Chapter I
Chapter - I.

Dynamics of AM fungi association and spore density in

*Elettaria cardamomum* and *Piper nigrum*

**Introduction**

Organic matter plays a vital role in the productivity and soil conditioning. Soil is a substrate for the growth and multiplication of the microbes. It serves as a source of food for bacteria and fungi, which are responsible for converting complex organic materials into simple substances readily used by the plants. The increased microbial activity in the rhizosphere leads to an increased competition between the component species for nutrient and space, and these may affect the plants either adversely or favourably depending on the type of microbes inhabiting the rhizosphere, which may either directly or indirectly influence the growth of the plants. Roots of the plants are known to be intimately associated with mycorrhizal fungi. The mycorrhizal fungi are important in sustainable agriculture due to their role in plant and soil nutrition acting as agent in transporting mineral nutrients to the plant and C compounds to the soil and its biota (Reide, 1990). These fungi are known to occur in all soil types and to enhance the uptake of diffusion-limited nutrient such as phosphorus (P) in soil with low availability in spite of their high P retention capacity. Arbuscular mycorrhiza (AM) fungi are not host specific. They differ in their ability to enhance nutrient uptake and plant growth (Mallsha *et al.*, 1994 and Joseph *et al.*, 1998). Differences among host plants and soil fertility stimulate differential sporulation by AM fungi species in the field (Hayman, 1975; Schenck and Kinlock, 1980; McGraw and Hendrix, 1984) and spore production is seasonal in several habitats (Brundett, 1991). However in studies on distribution of AM fungi, the difference in edaphic and climatic factors and host plants
have been so great that is too difficult to explain what factors determine the presence and absence of a particular species (Weller et al., 1982).

The purpose of the present study is to estimate the microbial status of *Elettaria cardamomum* and *Piper nigrum* on plantations. Hence an investigation was undertaken in *E. cardamomum* and *P. nigrum* with following objectives,

a) To estimate the microbial status of the host plants;
b) To find out the influence of edaphic and climatic factors on the degree of AM fungal association in the host; and
c) To identify the occurrence and dominance of microbial spores, particularly by AM fungi present in the soil at a particular time.

**Materials and methods**

**Study area**

A survey was carried out to assess the seasonality of AMF in *Elettaria cardamomum* and *Piper nigrum* in the Western Ghats ecosystem, Southern India. Seven-year-old plantations of *Elettaria cardamomum* and 15-year-old plantations of *Piper nigrum* were selected. The root and rhizosphere soil samples of *E. cardamomum* were collected at Indian Cardamom Research Institute, Regional Research Station, Thadiankudisai located at lower pulney hills in Kodaikanal, Tamil Nadu at an elevation of 1180 m a.s.l. and of *P. nigrum* root and rhizosphere soils samples were collected at Planters’ field at Thandikudi located in lower pulney hills in Kodaikanal, Tamil Nadu at an elevation of 1400 m a.s.l.

**Climatic data**

Climatological data [minimum and maximum temperature, relative humidity (RH%) and rainfall] were obtained from Indian Cardamom Research Institute, Regional Research Station, Thadiankudisai and Regional Coffee Research Station, Thandikudi, Kodaikanal, Tamil Nadu, India.
Sampling

Root and rhizosphere (upto 30 cm soil depth) soil samples were collected from *E. cardamomum* and *P. nigrum* plantations. The samples were collected at monthly intervals for a period of 12 months from November to October 2001. During each sampling five individuals were selected. The roots were dug out, washed free of soil and fixed in formalin-acetic acid-alcohol. The rhizosphere soils (1 Kg) from the individuals were mixed to form a composite soil sample; air-dried, packed in polythene bags and kept at room temperature (20-30°C) until further analysis.

**pH**

Ten gram of dry soil was taken in a beaker and 100 ml of water added to make a suspension of 1:10 (w/v) dilution and the pH was determined with a digital pH meter.

**Electrical conductivity**

Ten gram of dry soil was taken in a beaker and 100 ml of water was added to make a suspension of 1:10 (W/V) dilution and the electrical conductivity was measured with a digital electric conductivity meter.

**Analysis of soil nutrient content**

The total nitrogen (N) and available phosphorus (P) were determined respectively by micro-Kjeldahl and molybdenum blue methods of Jackson (1973). Exchangeable K was extracted from the soil in ammonium acetate solution (pH 7) and measured with a digital flame photometer (Jackson, 1973). Soil organic carbon was determined according to Piper (1966).

**Preparation of roots and AM assessment**

Fixed roots were washed free of FAA, and observed under a dissection microscope (X 20) for AM fungal spores attached to them. After examination, the roots were cut into 1 cm bits, cleared in 2.5% KOH (Koske and Gemma, 1989), acidified with 5 N HCl and stained with
trypan blue (0.5% in lacto glycerol). The roots were kept overnight immersed in stain for staining. The stained roots were examined with a compound microscope (X 200 – 400) for AM fungal structures and the percentage of root length colonization was estimated according to magnified intersection method (McGonigle et al., 1990).

**Enumeration and isolation of AM fungal spores**

One hundred gram soil was dispersed in 1L water and the suspension was decanted through 710- to 38-μm sieves. The residues in the sieves were washed into beakers. The sieves were dispersed in water and filtered through grided filter papers. Each filter paper was spread on a petridish and scanned under a dissection microscope X 40 magnification and all intact spores were counted. Sporocarps and spore clusters were considered as one unit. Intact AM fungal spores were transferred using a wet needle to polyvinyl alcohol-lacto glycerol with or without Melzers reagent on a glass slide for identification. Spores were identified based on spore morphology and sub-cellular characters and compared with original descriptions (Schenck and Perez, 1990). Spore morphology was also compared with the culture database established by INVAM (http://invam.cag.wvu.edu).

**Statistical analysis**

All data were subjected to Analysis of Variance (ANOVA) and the means were separated using Duncan’s Multiple Range Test (DMRT). Data on AM colonization and spore numbers were arcsine and log transformed \[\ln (1 + X)\] respectively prior to statistical analysis. Pearson’s correlation analysis was used to assess the relationships between edaphic-climatic factors, spore number and root colonization (Zar, 1984).

**Results**

The study on relationships between environmental factors and AM mycorrhizal variation was carried out in the rhizosphere of the *E. cardamomum* and *P. nigrum*. The edaphic factors showed in both
plantations that the soils were acidic in nature. The organic carbon ranged between 2.2 to 4.6 % and 1.6 to 4.3 in the rhizosphere of *E. cardamomum* and *P. nigrum* respectively and there was no appreciable difference in available nutrients. The percent root colonization of AM fungi in *E. cardamomum* ranged between 19.0 to 86.10 and 21.10 to 88.33 in *P. nigrum*. It was recorded maximum during February and minimum during July in *E. cardamomum*. Whereas in *P. nigrum* it was maximum during September and minimum during March (Fig. 1.1-1.4).

The population of AMF spores was calculated. The total AMF spore number varied from 18.66 to 67.00 and 12.00 to 90.30 g\(^{-1}\) soil in *E. cardamomum* and *P. nigrum* respectively. The number of spores in *E. cardamomum* was maximum during June and minimum during September. Likewise maximum and minimum spore number was recorded during March and August respectively in *P. nigrum*. AMF spores belonging to five species were recorded from rhizosphere of *E. cardamomum* (*Acaulospora* sp., *Gigaspora* sp., *Glomus aggregatum*, *G. fasciculatum*, *G. intraradices*) and in *P. nigrum* (*Acaulospora* sp., *Gigaspora* sp., *Glomus aggregatum*, *G. fasciculatum*, and *G. geosporum*).

The relationship between climatic, edaphic and biotic factors in *E. cardamomum* was studied and presented [Table 1.1]. Temperature was significantly and positively correlated with EC and negatively with soil N. Rainfall was correlated significantly to soil P and negatively to temperature. Soil moisture was correlated positively to %RLH and %RLC. Rainfall also significantly correlated with %RLA and negatively with spore density. Percent root length hyphae (RLH) was significantly positively correlated with %RLC [Plate 1. A-B].

The results of relationships among and between environmental factors and mycorrhizal variations in *P. nigrum* are presented [Table 1.2]. Temperature was significantly and positively correlated to EC but negatively to RH. Likewise rainfall was significantly correlated to soil
PLATE-1

A-D. Arbuscular mycorrhizal colonization in *Eletteria cardamomum* and *Piper nigrum*

A. Intraradical hyphae and vesicles in roots of *E. cardamomum* (X 200).

B. Arbuscules in root cortical cells of *E. cardamomum* (X 400).

C. Intraradical spores in *P. nigrum* (X 200).

D. Arbuscules in *P. nigrum* (X 400).
Table 1.1 Correlation coefficients of climatic, edaphic and mycorrhizal variables in *Elettaria cardamomum*.

<table>
<thead>
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<th>Climatic factors</th>
<th>Soil factors</th>
<th>Arbuscular mycorrhiza</th>
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<td>Mini. Tem.</td>
<td>RF</td>
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<td>%RLA</td>
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*Maxi. Tem., Maximum Temperature; Mini. Tem., Minimum Temperature; RH, Relative humidity; RF, Rainfall.*

SM, Soil moisture; pH, Electrical conductivity; N, Nitrogen; P, Phosphorus; K, Potassium; OC, Organic carbon.

*Spore, AMF spore No.; %RLC, Root length colonization; % RLH, Root length hyphae; % RLV, Root length vesicles; %RLA, Root length arbuscules

** *, * - Significantly at the 0.01, 0.05 level respectively.
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<th>0.543</th>
<th>0.060</th>
<th>0.237</th>
<th>0.017</th>
<th>-0.844**</th>
<th>0.788**</th>
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**Maxi.Tem., Maximum Temperature; Mini.Tem., Minimum Temperature RH, Relative humidity; RF, Rainfall.

SM, Soil moisture; pH, Electrical conductivity; N, Nitrogen; P, Phosphorus; K, Potassium; OC, Organic carbon.

Spore, AMF spore No.; %RLC, Root length colonization; %RLH, Root length hyphae; %RLV, Root length vesicles; %RLA, Root length arbuscules

**, * - Significantly at the 0.01, 0.05 level respectively.
Figure 1.1. Climatic data of the study sites during the period studied.
Figure 1.2. Edaphic factors of the study sites during the period studied.

Soil moisture (\%)

pH

EC (mS cm\(^{-1}\))

*Elettaria cardamomum*

Month (2000-2001)

*Piper nigrum*
Figure 1.3. Edaphic factors of the study sites during the period studied.

- **N** (mg kg⁻¹)
- **P** (mg kg⁻¹)
- **K** (mg kg⁻¹)
- **OC** (%)

**Elettaria cardamomum**

**Piper nigrum**

Month (2000-2001)
Figure 1.4. Incidence of AM fungal spore numbers and structures in *Elettaria cardamomum* and *Piper nigrum* during the period studied.
nitrogen, %RLC, %RLH and %RLV. Rainfall had significantly negative correlation with AM fungal spore density. Soil nitrogen was observed to have a significantly positive correlation with %RLA and %RLV. Spore population had significantly negative correlation to %RLC, %RLH, and %RLA [Plate 1. C-D].

**Discussion**

The variations in abundance of AM fungi as assessed by spore number and root length colonization throughout the year has attracted considerable attention (Abbot and Rabson 1991). Many such studies on the difference in mycorrhizal formation and spore number have been concentrated on short-lived annuals but the result for long lived may be considerably different (Hayman, 1970: Sutton, 1993: Smith, 1980). The present investigation clearly indicates the periods and factor that favour root colonization and AMF associated with *E. cardamomum* and *P. nigrum*. During the study period low spore count was recorded in the rhizosphere of these crops. Moreover the low spore density of AMF is due to the acidic nature of the soil. This above finding is supported by Udaiyen *et al.* (1996). The other reason for low spore number can be the presence of the AMF propagules like the intraradical structures persisting in root which are the main source of inoculum for perennial plants (Baylis, 1969). Generally tropic soils are characterized by low available nutrient especially P (Kang and Wilson, 1987).

During the study period the variation in AMF colonization and spore population in *E. cardamomum* and *P. nigrum* may also be due to seasonal influence. The higher AMF colonization and low spore numbers observed is in agreement with Udaiyen *et al.* (1996) and Rajeshkannan (2002). The reduction in spore numbers may result from spore germination and limited spore life span on the activity of antagonistic soil microorganisms, which may coincide with root growth (Mosse and Brown, 1968; Sutton and Barron, 1972). Sporulation has also been
reported to be depressed by hyperparasitic fungi (Schenck and Nicolson, 1977). Seasonal variations in mycorrhizal spore number were studied previously (Ebbers et al., 1987; Dhillion et al., 1988; Gemma and Koske, 1988). In most cases, spores were less abundant during the periods of mycorrhizal formation and became more numerous during periods of roots senescence and or of the growing season (Brundrett, 1991). Spore density had significantly negative correlation to %RLC, %RLH and %RLA which contrast the observations of Hetrick and Bloom (1996), Coltman et al. (1988) and Khalil et al. (1992) as wide range of host, fungal and environmental factors are known to influence AMF formation and subsequent spore production. Higher AMF colonization and low spore number observed in host species, in the present study is in agreement with those noted by Udaiyan et al. (1996) in Acacia farmesiana and A. planifrons and Rajeshkannan (2002) in Casuarina equisetifolia and Dalbergia latifolia.

The results revealed an inverse relationship between %RLC and spore number in E. cardamomum and P. nigrum, which agree with previous studies where higher AM fungal colonization and a low spore number have been noted (Udaiyan et al., 1996). Under environmental conditions where root growth is continuous the vegetative phase of AM fungi may be actively involved in initiating infection and spreading onto new roots. Further, the mycorrhizal dependency of the activity-growing host may delay the fungal reproductive phase to a certain extent. Root colonization was highest during February and September in E. cardamomum and P. nigrum respectively, when the soil moisture was moderate in these plants. The hosts’ dependence on AM fungi for nutrients might have enhanced mycorrhization with decreasing soil moisture. The reduction in soil moisture level adversely affected the root colonization but enhanced sporulation. However, spore numbers also tended to reduce at very low soil moisture. A similar increase in percent
of root colonization as well as spore population at moderate soil moisture level has been observed by Sangeeta Kaushal (2000). The presence of arbuscules during these periods further confirms an active growth of host and its dependence on mycorrhizal fungi (Jasper et al., 1989; Michelson, 1993). Arbuscules are the site of nutrient transfer from fungus to host and their presence are an indication of mutualism (Koske et al., 1992). Reinhadt and Miller (1990) also reported higher arbuscular infections during the host's active growth phase. Studies by Sparling and Tinker (1978) and Koide and Li (1990) have clearly demonstrated that the development of arbuscules in plant is controlled by host nutrient demand. In *P. nigrum* soil moisture was significantly correlated with percent length arbuscules (%RLA). Soil moisture conditions have also been proposed to influence root growth and AMF colonization (Rabati, 1977; Allen, 1983)

The extrametrical phase of the AM fungi in the soil, its growth and development is influenced by the edaphic factors. A study on the effect of pH on AM fungi has shown that germination of AMF spores is sensitive to pH. However, the effect of pH on root colonization tends to vary with strains of AMF species (Medeiros et al., 1994)

Plant's survival in the natural ecosystems depends upon the ability of plants to take up water under fluctuating soil moisture conditions (Sala and Lananroth, 1982). Information on the effect of soil moisture on mycorrhizal formation and spore abundance is very limited (Smith and Gianinazzi-Pearson, 1988; Sylvia et al., 1993) although the role of AM fungi is enhancing the water uptake by plants under fluctuating soil moisture conditions is well documented. Douds and Schenck (1991) found that water availability was an important determinant of AMF spore germination. However, either a rapid root growth greater than the infective capacity of AM fungi (Abbott and Robson, 1991) or the higher flush of nutrient released from accumulated soil organic matter following
moisture induced decomposition (Swift et al., 1981) may affect variations in mycorrhizal formation.

The %RLC was positively correlated with soil moisture, indicating the response of AMF colonization in roots of fluctuating soil moisture levels (Allen and Allen, 1984). Soil N was correlated positively with spore density and negatively to % RLC in *E. cardamomum*. Soil N plays an important role in influencing the mycorrhizal formation and function mainly through changes in soil pH. Soil N was positively correlated with spore number in *Accacia planiferons* (Udaiyan et al., 1996), however, the effect of N on AM fungal spore abundance is related to other soil factors and to the host with which they are associated (Hayman, 1982). But soil N was correlated negatively with EC and temperature and positively with spore population and % RLC in *D. latifolia* (Rajeshkannan 2002). Similar observation was made in *A. farnesiana* and spore number of *A. planifrons* (Udaiyan et al., 1996). The stimulatory effect of soil N on root colonization has also been reported by Aziz and Habte (1989) and Hepper (1983)

In *E. cardamomum* and *P.nigrum*, spore density was negatively correlated with rainfall. These observations agree with those of Udaiyan et al. (1996) and RajeshKannan (2002) and emphasize that climatic factors can strongly influence AMF colonization (Furlan and Forti,1973; Hayman,1974) The influence of soil P on AMF structures is in accordance with the observations of Udaiyan et al. (1996) where soil P tended to influence root colonization in *A. planifrons*. It is well established that increasing soil P can reduce AMF formation and the inhibition may be due to the direct effect of P on the external hyphal network development or indirectly associated with host P status (Sanders, 1975). However, the effect of P on spore density tends to vary with host species.

Native soil often contains AMF spores of more than one species and spore belonging to three AMF genera were isolated from the two host species of rhizosphere soil. Generally more than one AMF species are
quite common in the rhizosphere of perennial hosts (Thapar and Khan, 1985), which is substantiated by the recovery of spores belonging to different species in each host. The possibility of environmental factors in addition to host factors is important in influencing AMF distribution (Khali, 1992).

The rhizosphere is the natural reservoir of a myriad of microorganisms, which are activated to grow around developing plant roots. Factors such as soil type, pH, temperature, age and conditions of plants are known to influence rhizosphere (Rovira, 1965). In the present study highest population of non-mycorrhizal fungi was recorded in the rhizosphere of *E. cardamomum* and *P. nigrum*. It is evident that the conducive climatic and edaphic factors that prevailed during that period might have favoured the high microbial activity. However species of *Trichoderma* and *Aspergillus* occurred as common during the entire period. The fungus such as *Phytophthora*, *Pythium* and *Fusarium* were also recorded.