Fig. 4.1).

**Severity of rhizome rot incidence**

The severity of rhizome rot was recorded maximum in T1 and minimum incidence in almost all the treatments (Fig. 4.2). However, the plants in T12 and T16 recorded minimum disease severity. The percent disease reduction over control was 64.2 and 58.6, respectively at 30 DAI. The severity also recorded at 60 DAI. Results showed that maximum severity of disease occurred in T1 (control). The minimum was in T16 plants. The percent severity of reduction over control was 80.0%. In general severity of rhizome rot was reduced in all plots. Many of the plants in T3, T4, T5, T6 and T13 were recorded maximum incidence. Minimum incidence was recorded in T9 and T16. The percent reduction over control was 68.4 and 63.1, respectively [Plate 2].

**Capsule rot**

The infection on capsule of *E. cardamomum* was recorded at 30 DAI. The maximum disease incidence was recorded in control plants (53.15%). The less incidence occurred in T16. The disease reduction in the trial plot was calculated as 80.0% over control at DAI. In contrast the disease incidence on capsule rot was recorded maximum in T9 plants compared to control. However other treated plants were recorded less
PLATE-2

A-E. Rhizome rot disease of *Eleteteria cardamomum*

A. Infected clump.

B. Healthy (left) and infected (right) tillers.

C. Infected rhizome.

D. Cultured colony of *Fusarium oxysporum*.

E. Hyphae and conidia of *F. oxysporum* (X 400).
Figure 4.1. Effect of bioagents and copper oxychloride on rhizome rot disease of *Elettaria cardamomum* under field conditions.
Figure 4.2. Effect of bioagents and copper oxychloride on severity of rhizome rot disease of *Elettaria cardamomum* under field conditions.
incidence over control at 60 DAI. Minimum incidence was recorded at T_{16}. The disease reduction over control was 60.2%. At 90 DAI, the occurrence of disease incidence was similar with many of the treatments. However disease incidence was reduced in the plants treated with combined inoculation of the bioagents and fungicide (T_{16}). The disease reduction over control was 61.0% (Fig.4.3, Plate 3).

**Stem rot**

The incidence of stem rot of *E. cardamomum* was recorded at 30, 60 and 90 DAI. Results showed that maximum incidence was recorded in T_1 and minimum in T_{16}. The disease reduction over control plant was 78.9% at 30 DAI. Similarly the incidence was more in T_1 and less in T_{16} at 60 DAI. However in 90 DAI more incidences recorded in T_1 followed by T_2 and T_5 plants. The low incidences were recorded in T_{16} at 90 DAI. The disease reduction over control was 87.7% (Fig.4.4, Plate 4).

**Population of biocontrol agent**

**Trichoderma**

The density of the *Trichoderma* was maximum in T_{16} and minimum in T_1 plants at 90 DAI. There was varying difference among the treatments but higher than the control (Fig.4.5).

**Pseudomonas**

The population of the *Pseudomonas* was recorded maximum in T_{16} followed by T_{13} and T_{14} and minimum occurred in T_1 plots at 90 DAI (Fig.4.5).

**AMF population**

The spore population of AM fungi was recorded in rhizosphere of *E. cardamomum*. It was observed that maximum was recorded in T_{16}. The spore count was not much varied from T_{11} to T_{15}. However minimum spore density occurred in T_1 and T_4 soils (Fig.4.5).
PLATE-3

A-E. Capsule rot disease of *Eletteria cardamomum*

A. Infected capsules.

B & C. Infected leaves.

D. Cultured colony of *Phytophthora meadii*.

E. Hyphae and sporangium of *P. meadii* (X 400).
PLATE-4

A-F. Stem rot disease of *Eletteria cardamomum*

A & B. Lodging of the pseudostem.

C. Lesions on pseudostem.

D. Symptoms on leaves and root.

E. Cultured colony of *Fusarium oxysporum*.

F. Conidia of *F. oxysporum* (X 400).
Figure 4.3. Effect of bioagents and copper oxychloride on capsule rot disease of *Elettaria cardamomum* under field conditions.
Figure 4.4. Effect of bioagents and copper oxychloride on stem rot disease of *Elettaria cardamomum* under field conditions.

![Graph showing disease incidence for 30, 60, and 90 DAI across different treatments.](image)
Figure 4.5. Effect of bioagents and copper oxychloride on population of biocontrol agents in *Elettaria cardamomum* under field conditions at 90 DAI.
Figure 4.6. Estimation of disease potential index in *Elettaria cardamomum* under field conditions.
Disease potential index

Disease potential index of capsule rot incidence was recorded in *E. cardamomum*. It was maximum in T1 to T7 soils. However minimum occurred in T13 at 30 DAI. In contrast the DPI was maximum T1 and T2 compared to other treatments. Minimum DPI was recorded in T13 soil at 60 DAT. Likewise at 90 DAT the DPI was recorded maximum in T1 soil and minimum in T13 soil (Fig.4.6).

*Piper nigrum*

Foot rot incidence

This disease was noticed in all parts of the *P. nigrum*. Generally the leaf infection was maximum compared to spike and were maximum incidence was recorded in the control plants and minimum was in T16 at 30 DAI. However the disease incidence was less at 60 DAI compared to 30 DAI. The disease reduction over control was 70.00, 63.15 and 76.0 in leaf, spike and wine, respectively at 60 DAI (Fig.4.7, Plate 5).

Population of bioagent

*Trichoderma*

The density of the *Trichoderma* was maximum in T7 and minimum in T1. The minimum population count was also recorded in T10 and T11 soil. However it was higher than control (Fig.4.8).

*Pseudomonas*

The population of *pseudomonas* was maximum in T16. The minimum count was recorded in T4 at 90 DAI.

AMF spore population

The spore population of AMF was recorded in rhizosphere of *P. nigrum*. The maximum spore count was recorded in T16 and minimum was in T1 soil.
PLATE-5

A-F. Foot rot disease of *Piper nigrum*

A. Completely infected vine.
B, C & D. Infected spikes, vine and leaves respectively.
E. Cultured colony of *Phytophthora capsici*.
F. Hyphae and sporangium of *P. capsici* (X 400).
Plate 5
Figure 4.7. Effect of bioagents and copper oxychloride on foot rot disease of *P. nigrum* under field conditions.
Figure 4.8. Effect of bioagents and copper oxychloride on population of biocontrol agents *P. nigrum* under field conditions at 90 DAI.
**Disease potential index**

Disease potential index of foot rot incidence was recorded in *P. nigrum* correlate with disease incidence. DPI was maximum in *T*_1 and occurred minimum in *T*_16 at 30, 60 and 90 DAI (Fig.4.9).

**Discussion**

Several fungal antagonists have potential to become biocontrol agents under common agricultural practices. The use of biocontrol agents has become an integral part of organic farming especially in an eco-friendly manner. Spice crops such as small cardamom, vanilla, black pepper, ginger and herbal spices are being affected by a number of soil borne fungal diseases and as a result a considerable quantity of crop being lost every year. Most of these fungal pathogens such as *Phytophthora*, *Pythium Rhizoctonia* and *Fusarium* etc. have been controlled by using chemical fungicides. However, the fungicidal control has its own constraints such as residual effect in plants and fungus developing resistance (Joseph Thomas and Vijayan, 2004). The biocontrol efficacy of *T. harzianum* has been studied by several workers (Smith and Wehner 1987; Marshall, 1982). Similar biocontrol activity of *Trichoderma* spp. has been clearly demonstrated in other crops (Tso, *et al.*, 1988) and also in *E. cardamomum* (Bhai, *et al.*, 1992; Joseph Thomas, *et al.*, 1993).

In the present study, the degree of incidence of rot diseases of *E. cardamomum* were varied among the treatments. However, rhizome rot was in minimum level in the plants treated with *T. harzianum* + *P. flourescens* + *G. fasciculatum* + Copper oxychloride. Similarly rhizome rot, capsule rot and stem rot were also reduced in the plants treated with combined inoculation of the bioagents and copper oxychloride. The percent disease reduction over control was 72.7%, 61.0 and 87.70 in rhizome rot, capsule rot and stem rot respectively at the end of the season. This finding is in agreement with Suseela Bhai *et al.* (2000) and Vijayan (2002) who reported that combined application of *T. viride* in
Figure 4.9. Estimation of disease potential index in *Piper nigrum* under field conditions.
combination with Bordeaux mixture or copper oxychloride reduced the rot diseases of \textit{E. cardamomum}, reduced the disease potential index and increased the population level of \textit{Trichoderma}.

Divya and Nair, (2003) also studied that in \textit{P. nigrum}, foot rot incidence was completely controlled in plants applied with \textit{G. fasciculatum}, \textit{T. harzianum} + \textit{G. fasciculatum} and Copper oxychloride. The antagonistic effect of \textit{T. harzianum} on growth and proliferation of foot rot pathogen (\textit{Phytopthora capsici}) in the plant rhizosphere and in disease incidence in \textit{P. nigrum} were observed by many authors. AM fungi has also been reported earlier by Tsao et al. (1988), Anandaraj et al. (1993), Anandaraj and Sarma (1994), Sivaprasad et al. (1995) and Dare (1996).

Therefore, \textit{P. fluorescens}, \textit{B. subtilis} and \textit{Trichoderma} are commonly used as effective biocontrol agents in many of the crop plants for growth promotion and disease control. \textit{T. harzianum} was found to be compatible with \textit{P. fluorescens} (Saju et al., 2003). Combined application \textit{P. fluorescens} + \textit{Trichoderma} sp. has been the best to reduce the wilt disease of grape vine (Karunakaran, 2003).

The synergistic effects of one or more bioagents have been reported. AM fungi, \textit{T. harzianum} and \textit{P. fluorescens} when used together in a consortium has increased the seedling stand, plant growth nutrient uptake and dry matter production and controlled characol stump rot disease in Tea (Hazarika and Phookan, 2003) in geranium (Mansoor Alam et al., 2003) and sunflower (Prasad et al., 2003).

Bioagents must be used with compatible fungicides for increasing the efficiency in disease control. The integrated approach of basal application of \textit{Trichoderma} with potassium phosphonate (0.3%) not only reduce the diseases significantly but also enhance the population of \textit{Trichoderma} in the field (Joseph et al., 2004). Similarly, application of copper oxychloride (0.2%) reduced the rhizome rot incidence in \textit{E. cardamomum} and increased the population of \textit{Trichoderma} (Vijayan, 2002).
It is proved, in the present study also, that population level of the biocontrol agents were maximum in all the inoculated plants compared to control. *P. fluorescens* was highly compatible with many of the pesticides (Mathew, 2000). The combination of *P. fluorescens* + AM fungi + fungicide resulted better establishment and growth of black pepper (Thankamani *et al.*, 2003).

**Piper nigrum**

In *P. nigrum*, disease management practice with chemical fungicide, though not at all successful, are matters of major concern in view of residual toxicity problems. Antagonistic microorganisms present in the soil play a major role in keeping the population of soil borne pathogens at low level. Antagonistic microorganism, mostly fluorescent *Pseudomonas* and hyperparasitic fungi become successful as biocontrol agent and effective against infection of different soil borne pathogens (Papivizas and Lumsden, 1980). Application of such beneficial bioagents has shown potentiality for controlling soil borne root infecting fungi and other pathogens through diverse mechanism (Pandey and Kumar, 1990). Some other agents of bacterial and fungal origin including AM fungi were also proved as biocontrol agents (Anandaraj and Sarma, 1994). In the present study, *P. nigrum* vines were inoculated with *P. fluorescens*, *T. harzianum* and *G. fasciculatum* and in combination. Spraying of copper oxychloride (0.2%) also was included with this application. The foot rot incidence of *P. nigrum* was varied among the treatments. In general, the infection on leaf and spike was maximum compared to vine infection.

Application of *Trichoderma harzianum* and *Pseudomonas fluorescens* was recommended for promoting growth and suppression the disease caused by pathogen in black pepper, ginger and cardamom (Jisha *et al.*, 2002). It is in line with the present study that foliar spray can be given to all the vines with copper fungicides control the diseases of *P. nigrum* (Sarma, 2000).
In the present study, the population level of bioagent was increased in inoculated plants compared to control. This finding is in agreement with Hubbard et al., (1983) that when *T. hamatum* was inoculated to soil containing a *Pseudomonas* sp. The population level of the microbial inoculants was maximum in rhizosphere of *Casuarina equisetifolia* and *Dalbergia Latifolia* (Rajeshkannan, 2002).

In the present investigation there was significant compatibility of bioagent with copper oxychloride. The fungicides such as potassium phosphonate, mancozeb and copper oxychloride did not inhibit *T. harzianum*. In general, fungicides did not inhibit the growth of *P. fluorescense*. These bioagents can be used with chemical fungicides for integrated disease management as these are mutually compatible (Vijayan, 2004).

It is concluded that integration of compatible copper oxychloride and bioagents not only increased the disease control but also reduced the cost of plant protection. However, while selecting the suitable combinations, great care has to be taken to see that the components of integration are mutually compatible and contribute towards added advantages in promoting plant growth, productivity as well as protection from pathogenic organisms.