ECOLOGY OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI IN RUBBER GROWING SOILS OF KERALA

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By

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Dedicated to my Parents

Contents

Chapter 1

INTRODUCTION

1. INTRODUCTION

Natural rubber (*Hevea brasiliensis* Muell. Arg.) is an important plantation crop in India. It is the source of 99 per cent of the natural rubber (NR) produced in the world. *Hevea* is predominantly grown in the tropics where an equatorial monsoon climate prevails. The fundamental factors that influence rubber cultivation are rainfall, temperature, sunshine, relative humidity and wind velocity, of which the former two play a very important role in the selection of rubber planting area. Commercial planting of *Hevea* in India was started during the beginning of 20th century. Within a century it has occupied an important position with regards to its area, production potential and socio - economic status of people. Today, India is the third highest producer of NR in the world, having a total area of 5,66,558 ha with an annual production of 6,31,400 tonnes of various grades of rubber (Indian Rubber Statistics, 2002). At present, Kerala is the most important rubber producing state in India. Eighty four per cent of area under *Hevea* and 92 per cent of production are concentrated in the state. Geographical and agroclimatic suitability proved congenial for NR cultivation in the state of Kerala. Further, the first NR plantation in India was started in Kerala and now rubber is the third largest crop next to rice and coconut in this state. Rubber plantations occupy about 16 per cent of the geographical area and make significant contribution to the economy of the state.

In the beginning, rubber planting was mostly confined to newly cleared forests, rich in plant nutrients. Over a period of time, the situation has changed and the soil became unproductive. To ensure optimum growth and yield and to protect sustainability of the system, maintenance of soil fertility through regular application of fertilizer is being carried out. *Hevea* is cultivated mainly in hilly terrain lands with varying degrees of slope and fertility. Disturbances of soil by various agro-management practices have lead to erosion of soil as well as nutrients.

Next to fertilizers, plant protection chemicals especially copper-based fungicides are extensively used in rubber cultivation. On an average 4.0 kg of copper per ha is reaching the soil every year. Other fungicides like sulphur and modern synthetic fungicides, pesticides as well as weedicides are also extensively used.

Rubber growing soils are generally acidic with pH ranging from 4.0 to 6.5, but the crop tolerates pH in the range 3.8 to 8.0 (Dijikman, 1951). In the initial stages of cultivation, rubber was never a monocrop. Leguminous cover crops are grown for multifarious benefits like improvement in soil organic carbon and fertility, reducing soil temperature, improving soil moisture and preventing soil erosion. Annual leaf fall of *Hevea* also adds to the organic carbon value of soil and helps nutrient cycling. Nowadays, intercropping with various annual and perennial crops is also practiced (Punnoose *et al.*, 2000).

Needless to mention that all these factors have profound impact on the ecobiology of rubber growing soils. Deforestation leads to a change in the flora and fauna but also the soil microorganisms. A mature rubber plantation is a dynamic and self-sustaining ecosystem and a renewable source of rubber with minimum external agronomic inputs (Goldthorpe and Tan, 1996). Many rubber plantations in India are now under the third cycle of cultivation (Jacob, 2000). Continuous cultivation of rubber in these areas for the past several decades had changed the soil fertility and ecosystem. Latest reports indicate that in rubber growing soils, there are changes in the population of actinomycetes, bacteria and fungi quite distinct from that of native forest soils (Deka *et al.*, 1998).

Many living plant roots have a symbiotic association with a group of fungi and such association is called mycorrhizae (fungus root). Based on the morphology and anatomy of mycorrhizal roots, they are classified into ectomycorrhizae and endomycorrhizae (Subba Rao, 1977). Ectomycorrhizae is confined to coniferous plants in general and such plants do not establish themselves without mycorrhizal association. The symbiotic fungi that predominate in the roots and soils of agricultural crops and weed plants are of endomycorrhizal (Hayman, 1980; Trappe, 1981). Fungi forming endomycorrhizae have vesicles, the food storage organ and arbuscules, the site of nutrient transfer and hence they are called Vesicular Arbuscular Mycorrhizae (VAM). Later this group is known simply as Arbuscular Mycorrhizae as two of the constituent genera *viz. Sclerocystis* and *Gigaspora* do not form vesicles (Sylvia,

1998). It is an important symbiotic association, which helps in proper plant growth and yield (Abbott and Robson, 1984). The mycorrhizal fungi themselves are sustainable, remaining in the soil as spores or in association with roots. The factors, which influence the quantitative and qualitative occurrence of VAM under different agro-climatic and agro-managment conditions, root colonisation, survival of spores and dispersal, are only partially understood (Daniels, 1984). For exploiting the mycorrhizal fungi, to increase crop yield, a more thorough understanding of these factors are essential.

Development of VAM is an important factor, which has profound influence on the adaptation of any introduced plant, and such is the case with *Hevea*. VAM association in *Hevea* was established as early as 1965 (Wastie, 1965). This association is reported to eradicate root disease and foliage disease of *Hevea* (Jayaratna *et al.*, 1986; Feldman *et al.*, 1988). Ikram and Muhmud (1984) studied the distribution of VAM fungi in Malaysian soils, however, not much information is available on the nature and distribution of VAM fungi associated with *Hevea* in India. VAM association with plants is considered to be more essential with respect to phosphate and some micronutrient absorption by plants. The benefits of VAM on growth enhancement, improvement of nutrient uptake and diminishing abiotic stresses have been reported in a wide range of host plants from annual crops to woody perennials (Smith and Gianinazzi, 1988).

It is generally accepted that mycorrhizae can make plants more productive, although uncertainty remains about their role in managed ecosystems (Miller *et al.*, 1995). A study on the ecology of VAM in rubber growing soils of Kerala will be of immense importance in planning fertilizer schedule, application of plant protection chemicals *etc.*

Therefore, a study was carried out with the following objectives: -

- Qualitative and quantitative distribution of VAM in rubber growing soils of Kerala and variation in *Hevea* root colonisation.
- Effect of seasonal changes on VAM spore population and root colonisation in selected rubber plantations.
- Understanding the dissemination of VAM fungi through different agencies.
- Influence of soil temperature, moisture and organic matter on VAM population and root colonisation under controlled conditions.
- Changes in VAM population and their root colonisation under the influence of the application of fertilizers and plant protection chemicals.

Chapter 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Root systems of most living plants have developed an endophytic symbiotic association designated as Vesicular Arbuscular Mycorrhizae (VAM). Nonseptate, zygomycetous fungi belonging to genera, *Glomus*, *Gigaspora*, *Acaulospora*, *Sclerocystis*, *Scutellospora* and *Enterophospora* of the family Endogonaceae, form VAM association. VAM fungi are ubiquitous in nature and they colonise the roots of nearly all plants (Barea, 1991; Bolan, 1991). The relevance of mycorrhizal endophytes has been described as that of a fundamental link between plant and soil (Miller and Jastrow, 1994). VAM fungi are mainly distributed in the upper layers of soil and their highest density is encountered in the rhizosphere of plants (Schwab and Reeves, 1981; Bellgard, 1993). The importance of VAM fungi has been intensively investigated over the past four decades. It has been established that VAM fungi improve plant growth in terms of better nutrient uptake (Mosse, 1973a; Powell, 1982; Jeffries, 1987; Kumar *et al.*, 2002), water potential (Sanchez-Dias and Hornrubia, 1994) and lesser chances of root diseases (Jalali and Thareja, 1981; Newsham *et al.*, 1995). The symbiotic association increased the uptake of relatively immobile nutrients such as P, Cu, and Zn (Faber *et al.*, 1990; Kothari *et al.*, 1991). Mycorrhizae also enhanced the uptake of available nitrogen (Hamel *et al.*, 1991; Tober *et al.*, 1994) and in contrast often reduced Mn uptake (Kothari *et al.*, 1991; Posta *et al.*, 1994).

The symbiosis between VAM fungi and plant roots play a key role in plant ecology and productivity (Abbott and Robson, 1984). Ecological importance of VAM fungi had been studied in temperate regions but very little information is available on the tropical.

Hevea brasiliensis is a perennial tree crop grown extensively in the western side of the Western Ghats of India. *Hevea* was introduced into India a century ago and assumed an important position in the agricultural scenario of Kerala and plays a major role in the socio-economic condition of the state. Deforestation and planting of *Hevea* has lead to a change in the ecology of VAM fungi considerably. Nair and Girija (1988) recorded highest VAM infection in rubber compared to other tree crops of economic importance. Leguminous cover crops grown in the interspaces of *Hevea* are also found to be mycorrhizal (Joseph *et al.*, 1988).

The factors, which influence the survival, infectivity, spread, and persistence of VAM inoculum in nature were only partially understood (Bagyaraj, 1991). If one moves closer to using mycorrhizal fungi to increase agricultural plant yield, a more thorough understanding of these factors will be essential. In this chapter effect of various factors, which influence the colonisation and spore population of VAM fungi are reviewed.

2.1. Effect of location on VAM distribution

The physico-chemical characters of soils vary considerably with respect to the geographical location. VAM occurs in plants in arctic, temperate and tropical regions including dense forests, open woodlands, scrub, savanna, grasslands, heaths, sand dunes and semideserts. VAM occurrence within these systems varies according to localised environmental conditions and plant cover (Hayman, 1982a). There are a number of reports of VAM endophyte from India (Godse *et al.*, 1978; Bagyaraj *et al.*, 1979; Kehri *et al.*, 1987; Kaushal, 2000). However, very few attempts have been made to undertake regional surveys of mycorrhizal association with tree crops, soil and agroclimatic conditions in Kerala. Joseph (1997) studied the distribution of VAM fungi in rubber plantation soils from 16 localities spread over from Nagercoil in the south and Mangalore in the north on western side of Western Ghats and observed a wide variation of VAM population in these soils. Harikumar and Potty (1999) reported variation in the distribution of VAM species in southern and northern regions of Kerala. According to them the spore density, species richness and diversity index differed in these regions with a general increase in southern Kerala. Mohanan and Sebastian (1999) made a survey on the mycorrhizal status of bamboos in Kerala and could not observed any correlation with their ecological range of distribution.

2.2. Effect of season on VAM colonisation and spore population

Root colonisation, growth and sporulation of VAM depend on many environmental factors (Daniels, 1984). It was thought that spore production increased after periods of extensive root growth or as the host matured or senescenced (Mason, 1964; Mosse and Bowen, 1968). Many surveys had documented seasonal variations within VAM fungal populations (Daniels and Bloom, 1983). Hayman (1970) observed higher VAM spore numbers and root infection during summer in wheat under irrigated conditions. Sutton and Barron (1972) reported an increased VAM spore density in April in strawberry and maize field soils. Sparling and Tinker (1975) found that the root weight and per cent colonisation were high in summer. In California soils, chlamydospores were most abundant from November to May and least from June to September (Menge *et al.*, 1981). According to Rolden *et al.* (1982) the extent of VAM infection and the number of endogonaceous spores in the rhizosphere of almond trees were low in winter, while Jakobsen and Nielsen (1983) reported that mycorrhizal infection levels in winter wheat, rye and barley were low in spring season. Michael (1983) reported that in *Atriplex gardneri* arbuscles were observed in April but were not found in June and the total VAM colonisation was highest (78 per cent) in April, 36 per cent in June and 3 per cent in July at kemmerer coal mine in Wyoming.

According to Daniels (1984) enough spores survived the winter season to initiate colonisation in spring. He also observed an increase in spore population during the active growth season, which declined by the following spring season. But Mohankumar and Mahadevan (1988) recorded an increased spore count from September (rainy season) to April (summer season) in Kalakad Reserve Forest, a tropical forest located in the Western Ghats of Tamil Nadu. They also observed a decrease in root colonisation as the summer progressed.

Iyer *et al.* (1988) observed a reduced root colonisation in the premonsoon samples of cardamom while the infection grading increased after the rains. A marked decrease of VAM mycorrhizal infection and spore number in summer was noticed in the study of seasonal variation of VAM in maritime sand dune at Pisa in Italy (Giovanetti, 1985).

The number of spores in the soil and infection of the roots of different plant species in Madras were more in summer than in the rainy season (Parameswaran and Augustine, 1988). According to Siguenza *et al.* (1996) root colonisation and arbuscle development started with the rainy season and as the soil dried the number of arbuscles declined and vesicle numbers increased. At this point, the levels of colonisation were also high and the fungus produced spores many of which remained in the soil until adequate moisture initiated their germination in the next growing season. It was found that post rainy (November) and winter (February) months soil

samples had more number of VA mycorrhizal spores than those of summer (May) and rainy (August) months of Tarai soils in Uttar Pradesh (Trimurtulu and Johri, 1998). Lakshman *et al.* (1999) while studying the seasonal influence of VAM fungi in some commonly cultivated crops of Dharwad district in Karnataka, observed high spore population in summer and low in rainy season. Schwob *et al.* (1999) reported a higher number of VAM spores in the rainy season than in dry season, although VAM colonisation of roots was unaffected by season. A seasonal study on AM fungi associated with *Ipomoea pes-caprae* established on the coastal sand dunes of the west coast of India reported highest spore density during summer season (Beena *et al.*, 2000). But Kaushal (2000) observed that in Rajasthan soils the spore count was minimum in summer season, *i.e.* April, May and June and maximum in August and September.

2.3. Comparison of forest and rubber growing soils

In forests, the continued deposition of leaves and other debris creates a kind of organic mulch in surface soils, which results in a more stable microclimate and provides conditions for a wider spectrum of soil animals and microorganisms. Prichett (1979) had compared forest soils with agricultural soils. Under forest ecosystem, several hard wood trees are naturally infected with VAM as a microsymbiont (Kormanic, 1986). Mohankumar and Mahadevan (1987) observed an increased VAM spore population and per cent infection in an evergreen forest soil in

Tamil Nadu. The use of VAM can substantially favours growth in tree plantations (Bagyaraj *et al.*, 1989; Behl, 1990). Various agromanagement practices in agriculture, inhibit colonisation of plant roots by VAM thereby inhibiting the production of VAM spores (Douds, 1994). Deka *et al.* (1998) also reported that the number of VAM spores was less in a rubber plantation soil in Tripura state, compared to those occurring in a nearby natural forest.

2.4. Effect of soil factors on the distribution of VAM fungi

2.4.1. Soil temperature

Both temperature and light have been shown to have a significant influence on colonisation and sporulation by VAM fungi. Higher temperature generally resulted in greater root colonisation (Hayman, 1974) and increased sporulation. Studying the effect of soil temperature on VAM establishment, Schenck and Schroder (1974) observed maximum arbuscule development near 30°C while the mycelial colonisation of the root surface was greatest between 28 and 34°C and sporulation and vesicle development at 35°C. Periods of cold stress followed by maintenance of high soil temperature had also been shown to increase colonisation and sporulation (Ferguson, 1981). According to Daniels and Bloom (1984) the optimum temperature for root colonisation by VAM in wheat and red clover is 20-25°C. While investigating the factors influencing VAM in scrub jungle soil, Parameswaran and Augustine (1988)

found a positive influence of soil moisture and temperature on VAM activity. The number of VAM spores in a Brazilian rubber plantation was found to be greater at a temperature above 25°C (Schwob *et al.*, 1999).

Schenck *et al.* (1975) reported that *Gigaspora coralloidea* had a higher optimum temperature (34°C) for its germination where as maximum germination of *Glomus mossae* occurred at 20°C. Daniels and Trappe (1980) observed an optimum temperature of 20-25°C for the germination of *Glomus epigaeus* spores. According to Koske (1981) the most rapid germination of *Gigaspora gigantea* occurred at 30°C. These studies suggested that increased soil temperature hastened the germination of spores of *Gigaspora* spp.

2.4.2. Soil moisture

Soil moisture had profound influence on VAM spore population as well as colonisation of host plants (Daniels and Trappe, 1980). Allen and Allen (1990) suggested that mycorrhizal status and succession could vary depending on the moisture and nutrient conditions of soil. High moisture resulted in decreased spore population and percentage of root colonisation by VAM fungi (Trinick, 1977; Vyas and Srivastava, 1988). Keeley (1980) reported that upland plants of black gum (*Nyssa sylvatica*) established fewer endomycorrhizae and survived flooded conditions poorly. Most wetland plants are reported as not infected or poorly infected (Sondergaard and

Laegaard, 1977; Anderson *et al.*, 1984; Clayton and Bagyaraj, 1994). Stevens and Peterson (1996) while studying the effect of water gradient on vesicular arbuscular mycorrhizal status of *Lythrum salicaria* revealed that hyphal colonisation levels were significantly higher in the dry plots than in the wet regions.

Glomus epigaeus spores germinated best at moisture contents between field capacity and soil saturation (Daniels and Trappe, 1980). Below field capacity germination declined with no germination occurring below -31 bars. Koske (1981) made a detailed investigation on the germination of *Gigaspora gigantea* spores placed on sand to which concentrations of polyethylene glycol (PEG) were added and observed that in *Gigaspora gigantea* germination was strongly inhibited at -10 bars. He also observed that germ tube length was reduced at low water potential levels. These findings indicated that *Gigaspora* sp. delayed germination and reduced the hyphal growth from germinated spores at low water potential levels.

Maximum VAM colonisation and spore count of cowpea was observed when soil moisture was maintained at field capacity. Progressive stress reduced both spore count and colonisation and it was least when soil moisture was maintained at 50 per cent field capacity (Pai *et al.*, 1993). Per cent infection and number of resting spores in the rhizosphere increased in sunflower plants grown under water stress condition of 10 per cent soil moisture level (Bolgiano *et al.*, 1983 and Reddy *et al.*, 1997).

2.4.3. Soil organic matter

Soil organic matter is an important edaphic factor that influences soil moisture, pH and water holding capacity, which in turn directly or indirectly influence VAM development and its efficiency. In rubber growing soils, the organic carbon status is high (1-2.5 per cent). The rubber plantations, being a closed ecosystem, recycles enormous biomass through litter decomposition which takes place rapidly thus producing considerable organic carbon in the surface layers of soil (Krishnakumar *et al.*, 1991). Nicolson (1960) reported a decrease in mycorrhizal infection in the plants of graminae with increasing soil organic matter. According to Sheikh *et al.* (1975) endogenous spore population was closely correlated with the level of organic matter in soil. Maximum spore numbers were recovered from soils containing 1-2 per cent organic matter and spores were sparse in tropical soils with 0.5 per cent organic matter. No such correlation had been observed in temperate soils with higher (2-18%) organic matter content although organic manure often enhanced mycorrhizal development in tropical soils (Johnson and Michelini, 1974; Sreelakshmi *et al.*, 1999). Read (1993) reported that VAM predominated in ecosystem where the mineralisation is rapid enough to avoid the accumulation of organic matter.

Farmyard manure favoured VAM compared to inorganic fertilizers, as increased soil organic matter levels encouraged VAM (Harinikumar and Bagyaraj, 1989). Geethakumari *et al.* (1990) observed that application of organic amendment

(finger millet husk and farmyard manure mixed in the ratio 1:1 by weight) in addition to mycorrhizae increased the per cent mycorrhizal colonisation in finger millet. Wheat grown under organic farming was found to have VA mycorrhizal colonisation levels consistently 2 to 3 times higher than in the conventional farming (Ryan *et al.*, 1994). Baby and Manibhushan Rao (1996) established that in rice plants VAM spore density and per cent infection were increased by organic matter amendments in soil especially green leaf manure. But in Rajasthan soils, the spore population and root colonisation were not favoured where organic carbon level in soil was high (Kaushal, 2000).

The mycorrhizal infectivity and spore density were greatly increased by the addition of composted farmyard waste (Noyd *et al.*, 1996). A decreased VAM colonisation of roots as a result of high proportion (50-100 per cent) of vermicompost in the growth substrate was noted by Sainz *et al.* (1998) because of the enchanced P availability in vermicompost. Scullion *et al.* (1998) observed higher mycorrhizal infectivity under organic management, while intensive farming practices reduced the effectiveness of VAM population in *Allium* sp. The addition of organic matter reduced the density of spores and colonisation in an agro forestry system and increased in a monoculture system with *Zea mays* (Boddington and Dodd, 2000).

2.4.4. Soil pH

Soil pH significantly influenced VAM spore production and VAM activity (Kruckelmann, 1975; Vyas and Sreevastava, 1988). Hayman and Mosse (1971)

observed VAM formation in *Coprosoma robusta* in soils with pH of 3.3 to 4.6. Mosse (1972) obtained considerably high efficiency of VAM by increasing soil pH. However, very high and low pH decreased VAM infection. Abbott and Robson (1977) reported that the distribution of different types like honey coloured sessile and yellow vacuolate spores in western Australia was related to soil pH. Much VAM infection but few spores were reported in acid hill grasslands in Northern England (Sparling and Tinker, 1978), Mid-wales (Hayman and Mosse, 1979), Western United States and Canada (Molina *et al.*, 1978). However, Mohankumar and Mahadevan (1987) could not find any defenite correlation between soil pH and VAM development and sporulation. Zhaobin (1988) reported that *Glomus* sp. infected cotton root ramified extensively and formed more hyphae at high soil pH conditions. The intensity of colonisation and spore density has shown to depend on soil pH (Sylvia and Williams, 1992). Rathore and Singh (1995) also observed an increase in the VAM population with increasing pH. VAM colonisation of roots increased with increase in soil pH from 4.3 to 5 and further increases in pH did not influence colonisation (Soedarjo and Habte, 1995; Begho and Asawalam, 1999).

VAM symbiosis was hardly affected by a decrease in soil pH in heathland herb species (Heijne *et al.*, 1996). A survey of different localities of Rajasthan revealed that VAM spore population and root colonisation in *Acacia nilotica* were positively correlated with soil pH (Kaushal, 2000).

The effect of soil pH on VAM spore germination and root colonisation has been studied in depth and documented profoundly. The influence is either direct or indirect. Green *et al.* (1976) reported that the influence of pH on spore germination was different for different species of the fungus. On the other hand, Green *et al.* (1976) observed an increased germination of *Gigaspora coralloidea* and *Gigaspora heterogama* in soil of acidic pH range. Most favorable pH for the germination of the above two species was found to be 4 to 6. There are also reports that the germination of spores occurred at a wide range of pH as in the case of *Glomus epigaeus.* Siqueira *et al.* (1982) reported that a soil pH of 6 was optimum for the spore germination in agar medium.

Even though considerable work has been done on the role of soil pH on VAM spore germination, the actual mechanism of pH affecting spore germination is yet to be understood. Daniels and Trappe (1980) suggested that the pH induced differences in nutrient availability in soil are responsible for stimulation or inhibition of VAM fungal spore germination.

2.5. Effect of cultural operations on VAM colonisation and spore population

Disturbances of soil started as cultivation of plants began. Tillage is the main cultural operation that disturbs the soil. Yocom *et al.* (1985) found that mycorrhizal

colonisation of winter wheat was considerably lower in fields subjected to intensive tillage. O'Halloran *et al.* (1986) observed an increased root colonisation of VAM in undisturbed soil. While having a direct influence on root colonisation, tillage also had an indirect influence. Tillage reduced soil fauna that influenced the dispersion of VAM fungi (Rabatin and Stinner, 1989). Kruckelmann (1975) and Douds *et al.* (1995) reported negative effect of tillage on mycorrhizal sporulation in pot culture experiments. Independently conducted field studies have established that soil disturbance by tillage or by hand reduced mycorrhizal colonisation of plants (Anderson *et al.*, 1987; Vasatka and Dodd, 1998; Evans and Miller, 1990; Mc Gonigle and Miller, 1993; Douds *et al.*, 1995; Kabir *et al.*, 1997). Entry *et al.* (1996) also observed that corn plants in no tillage treatments had higher root biomass infected with mycorrhizae than those in conventional tillage. But according to Gavito and Miller (1998) tillage had little effect on intraradical colonisation. However, the effect of tillage on mycorrhizal colonisation has been inconsistent in both pot and field studies (Miller *et al.*, 1995; Addy *et al.*, 1997). McGonigle and Miller (1993) and Boddington and Dodd (2000) also observed a negative effect of soil disturbance on VAM hyphal growth.

2.5.1. Fertilizer application

Changes in soil fertility due to application of mineral fertilizers or organic matter markedly affected the activity of soil mycorrhizal population in terms of the

amount of root infection and number of resting spores produced (Mosse, 1973b; Hayman, 1982a). Mycorrhizae were abundant under the entire natural range of soil fertility, although the degree of mycorrhizal colonisation increased as fertility declined (Jasper *et al.*, 1979).

There are contradictory reports on the role of fertilizers in influencing VAM fungi in soil. Many reports indicated a negative influence (Hayman, 1975; Jensen and Jacobsen, 1980; Plenchette and Corpron, 1987; Vivekanandan and Fixen, 1991) while a few reported a positive influence of fertilizers on VAM (Dehne, 1987; Gryndler *et al.*, 1990). Johnson and Pfleger (1992) summarized the effect of fertilizers on VAM and attributed the variability due to the type and quantity of fertilizer, fertility of soil and the crop. The adverse effect of fertilizer is considered to be due to higher fertility of soil (Jasper *et al.*, 1979; Hayman, 1982a; Dehne, 1987). Conversely, when soils were poor in nutrients, mycorrhizal colonisation and spore population were limited by poor growth of host plant and small additions of fertilizer can stimulate mycorrhizal colonisation, whereas large additions of fertilizer suppressed it (Porter *et al.*, 1978; Bagyaraj and Sreeramulu, 1982; Hao and Lin, 1987; Thingstrup *et al.*, 2000; Rajeswari *et al.*, 2001).

The ratio of major nutrients in fertilizers was considered to have more influence on VAM. Among the major nutrients of plants, P is an important nutrient having a profound influence on VAM colonisation. The level and type of phosphatic

fertilizer influenced VAM spore population as well as root colonisation. Higher the level of phosphorus in soil, lower was the population of VAM spores (Hayman, 1975). Jasper *et al.* (1979) observed reduced mycorrhizal infection under high phosphatic fertilizer. Excess phosphorus reduced the root infection and spore production by VAM in *Abelmoscus esculentus* (Krishna and Bagyaraj, 1982), cardamom (Iyer *et al.*, 1988) and black gram (Umadevi and Sitaramaiah, 1990). Kruckelmann (1975) while studying on VAM in soil reported a positive effect of fertilizers on spore population of VAM in soil and root infection .He also found that application of phosphate (220 kg P ha^{-1}) for seven subsequent years did not affect the frequency of VAM spores in soil. Powell and Daniel (1978) reported that P from poorly soluble rock phosphate only was available to mycorrhizal plants and colonisation increased with increase in the availability of P.

Application of rock phosphate at the graded levels of 1.5, 2.5, and 3.5 g kg^{-1} soil significantly increased the per cent root colonisation (Veeraswamy *et al.*, 1992). Phosphorus fertilization however reduced the mycorrhizal colonisation of maize in the field at early (Khan, 1972; Lu and Miller, 1989; Vivekanandan and Fixen, 1991) and late development stages (Guttay and Dandurant, 1989) but accroding to Gavito and Miller (1998) P fertilization had no effect on mycorrhizal development. A reduction in mycorrhizal colonisation by P fertilization was also observed by Chandrashekara *et al.* (1995) and Thingstrup *et al.* (1998). In a study of mycorrhizal colonisation of corn

hybrids with three P rates (0, 40 and 80 mg P kg^{-1} soil), Liu *et al.* (2000) observed that the colonisation was greatest at the lowest P level.

Gryndler *et al.* (1990) showed that a balanced fertilizer application stimulated mycorrhizal colonisation of corn while fertilization with unusually high or low levels of nitrogen decreased root colonisation. Saif (1986) showed that application of P alone reduced mycorrhizal infection in forage species, on the other hand NPK fertilizer did not show any adverse effect. The nutrients N, P and K interacted with the plants and helped VAM colonisation. N status of host plants influenced VAM in P absorption (Sylvia and Neal, 1990) and the same is mediated by K (Plenchette and Corpron, 1987). They also observed a negative influence of P and K when applied individually and a positive effect upon combined application of P and K.

Different species of VAM fungi differed in their response to fertilizers. *Gigaspora margarita* and *Scutellospora calospora* are highly sensitive to fertilizers while *Glomus intraradix* was insensitive to fertilizers (Sylvia and Schenck, 1983; Thomson *et al.*, 1986; Douds and Schenck, 1990).

2.5.2. Fungicide application

Fungicides include a large number of synthetic compounds differing greatly in their mode of action and effects on VAM fungi and conflicting reports are common,

even within a single class of fungicide (Johnson and Pfleger, 1992). The effe fungicides on VAM depends considerably on the rate at which they are ap Fungicides typically delayed or reduced VAM infections but rarely elimina altogether (Menge, 1982). Benomyl, ethirimol and thiabendazole applied as treatment had inhibitory effect on the development of VAM on wheat roots, ev the lowest concentration (Jalali and Domsch, 1975). The inhibitory effect of P and benomyl was reported (Menge *et al.*, 1979; Ocampo and Hayman, 1980). N (1980) reported the adverse effect of captan at higher concentration (9 kg ha^{-1}) in orange. On the other hand Sreenivasa and Bagyaraj (1988) reported an increasec colonisation and spore production of VAM by captan at lower concentrations.

Benzimidazole fungicides such as benomyl, carbendazim, thiabenda thiophanate and thiophanate-methyl were highly detrimental to VAM irrespecti mode of application (Hale and Sanders, 1982; Manjunath and Bagyaraj, 1984; K *et al.*, 1987). Non-systemic fungicide like thiram was consistently tox mycorrhizal fungi (Kumar and Jayaraman, 1987). Lu and Miller (1989) reporte application of fungicides generally reduced and disrupted VAM root colonisatio hyphal P transport. Both benomyl and Bavistin significantly reduced the degr colonisation of cowpea by VAM (Gunasekaran *et al.*, 1987; Pedersen and s 1997). Kehri and Chandra (1993) reported a damaging effect of Bavisti mycorrhizal formation in green gram.

Benomyl was used at high (3.0 g l^{-1}) application rates as a control treatment in VAM fungal experimentation (Fitter and Nichols, 1988) and there is some evidence of adverse effects even at rates normally used in agriculture (Johnson and Pfleger, 1992). Benomyl inhibited mycorrhizal formation (Boatman *et al.*, 1978; Carey *et al.*, 1992) and both benomyl and carbendazim inhibited hyphal P transport and metabolic activity (Larsen *et al.*, 1996; Kling and Jakobsen, 1997) and function (Thingstrup *et al.*, 2000).

Fosetyl AL and metalaxyl, the antioomycete fungicides were reported to augument mycorrhizal colonisation (Jabaji-Hare and Kendrick, 1987). Other workers also reported the beneficial or neutral effect of metalaxyl on mycorrhizae (Groth and Martinson, 1983; Afek *et al.*, 1990; Daniels and Wilson, 1991). But Anusuya and Dhaneswari (1995) reported a significant reduction in VAM infection at 500 and 1000 mg l^{-1} of metalaxyl sprayed groundnut plants. Ridomil-72 WP application stimulated the colonisation and spore production of *Sclerocystis coremioides* in the rhizosphere of black pepper (Robert *et al.*, 1995).

Vijayalaxmi and Rao (1993) reported that carbendazim and copper oxychloride treatment inhibited the mycorrhizal infection in sesame. But according to Vyas and Vyas (1995) carbendazim, fosetyl-Al and mancozeb significantly increased both mycorrhizal infection and chlamydospore number and triadimefon adversely affected the growth of mycorrhiza. Anusuya (1995) reported that soil application of

carbendazim did not deleteriously affect VAM fungi. Carbendazim, a systemic soil fungicide had inhibited both VAM root colonisation and formation of resting spores in the earlier stages of growth in *Albezzia lebbeck*, but at later stages the percentage of infection recorded was almost at par with the control (Kumar *et al.*, 1999).

2.5.3. Pesticide application

Modern cropping technology involves the use of a number of pesticides for controlling the insects and nematodes. While affecting the target fauna they also had influence on the soil microbial population including VAM fungi (Hayman, 1982b; Moorman, 1989). Most of the pesticides inhibited the infection and development of VAM (Hayman *et al.*, 1978: Menge *et al.*, 1979; Trappe *et al.*, 1984; Parvathi *et al.*, 1985), although some increased mycorrhinal colonisation and development (Bird *et al.*, 1974).

VAM populations in field grown potatoes were reduced by the insecticide aldrin and in tomatoes by the insecticide metasystox (Kruckelmann, 1973). The nematicide, dibromo chloropropane (DBCP) is an example of a pesticide that enchanced mycorrhizal colonisation (Bird *et al.*, 1974; Menge *et al.*, 1979). Aldicarb also seemed to stimulate both spore and mycorrhiza formation in barley (Ocampo and Hayman, 1980; Trappe *et al.*, 1984). It is possible that these pesticides indirectly

stimulated mycorrhizae by reducing population of nematodes or other predators, parasites or competitors of VAM fungi.

Backman and Clark (1977) reported a reduced mycorrhizal colonisation by carbofuran at recommended dose while at half dose the root colonisation increased (Sreenivasa and Bagyaraj, 1988). However, organophosphorus insecticides and nematicides like chlorfenvinphos, carbaryl, malathion and parathion generally had no effect or a slightly detrimental effect on VAM fungi (Ocampo and Hayman, 1980; Spokes *et al.*, 1981; Parvathi *et al.*, 1985). Similar is the case with carbofuran and oxamyl (Spokes *et al*, 1981; Nemec, 1985). But Venketeswarlu *et al.* (1994) reported that VAM colonisation and sporulation by *Glomus clarum* enhanced significantly at the recommended field dose of carbofuran and higher dose inhibited the mycorrhizal status of groundnut. The number of spores in the soil was reduced significantly at recommended and higher level of carbofuran application. Among the plant protection chemicals insecticides were least toxic to mycorrhizal fungi and spore production (Vyas and Vyas, 1995). According to them the descending order of toxicity towards mycorrhizae in maize was Thimmet > carbaryl > methylparathion > chlorpyriphos > quinalphos. Quinalphos has been found to enchance, both the per cent of mycorrhizal infection and the number of propagules (Kumar *et al.*, 1999).

2.5.4. Weedicide application

Application of weedicides is a common practice in modern agriculture. Unlike insecticides and fungicides most of the weedicides are systemic and translocated to all parts of plants. They inturn influenced VAM association .The role of herbicides on VAM was reviewed by Trappe, *et al.* (1984).

The herbicide, simazine when applied at field rate had no effect on VAM formation in corn (Kruscheva, 1971), decreased VAM formation in *Citrus* species (Nemec and Tucker, 1983) and increased VAM formation in *Chenopodium quinona* (Schwab *et al.*, 1982). Nemec and Tucker (1983) also found that in Florida citrus diuron and bromacil had no apparent effect on *Glomus etunicatus*. But application of diuron increased VAM fungal spores in soil (Smith *et al.*, 1981). At recommnded dose diuron did not have any adverse effect either on sporulation or root colonisation (Dodd and Jeffris, 1989).

Accroding to Pope and Halt (1981) paraquat applied at 2 kg active ingredient/ha significantly reduced the mycorrhizal infection, vesicle development and chlamydospore formation in white ash seedlings, while paraquat applied at 0.5kg ha^{-1} promoted the hyphal development and spore production. Atrazine was reported to enhance mycorrhiza formation in *Liquidambar styraciflua* (Trappe *et al.*, 1984). Anusuya and Shobha (1995) reported a positive response with respect to VAM spore

numbers and number of infective propagules upon the application of glyphosate on green gram. Trifluralin had no effect on VAM infection in roots (South, 1981; Nemec and Tucker, 1983). The hyphae of VAM fungi could remove atrazine from the soil and transfer it to corn plants (Nelson and Khan, 1992).

Rachel *et al.* (1996) while working with sunflower reported that application of 2,4-D had shown a negative effect on the mycorrhizal association. But Kumar *et al.* (1999) reported its positive effect on VAM colonisation with reference to two tree species *viz. Sesbania grandiflora* and *Albizzia lebbeck* at nursery level.

2.6. Soil solarisation

There are only few reports on the effect of soil solarisation on VAM. The concept of soil solarisation stemmed from the possibility of raising soil temperature to lethal level, by mulching soil surface with plastic film (tarping) during hot summer months. Use of this method has been reported to control soil borne plant pathogens, weed seeds, insect larvae etc. (Katan, 1981; Morgan *et al.*, 1991; Chattopadhyay and Sastry, 2001). The effect of solarisation on mycorrhizal colonisation was examined by Pullaman *et al.* (1981) and reported that the cotton roots from tarped soil showed mycorrhizal colonisation to a lesser extent than nontarped soil. According to Sharma and Sharma (2002) the total microbial population decreased due to solarisation treatments.

2.7. Dissemination of VAM spores

VAM fungi dispersal is an important aspect in their survival and function. The dispersal may take place by root contact as well as by water and wind blown particles. Animals and crop management practices also play a role in the dispersal of VAM fungi. The dispersal may be either active or passive (Bagyaraj, 1991).

2.7.1. Active dissemination

Active dispersal of VAM occurs as mycelia grow through the soil either alone or along with roots though it is effective over a limited range. Powell (1979) determined that an efficient mycorrhizal fungus would advance 65m in 150 years or 0.43 m $year^{-1}$ under controlled conditions. Warner and Mosse (1982) have shown that plant species and root density significantly influenced the rate of VAM fungal spread. In clover the greatest rate of spread of *Glomus fasciculatum* was 1cm $week^{-1}$ while in fescue, *Glomus fasciculaum* spread at only 0.7 cm $week^{-1}$.

Mosse *et al.* (1982) conducted experiments in unsterile soil and demonstrated that *Glomus caledonicum* was able to spread 7 to 13 cm from an inoculation point after 13 weeks. No correlation was observed between rate of spread and plant size, but spread rate was greater in non sterilized plots than in sterilized soils.

Whether VAM fungi grow towards a root stimulus or randomly in soil has been debated. Powell (1976) using the buried slide technique in partially sterilized soil, demonstrated little or no attraction of VAM hyphae to root until random contact occurred, except with hyphae from honey coloured spores (*Acaulospora laevis*) which frequently grew towards the roots. Koske (1981) has demonstrated chemotactic attraction of hyphae of *Gigaspora margarita* to host roots *in vitro* and the attractant is probably a volatile substance. Whether such chemotactic substances are produced under field conditions and can direct the mycelial growth in the field has not been studied.

2.7.2. Passive dissemination

Passive dispersal occurred through biotic agents like rodents, worms, insects and birds or through abiotic agents like wind and water.

2.7.2.1. Biotic agents

Both flora and fauna contributed to the passive dissemination of VAM spores. Animals appear to be the major vectors for dispersal of VAM fungi in many habitats (Marx, 1975; Allen, 1987). A wide variety of animals were known to have VAM spores in their gut tracts or faeces. Thaxter (1922) was the first to observe VAM spores in the digestive tracts of millipedes. VAM spores were reported in grasshoppers and crickets (Hansen and Uckert, 1970) and in earthworms and ant casts (Mc Ilveen and Cole, 1976). VAM spores remained viable following earthworm

ingestion, since earthworm casts gave rise to typical VAM colonisation (Mc Ilveen and Cole, 1976). Mac Mohan and Warner (1984) recorded VAM spores in the gut tracts or feces of 42 different species of mammals. Fogel and Trappe (1978) observed a relationship between mammal size and size of sporocarps ingested. Shrews, jumping mice, mice, rats, lemmings, voles and pikas are potent vectors of spore dispersal. Bagyaraj (1991) observed VAM spores in the fecal matter of millipeds, earthworms and sphecid worms.

VAM association did not develop in the roots of sunflower plants grown in steam treated check soil. However, in soils amended with air-dried worm cast or wasp mud pots, the roots developed mycorrhizal colonisation. (Lakshman and Raghavendra, 1990).

2.7.2.2. Abiotic agents

Wind and water are the two major abiotic agents of passive dissemination of VAM spores. Ponder (1980) reported VAM fungal spores in the droppings of leaf feeders like grasshopper and rabbit. The presence of VAM fungal spores in their digestive tracts or feces, therefore, implies that spores were present on leaves prior to feeding, probably as a result of wind dispersal. Taber (1982) has observed VAM fungal spores in *Portulaca* seed capsules, which are oriented on the plant towards wind. Mac Mohan and Warner (1984) collected airborne VA mycorrhizal spores from modified sticky traps, thus giving direct evidence of wind dispersal of VAM spores. The wind dispersal of spores upto 2 km was demonstrated by Warner *et al.* (1987).

Water irrespective of the source flowed through the surface of land and carried soil particles and microorganisms including spores of VAM (Powel, 1980). The number of spores carried by water depends on quantum of water from the source, rate of flow and the source. Irrigation water is the most potential source of passive VAM dispersal.

Chapter 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Collection of soil and root samples

Soil samples and root samples were collected from twenty-one locations of rubber growing areas in Kerala during pre monsoon, monsoon, post monsoon and summer seasons, to study the natural distribution of VAM fungi. Soil was dug out with a trowel to a depth of 15 cm after scrapping away the top litter layer. Samples were collected from 10 different places in each area, pooled and homogenised. The samples contained feeder roots of *Hevea* also. The roots were separated from the soil, washed and stored in lactophenol for determining root colonisation. Representative soil samples were taken in the polythene bags, labeled and stored at 20° C until they were processed further. From this 50 g soil was used for wet sieving to take spore count.

3.1.1. Enumeration of VAM spore population

The spores of mycorrhizal fungi were separated by wet sieving method as detailed by Gerdemann and Nicolson (1963). A quantity of 50 g soil was suspended in 200 ml of luke-warm water. Heavier particles were allowed to settle for a few seconds and the suspension was decanted through a 710 μm sieve to remove the larger

particles of organic matter. The residue was resuspended in more water and sieving was repeated. The suspension that passed through this sieve was saved and stirred to resuspend all particles. The heavier particles were allowed to settle for a few seconds and the supernatant was decanted through 250 μm sieve. The suspension that passed through this sieve was again collected and the sieving was repeated using 105 μm sieve and 45 μm sieve. The larger particles of organic matter were caught on the top sieves of higher pore size. The soil particles and spores collected in 105 μm and 45 μm sieves were taken in 100 ml conical flasks separately. The suspension in each flask was shaken thoroughly and allowed to settle for 30 seconds. The spores present in these suspensions were trapped on a nylon mesh with 45 μm pore size placed on a marked petridish and the number of spores was counted by observing under a binocular stereo-microscope. Shriveled and desiccated spores were omitted. Only spores that appeared to be viable (based on colour, shape, surface conditions and examination of spore contents) were counted (Eom, *et al.*, 2001).

3.1.2. Identification of spores

Spores of common genera of VAM were identified using synoptic keys of Zygomycetous mycorrhizal fungi by Trappe (1982) and photographic slide collection illustrating features of endogonaceae of Hall and Abbott (1981).

3.1.3. Assessment of root colonisation (Phillips and Hayman, 1970)

The surface feeder roots were cut into 1 cm bits, washed gently in tap water without disturbing the external mycelium. The samples were heated to about 90°C for 1 h in 10 per cent potassium hydroxide solution in a water bath. It was rinsed four times in tap water and acidified by immersing for five minutes in 2 per cent hydrochloric acid. The acid was poured off and added 0.05 per cent cotton blue in lactophenol. The root bits were boiled in this stain for 3 minutes. The stain was poured off and added with lactophenol and kept overnight to destain the host tissues and examined under a stereomicroscope at x160 magnification for mycorrhizal infection. Mycorrhizal colonisation was expressed using the following formula:

$$\text{Per cent colonisation} = \frac{\text{Number of root segments with VAM}}{\text{Total number of root segments examined}} \times 100$$

The root segment was considered mycorrhizal even if one of the three structures, *i.e.*, hypha, arbuscule or vesicle was present.

3.1.4. Seasonal variability on VAM spore count in rubber growing soils and colonisation in *Hevea* roots

Soil samples and root samples were collected from twenty-one rubber growing areas in four different seasons *viz.* summer (January-March), premonsoon (April and May), monsoon (June-August) and postmonsoon (September-December) to study the variation in the distribution of VAM fungal spores and per cent root colonisation in

different seasons. About 500 g of soil sample was collected from each location along with feeder roots of *Hevea*. The root samples were thoroughly washed with water and stored in lactophenol solution for examining VAM colonisation.

3.2. Determination of organic carbon in soil

Walkley and Black's (1934) rapid titration method was employed to quantify organic carbon in the soil. Weighed exactly 0.5 g of finely ground soil into a 500 ml conical flask. Ten millilitre of 1 N potassium dichromate solution was added to the flask and swirled the flask to mix the dichromate with the soil. Then added 20 ml conc. sulphuric acid and mixed by gentle rotation for one minute to ensure complete contact of the reagents with the soil, taking care to avoid throwing the soil up into the sides of the flask. Left the flask to stand on a sheet of asbestos for 30 min.

Then added 200 ml of distilled water, followed by 2 to 3 drops of ferroin indicator. Titrated the contents against ferrous sulphate solution. The colour changed from blue to green and finally to grayish red (brown) at the end point. Carried out a blank titration using only the reagents. The per cent of organic carbon in each sample was determined by the formula :

$$\frac{(B-TV) \times 0.003 \times 100}{\text{Weight of soil}} \times \frac{10}{B}$$ where B = blank and TV = Titrate value

Reagents

1. Potassium dichromate I N

Weighed out accurately 49.04g of the dry potassium dichromate and transferred the salt quantitatively to a one liter-measuring flask using a small funnel to avoid loss. Dissolved the salt in the flask in water and brought to the mark by adding water.

2. Concentrated sulphuric acid (98% w/v AR grade)

3. Ferroin indicator

Dissolved 0.695 g of ferrous sulphate ($FeSO_47H_2O$) and 1.485 g *ortho* phenanthrolene monohydrate ($C_2H_8N_2H_2O$) in water and made upto 100 ml.

4. Ferrous sulphate solution (approximately 0.5 N)

Dissolved 196 g of ferrous ammonium sulphate in 800 ml of freshly boiled distilled water containing 20 ml concentrated sulphuric acid and diluted to one litre.

3.3. Determination of soil pH

pH of the soil samples was determined in 1: 2.5 (W/V) soil: water solution using a digital table top pH meter (Scientific Tech) fitted with combined electrode.

3.4. VAM spore population and root colonisation in *Hevea* plantations under different period of cultivation

Soil samples and root samples were collected from rubber plantations under four different duration of cultivation (5 years, 10 years, 20 years and 30 years) to study the population of VAM spores and per cent of VAM colonisation. The samples were collected from Mundakayam. Five replicate samples were collected from each plantation.

3.5. VAM spore population and root colonisation in rubber soils under 3 different cycles of cultivation

Soil samples and root samples were collected from rubber plantations under the first, second and third cycle of cultivation during premonsoon season for studying the spore population and root colonisation. Each cycle comprises of 32-35 years of plant growth. Seven replicate samples were collected from plantations at Kottayam.

3.6. VAM spore population at different soil depths

Soil samples were collected from an experiment laid out in a rubber plantation at Rubber Research Institute of India, Kottayam, using soil core method (Escamilla *et al.*, 1991) at depths of 0-15 cm, 15-30 cm, and 30-45 cm vertically to

study the difference in VAM population. The soil core used was of a 4.25 cm diameter and 15 cm long GI (galvanized iron) pipe with a sharpened edge.

3.7. Comparison of forest soil and rubber plantation soil for VAM spore distribution

Soil samples were collected from four different locations of rubber plantations and nearby undisturbed tropical rain forests for a comparative study on VAM spore count in forest soils and rubber growing soils. The locations selected were Mundakayam, Trissur, Palakkad and Kozhikode in Kerala state. The prominent tree species in these forests include *Lagestromia* sp., *Sapindus laurifolius*, *Alstonia scholaris*, *Terminalia bellerica*, *Ficus* sp., *Mangifera indica*, and *Tectona* sp.

3.8. Effect of soil factors on VAM spore count and root colonisation

The effect of different soil factors like temperature, moisture and organic matter on VAM spore population and root colonisation were studied.

3.8.1. Effect of temperature

Three kilograms of red loam garden soil sand mixture (1:1 W/W) was taken in polybags of 15 cm diameter for studying the effect of temperature on VAM spore

population and root colonisation. Four temperature treatments were made with five replications. The temperature treatments were 20°C, 30°C, 40°C and 50°C. For 20° C the soil samples were kept in an air-conditioned room. For obtaining 30° C, 40° C and 50° C the soil samples were incubated in ovens, the temperature of which were adjusted. Water with filter paper in a tray was kept at the bottom of the oven. After 15 days of treatment polybags were taken out and 50 g of soil from each polybags was used for determining the number of spores. Only the viable spores were counted. Then sorghum seeds were sown in each polybag and maintained in the glass house condition. They were watered regularly. After 45 days of plant growth they were uprooted and the roots after washing were stored for the determination of root colonisation.

3.8.2. Effect of soil moisture on VAM spore count and root colonisation

Soil sand mixture, sieved through a sieve of 1 mm^2 pore size was dried in an oven by keeping it in a tray, at 70° C for 4 h to reduce the moisture to below 10per cent. Two hundred g of dried soil was taken in plastic cups of 7 cm diameter. This soil was inoculated with VAM spores at room temperature and kept for 30 days. Then the moisture content was adjusted to 10, 20, 30, 40, 50, 60, 70 and 80 per cent on dry weight basis by adding sterile water and thoroughly mixing. Three replications were maintained for each moisture level. The moisture was maintained at required level by adding sterile water. After 30 days, 50 g of soil from each cup was taken out and used

for determining the number of viable spores. The moisture content of all the treatments was then adjusted to field capacity and sorghum seeds were sown in the cups and water was applied regularly. After 45 days of growth, plants were uprooted and observations for root colonisation were made.

3.8.3. Effect of different sources of organic matter in soil on VAM spore count and root colonisation

Three kilograms of unsterile soil sand mixture was taken in polybags of 15 cm diameter and were treated with different types of organic matter like cowdung, rubber leaf litter and compost. Control without any organic matter application was also maintained. After 15 days sorghum seeds, used as test plants were sown in the polybags. Five replications were maintained for each treatment. After 45 days of growth, plants were uprooted and per cent root colonisation and spore population in soil were observed.

3.9. Effect of cultural operations on VAM spore count and root colonisation

3.9.1. Effect of fertilizers

Three kilograms of unsterile soil in polybags (15 cm diameter) were applied with fertilizer mixture (NPKMg -10:10:4:1.5) prepared from urea, rock phosphate,

muriate of potash and magnesium sulphate at three different doses *viz.* recommended, half the recommended and double the recommended doses. Seeds of *Hevea* sprouted in sterilized sand were planted after 15 days of treatment. Seven replications of each dose with one plant in each bag were maintained in randomized block design. Control without fertilizer also was maintained. They were watered regularly and after 90 days of growth the plants were uprooted and observations for root colonisation and the population of viable spores in soil was recorded.

3.9.2. Effect of plant protection chemicals

The effect of different fungicides, insecticides and herbicides on VAM colonisation and spore population was studied in polybags of 20 cm long and 15 cm diameter using unsterile soil.

3.9.2.1. Fungicides

Unsterile soil and sand mixture, inoculated with soil containing large number of VAM spores and colonised root fragments of grass were taken in polybags with 3 kg soil in each bag. The soil in the bags was treated with different fungicides used in rubber plantations, at three different concentrations *viz.* half the recommended, recommended and double the recommended dose. The different doses of each

fungicide were represented in ppm. The fungicides used were Bordeaux mixture 1 per cent, mancozeb (Indofil M-45 WP), thiram (Thiride 75 WS), tridemorph (Calixin 80 EC), phosphorous acid (Akomin 40), wettable sulphur (Sulfex 80 WP), metalaxyl (Ridomil 8 WP), carbendazim (Bavistin 50 WP), benomyl (Benlate 50 WP) and hexaconazole (Contaf 5 EC). Control without fungicide application was also maintained for the comparison of the treatments. After 15 days, seeds of *Hevea* germinated in sterilized sand were planted. One plant in each bag was maintained in randomized block design (RBD) with three replications. They were watered daily. After 90 days of growth, plants were uprooted and observations were made for root colonisation and spore population in soil. Only the viable spores were counted.

3.9.2.2. Insecticides

The experiment was carried out using different insecticides in three different doses. The insecticides used were malathion (Malathion 50 EC), chlorpyriphos (Classic 20 EC), monocrotophos (Nuvacron 36 EC), fenvalerate (Sumicidin 20 EC), carbaryl (Sevin 50 WP), carbofuran (Furadan 3 G) and endosulphan (Spic sulfa 35 EC). The different doses were represented in ppm. The insecticides were dissolved in water and mixed with soil. Control without insecticide application was also maintained. Pre-germinated seeds of *Hevea* were planted. The treatments were replicated thrice; each polybag had one seedling in RBD. After 90 days, the plants

were uprooted and observations for root colonisation and the population of viable spores were made.

3.9.2.3. Weedicides

The weedicides studied included diuron (Klass 80 WP), paraquat (Gramoxon 24 SL) and glyphosate (Round up 41 SL). Three different doses of applications in ppm were made. Control also was maintained without any herbicide treatment. Three replicates with one *Hevea* plant in each polybag were arranged in RBD. After 90 days observations were made for root colonisation and the number of living spores.

3.9.3. Effect of soil solarisation on VAM

Solarisation study was conducted at Rubber Research Institute of India experiment station during summer months (January-March) with higher temperature, sunshine hours and low rainfall. Individual plots of 4 x 1.25 m were laid out for the treatment. These plots were irrigated to field capacity and were covered with transparent polyethylene mulch (tarp) of 50 μm thicknesses. Care was taken to minimize the gap between the tarps and soils to prevent air pockets that might retard the soil heating process. The edges of the tarps were buried and sealed with topsoil in

a 20 cm deep furrow. The plots remained covered for 45 days maintaining open lands as control.

Soil temperature at 10 and 20 cm soil depth was recorded daily between 14 and 15 hours, the period when the temperature reached the peak. Soil thermometer (Venus) was used for recording soil temperature.

Soil samples were collected from top 15cm soil from tarped and non-tarped areas on the day the tarps were removed. The samples were tested for VAM spore population. Then, three replicates of each soil was placed in 15cm diameter polybags and sown with seeds of sorghum. Plants were thinned to three per polybag after germination. After 45 days of growth, the roots were collected, stained and examined for mycorrhizal colonisation.

3.10. Studies on the dissemination of VAM fungi

3.10.1. Biotic agents

Earthworm, termites, ants and wasps were selected as biotic agents for studying the passive dissemination of VAM spores. Samples in the form of earthworm castings (plate 1), termitaries (plates 2 & 3), mounds of ants (plate 4) and nests of wasps (plate 5) were collected from different locations of the shady area.

Twenty replicate samples were done for each type and VAM spore counts were made following the wet sieving method, as mentioned earlier (3.1.1).

3.10.2. Abiotic agents

Water and wind were selected as abiotic agents for the study. To determine spore population in run off water, water samples of first monsoon rain were collected from rubber field at different intervals, *viz.* just after starting rain and two hours after starting rain (plate 6), from different locations. Soil samples were also collected from the same locations at the same intervals. The samples were pooled and used to estimate spore count.

To trap the spores disseminated by wind, locally built rectangular box type spore traps (plates 7&8) made of galvanized iron sheets, with either end open, of size 16 x 9 x 6 cm were used. A vane of 22 x 9 cm size was attached, vertical to its breadth, at one end. At the other end 5 cm away from the opening, on the base of the box, there is a slit running all along the width. This slit corresponds with a pair of holders fitted inside the box on either side for holding the slide.

The spore traps were fixed exactly at the center to rotate freely in wind. These were erected on 50 cm long stands. With this arrangement the mouth of the spore trap holding the slide (5x2 cm) was always directed towards the wind.

Twenty numbers of such spore traps were erected at different places in the rubber field at Rubber Research Institute of India, Kottayam during the summer season. Colorless vaseline coated slides were placed inside the spore traps. The slides were exposed for 24 hrs, collected and observed under a stereo microscope for the presence of VAM spores.

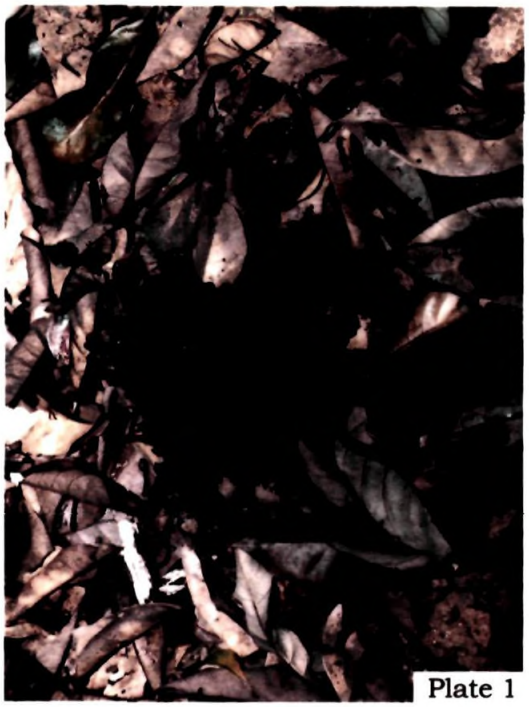


Plate 1

Plate 2

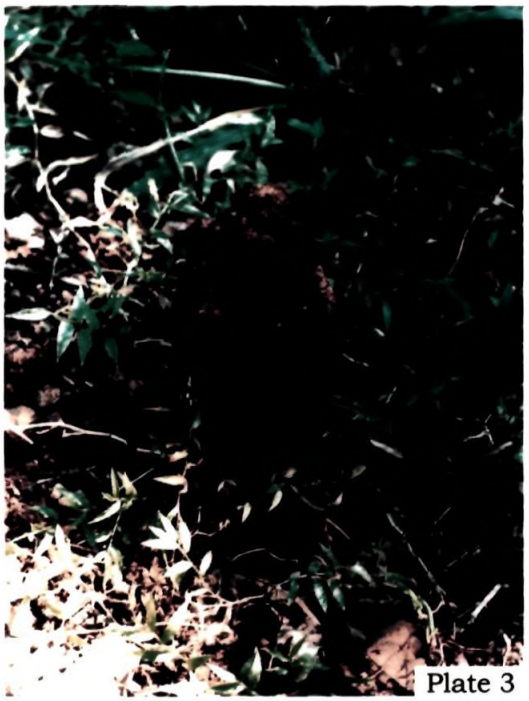


Plate 3

Plate 4

Plate 5

Plate 6

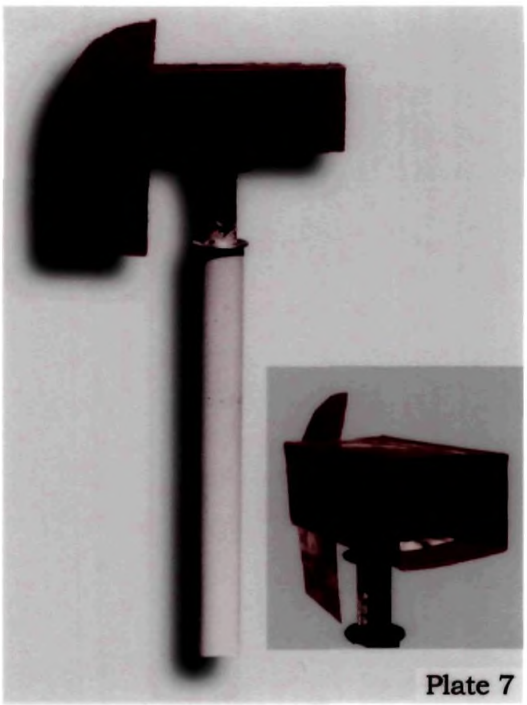


Plate 7

Plate 8

Chapter 4

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

4.1. Influence of location and season on VAM spore population in rubber growing soils of Kerala

The results of the survey on the distribution of VAM spores in different rubber growing soils of Kerala (Fig. 1) are given in Table 1. Wide variation in VAM spore population was recorded in different locations studied. In general, soils from rubber fields in summer registered more VAM spores than in other seasons. VAM population got reduced in the pre monsoon and further reduction was seen in the monsoon season. Thereafter the spore population started increasing reaching a maximum in summer.

In summer, pre monsoon and monsoon seasons maximum VAM spore count was recorded in soils of Venjaramood, Punalur, Palakkad and Kalpatta. But in the post monsoon season Punalur, Palakkad and Kalpatta soils showed maximum spore count. Highest spore count over all seasons was observed in Palakkad and the least spore count was observed in Kottayam region. Thiruvananthapuram, Venjaramood, Punalur, Muvattupuzha, Vaniampara, Palakkad, Kozhikode and Kalpatta recorded higher spore count and were significantly superior to other locations.

Rubber growing soils in general contained more of *Glomus* spp. spores (Plates 9, 10, 11 & 12). *Acaulospora* spp. (Plate 13), *Sclerocystis* spp. (Plate 14)

Map of Kerala State

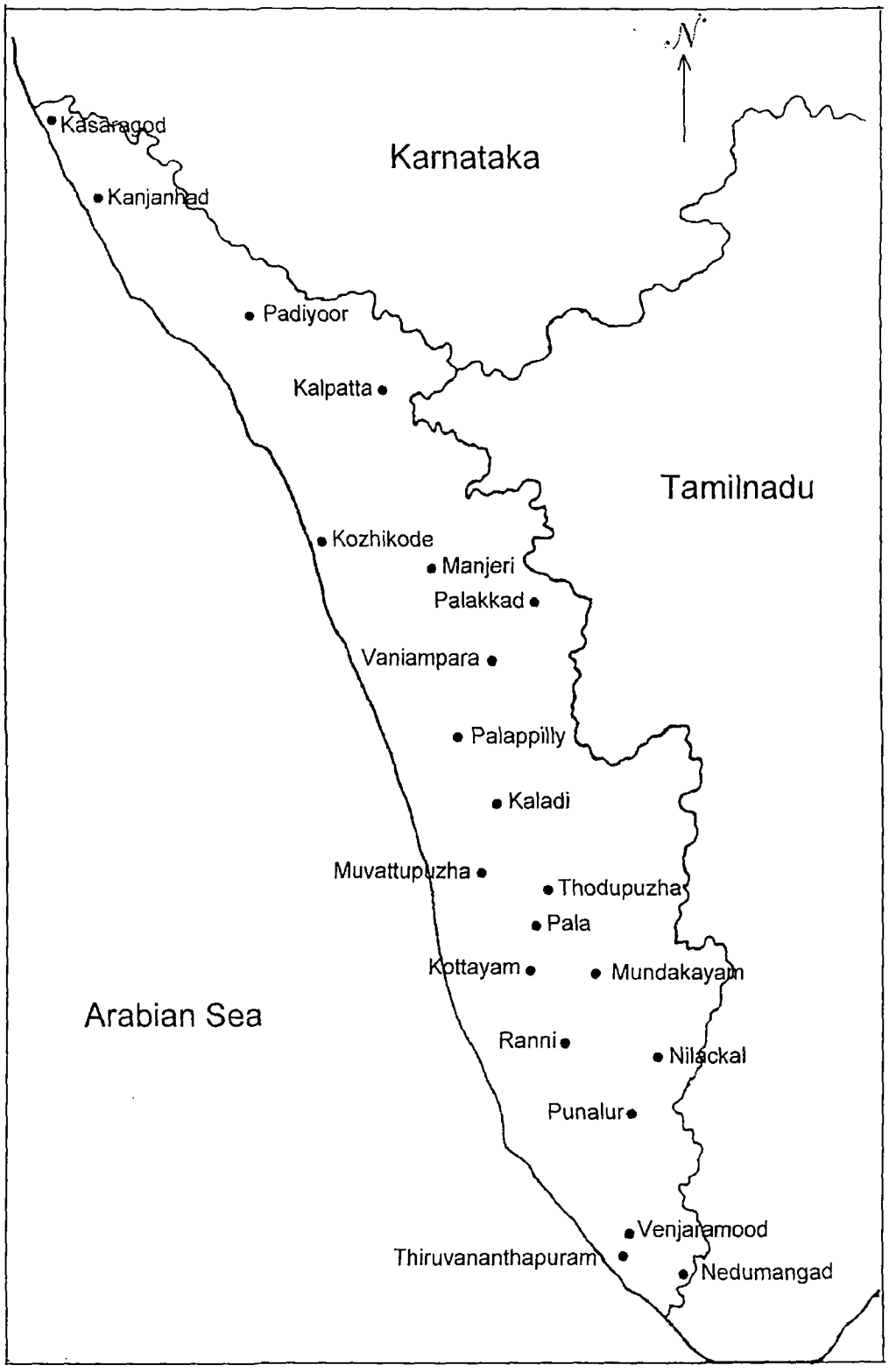


Figure 1. Sites of soil sample collection.

Table 1

Influence of location and season on VAM spore population in rubber plantation soil

Location	pH	Organic C (%)	Spore count/50 g soil.				
			Summer	Pre monsoon	Monsoon	Post monsoon	Mean
Thiruvanantha puram	5.3	1.18	482	409	367	409	416
Nedumangad	5.2	1.25	454	388	348	395	396
Venjaramood	5.6	1.87	508	429	385	413	434
Punalur	5.5	1.32	498	424	381	446	437
Ranni	4.9	2.22	406	348	312	381	362
Nilackal	5.4	2.15	447	368	345	406	392
Kottayam	4.5	1.50	352	299	267	305	306
Mundakayam	4.6	1.77	379	322	292	362	339
Pala	5.2	2.18	444	374	336	403	389
Thodupuzha	5.0	2.50	426	360	328	380	373
Muvattupuzha	5.0	1.62	483	407	364	426	420
Kaladi	5.4	1.50	453	382	349	395	395
Palappilly	5.2	2.32	436	371	337	411	389
Vaniampara	5.4	2.50	481	411	371	419	420
Palakkad	5.8	1.62	511	433	396	457	449
Manjeri	4.9	1.75	393	331	301	339	341
Kozhikode	5.0	2.32	454	403	363	414	409
Kalpatta	5.6	2.15	506	433	389	438	441
Kanjanhad	5.2	2.36	451	382	347	405	396
Kasaragod	5.4	2.40	428	362	328	394	378
Padiyoor	5.0	1.62	421	361	322	381	371
Mean			449	381	345	399	
CD (P = 0.05)	(Season) = 4.22 (Location) = 9.66 (Season x Location) = 19.32						

and *Gigaspora* spp. (Plate 15&16) followed this. The spores that could not be clearly identified were also present and they were very few in number. They were recorded separately as unidentified.

In summer, spores of *Glomus* spp. were significantly (at 5 per cent level) more than in other three seasons (Table 2). *Acaulospora* spp. and *Sclerocystis* spp. spores predominated in the monsoon season whereas in other seasons the count was lesser (Tables 3 & 4) with least in summer. Spores of *Gigaspora* spp. were least in monsoon season (Table 5). No significant change was noticed in the number of unidentified spores (Table 6) in all the four seasons.

Spore population of *Glomus* species in summer was more in the soils of Ranni, Nilackal, Mundakayam, Palappilly, Vaniampara and Palakkad and was least in Kottayam, Thodupuzha, Manjeri, Kozhikode and Kanjanhad. In the pre monsoon season, spores of *Glomus* spp. were more in the soils from Punalur, Ranni, Nilackal, Mundakayam, Kaladi, Palappilly, Vaniampara, Palakkad and Kasaragod. The lowest spore population was noticed in the soils of Thodupuzha and Muvattupuzha. During monsoon season, *Glomus* spp. spores were more in the soils of Punalur, Ranni, Nilackal, Mundakayam, Kaladi, Palappilly, Palakkad and Padiyoor. However, the spore count was least in Nedumangad, Kottayam, Muvattupuzha, Manjeri, Kozhikode and Kanjanhad soils. *Glomus* spp. spore count

was maximum in Ranni, Nilackal, Mundakayam, Kaladi, Palappilly, Vaniampara and Palakkad soil in post monsoon. At the same time the spore count was least in the soils of Venjaramood, Kottayam, Muvattupuzha, Manjeri, Kozhikode, Kalpatta and Kanjanhad (Table 2). In general, Ranni, Palappilly and Palakkad soil showed more *Glomus* spp. spore population irrespective of the season.

Kottayam soil harbored maximum *Acaulospora* spp. spores while soils of Thiruvananthapuram, Ranni, Palappilly, Vaniampara and Palakkad registered the least in summer. In the pre monsoon season also Kottayam soil recorded highest *Acaulospora* spp. spore count but soils of Punalur, Ranni, Palappilly, Kozhikode, Kasaragod and Padiyoor contained the least. Soil collected from Kottayam and Kanjanhad region showed uniformly more *Acaulospora* spp. spores in monsoon season whereas lowest spore count was recorded in most of the soils *viz.* Thiruvananthapuram, Nedumangad, Punalur, Ranni, Nilackal, Thodupuzha, Kaladi, Palappilly, Vaniampara, Palakkad, Kasaragod and Padiyoor. As observed in other seasons *Acaulospora* spp. spores were maximum in Kottayam soils in the post monsoon season and the count was less in soils of Thiruvananthapuram, Nedumangad, Palappilly, Vaniampara and Palakkad (Table 3). Significantly higher *Acaulospora* spp. spore count in all the seasons was observed in soils from Venjaramood, Kottayam, Thodupuzha, Muvattupuzha, Manjeri, and Kanjanhad than the other locations, the highest being in soil from Kottayam.

Table 2

Influence of location and season on *Glomus* spore population

Location	Spore count/50 g soil (%)				
	Summer	Pre monsoon	Monsoon	Post monsoon	Mean
Thiruvananthapuram	77.00	70.33	69.33	70.67	71.83
Nedumangad	73.67	67.33	66.67	72.33	70.00
Venjaramood	71.33	69.33	67.33	69.33	69.41
Punalur	75.33	72.33	71.00	72.67	72.83
Ranni	80.00	73.67	70.67	73.33	74.41
Nilackal	78.33	71.67	70.33	74.00	73.58
Kottayam	68.33	65.67	66.33	68.67	67.25
Mundakayam	79.33	71.67	71.33	73.33	73.91
Pala	74.67	69.33	67.33	71.33	70.66
Thodupuzha	69.33	65.33	70.00	71.33	69.00
Muvattupuzha	72.33	62.67	66.33	69.00	67.58
Kaladi	75.67	71.67	70.67	73.67	72.91
Palappilly	77.67	73.33	73.00	73.67	74.41
Vaniampara	77.33	72.33	70.00	73.33	73.25
Palakkad	79.67	74.33	72.33	75.67	75.50
Manjeri	68.66	66.67	64.33	69.00	67.16
Kozhikode	69.00	69.67	65.00	68.33	68.00
Kalpatta	71.33	69.67	68.67	70.33	70.00
Kanjanhad	70.33	69.33	65.33	67.67	68.16
Kasaragod	75.33	72.67	69.33	71.67	72.25
Padiyoor	74.67	71.33	71.33	71.67	72.75
Mean	74.25	70.12	68.88	71.47	
CD (P = 0.05)	(Season) = 0.63 (Location) = 1.44 (Season x Location) = 2.89				

Table 3

Influence of location and season on *Acaulospora* spore population

Location	Spore count/50 g soil (%)				
	Summer	Pre monsoon	Monsoon	Post monsoon	Mean
Thiruvananthapuram	9.67	12.33	13.33	11.67	11.75
Nedumangad	11.67	14.33	13.67	11.67	12.83
Venjaramood	14.33	13.67	16.33	14.33	14.66
Punalur	10.67	10.33	13.00	12.33	11.58
Ranni	8.00	11.00	13.33	12.67	11.25
Nilackal	10.33	12.33	12.67	13.67	12.25
Kottayam	19.33	19.67	18.67	18.67	19.08
Mundakayam	11.33	12.67	14.33	12.33	12.66
Pala	10.67	12.67	14.33	13.00	12.66
Thodupuzha	16.66	16.67	13.33	13.67	15.08
Muvattupuzha	11.00	17.33	14.67	14.33	14.33
Kaladi	11.67	13.33	13.00	13.33	12.83
Palappilly	9.67	12.00	12.33	11.67	11.41
Vaniampara	9.33	12.33	13.67	10.33	11.41
Palakkad	9.67	12.33	12.33	11.33	11.41
Manjeri	14.00	14.33	16.33	14.33	14.75
Kozhikode	11.00	12.00	14.33	12.67	12.50
Kalpatta	11.00	13.00	14.67	13.67	13.08
Kanjanhad	15.67	15.33	18.67	16.67	16.58
Kasaragod	11.67	12.00	14.00	12.67	12.58
Padiyoor	11.00	12.00	13.67	13.67	12.58
Mean	11.82	13.41	14.31	13.26	
CD (P = 0.05)	(Season) = 0.42 (Location) = 0.96 (Season x Location) = 1.94				

Soil from Kozhikode collected in summer contained more of *Sclerocystis* spp. spores while that from Thiruvananthapuram, Punalur, Ranni, Nilackal, Kottayam, Mundakayam, Kaladi, Palappilly, Palakkad and Padiyoor showed less. Interestingly in pre monsoon season *Sclerocystis* spp. spores were more in soils of Nedumangad, Pala, Muvattupuzha, Kozhikode and Kalpatta. At the same time least spore count was recorded in the soils of Ranni, Nilackal, Kottayam, Mundakayam, Palappilly, Vaniampara, Palakkad and Kasaragod. In monsoon season the difference in *Sclerocystis* spp. spores at different location was not much except the increase in Kozhikode soil and a decrease in Kottayam soil. As in the case of other seasons, in post monsoon season also soil from Kozihikode showed more *Sclerocystis* spp. spore count. The lowest spore count was registered in Kottayam, Palappilly, Palakkad, Manjeri, Kasaragod and Padiyoor (Table 4). Irrespective of the seasons Kozhikode soil showed highest *Sclerocystis* spp. spore count and the least was in Kottayam.

Table 4

Influence of location and season on *Sclerocystis* spore population

Location	Spore count/50 g soil (%)				
	Summer	Pre monsoon	Monsoon	Post monsoon	Mean
Thiruvananthapuram	06.66	10.67	11.33	10.00	09.66
Nedumangad	07.33	11.67	12.33	09.67	10.25
Venjaramood	09.00	09.33	10.67	11.00	10.00
Punalur	06.33	09.67	10.67	09.67	09.08
Ranni	07.00	08.00	09.67	09.33	08.50
Nilackal	07.00	08.67	10.33	09.33	08.83
Kottayam	06.33	07.33	07.67	06.67	07.00
Mundakayam	05.33	08.33	09.67	09.33	08.16
Pala	08.33	11.33	11.00	09.00	09.91
Thodupuzha	09.33	11.00	11.33	09.00	10.16
Muvattupuzha	09.00	13.00	12.33	10.00	11.08
Kaladi	06.67	10.67	11.33	09.67	09.58
Palappilly	07.00	09.00	09.67	07.67	08.33
Vaniampara	07.33	09.00	10.00	09.33	08.91
Palakkad	06.33	08.67	11.00	06.67	08.16
Manjeri	08.33	11.00	13.33	08.33	10.25
Kozhikode	13.33	12.33	15.33	13.00	13.50
Kalpatta	10.00	12.00	10.67	10.67	10.83
Kanjanhad	07.33	10.33	10.00	10.33	09.50
Kasaragod	07.33	09.00	11.67	07.67	08.91
Padiyoor	06.67	10.33	10.33	07.33	08.66
Mean	07.71	10.06	10.96	09.22	
CD (P = 0.05)	(Season) = 0.37 (Location) = 0.84 (Season x Location) = 1.68				

The occurrence of *Gigaspora* spp. spores was more in the soils of Punalur, Thodupuzha, Muvattapuzha, Manjeri and Kasaragod and in most of the other soils the *Gigaspora* spp. spore count was the least in summer season. In the case of pre monsoon season *Gigaspora* spp. spore count was significantly more in most of the soils except Kaladi, Palakkad and Padiyoor. In the monsoon season, the variation in *Gigaspora* spp. spore count was more in four different soils *viz.* Nedumangad, Nilackal, Kottayam and Muvattupuzha. In all other soils the count was very low. During post monsoon season the spore count was almost the same except in soils of Ranni, Nilackal and Kalpatta where the count was less (Table 5).

There was not much variation in the spore count of unidentified VAM among different locations during summer, pre monsoon, monsoon and post monsoon seasons (Table.6). The effect of season on the occurrence of different genera of VAM spores is shown in fig. 2.

4.2. Effect of location and season on *Hevea* root colonisation by VAM

Hevea root colonisation by VAM at 21 location during four different seasons presented in Table 7 showed that all the samples irrespective of location and season were colonised by VAM. In general, VAM colonisation in *Hevea* roots during summer season was comparatively low in all the locations. Thereafter the degree of colonisation started increasing.

Table 5

Influence of location and season on *Gigaspora* spore population

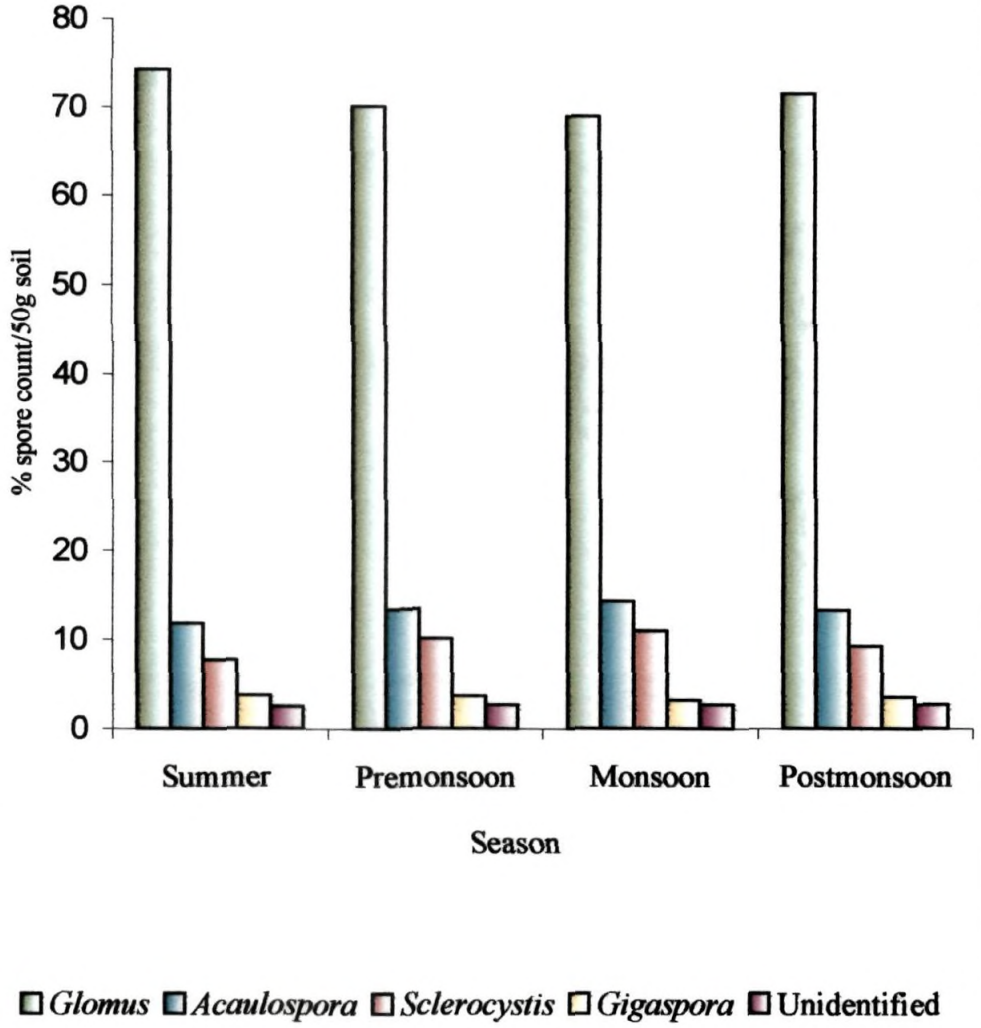
Location	Spore count/50 g soil (%)				
	Summer	Pre monsoon	Monsoon	Post monsoon	Mean
Thiruvanantha puram	2.67	3.33	2.33	3.33	2.92
Nedumangad	4.00	3.67	5.00	4.00	4.17
Venjaranmood	3.33	3.33	3.00	3.67	3.25
Punalur	4.67	4.67	3.00	3.33	3.92
Ranni	2.67	4.00	3.00	2.67	3.08
Nilackal	2.67	4.67	4.33	1.67	3.33
Kottayam	3.67	4.33	5.33	3.67	4.25
Mundakayam	3.00	4.67	2.67	3.33	3.33
Pala	3.00	3.33	3.67	3.33	3.33
Thodupuzha	4.33	3.33	2.67	3.67	3.50
Muvattupuzha	5.33	4.67	4.33	4.67	4.75
Kaladi	4.00	2.67	3.00	4.00	3.33
Palappilly	3.33	3.67	3.00	4.00	3.50
Vaniampara	3.33	3.33	3.33	3.67	3.42
Palakkad	2.67	2.67	2.33	3.33	2.75
Manjeri	5.67	4.00	2.67	4.00	4.08
Kozhikode	3.33	3.67	2.33	3.33	3.17
Kalpatta	4.00	3.67	3.33	2.67	3.42
Kanjanhad	4.00	3.33	3.00	3.67	3.42
Kasaragod	4.33	3.33	2.67	4.67	3.75
Padiyoor	4.00	2.67	2.00	4.00	3.17
Mean	3.71	3.67	3.19	3.56	
CD (P = 0.05)	(Season) = 0.32 (Location) = 0.72 (Season x Location) = 1.44				

Table 6

Influence of location and season on unidentified spore population

Location	Spore count/50 g soil (%)				
	Summer	Pre monsoon	Monsoon	Post monsoon	Mean
Tiruvananthapuram	3.67	3.33	3.67	4.00	3.67
Nedumangad	3.33	3.00	2.33	2.33	2.75
Venjaramood	2.00	3.33	2.67	2.00	2.50
Punalur	3.33	3.00	2.33	2.00	2.67
Ranni	2.33	3.33	3.33	2.00	2.75
Nilackal	1.33	2.67	2.33	1.33	1.92
Kottayam	2.67	3.00	2.00	2.33	2.50
Mundakayam	1.33	2.67	2.00	2.00	2.00
Pala	2.67	3.33	3.67	3.33	3.25
Thodupuzha	1.33	3.67	3.67	2.33	2.75
Muvattupuzha	2.67	3.67	2.33	2.00	2.67
Kaladi	2.00	1.67	2.00	2.00	1.92
Palappilly	2.00	2.00	2.00	3.00	2.25
Vaniampara	2.67	3.00	3.00	3.33	3.00
Palghat	1.67	2.00	2.00	3.00	2.17
Manjeri	3.33	3.33	3.33	4.33	3.58
Kozhikode	3.00	2.33	3.00	2.67	2.75
Kalpatta	3.67	1.67	2.67	2.67	2.67
Kanjanhad	2.33	1.67	3.00	2.00	2.25
Kasaragod	2.00	3.00	2.33	3.00	2.58
Padiyoor	2.67	1.67	2.67	3.33	2.58
Mean	2.48	2.73	2.68	2.62	
CD (P = 0.05) (Season) = 0.37 (Location) = 0.86 (Season x Location) = 1.71					

Fig.2 **Effect of season on the occurence of different genera of VAM spores in *Hevea* plantations**



In summer season, *Hevea* root colonisation was more in samples from Mundakayam, Pala and Palappilly, which were followed by samples from Nilackal and Thodupuzha. Lowest VAM infestation was recorded in samples of Thiruvananthapuram, Nedumangad, Punalur, Palakkad, Manjeri, Kalpatta, Kanjanhad and Kasaragod. During the pre monsoon season also VAM colonisation was maximum in the samples of Mundakayam and Pala in addition to Thodupuzha and the least infestation was recorded in samples from Nedumangad, Punalur, Palakkad, Kanjanhad and Kasaragod.

In the monsoon season root samples from Mundakayam, Pala and Thodupuzha registered the highest VAM colonisation whereas samples from Thiruvananthapuram, Nedumangad, Punalur, Palakkad, Kanjanhad and Kasaragod recorded the minimum colonisation.

Samples collected from Mundakayam, Pala and Thodupuzha during post monsoon season also showed more root colonisation. The lowest VAM colonisation was registered in samples from Thiruvananthapuram, Punalur, Palakkad, Kanjanhad and Kasaragod. Considerable variation in colonisation was recorded in other locations. VAM colonisation in *Hevea*, in general was poor in samples from Nedumangad, Punalur, Palakkad, Kanjanhad and Kasaragod and samples from Mundakayam, Pala and Thodupuzha showed higher root colonisation in all the seasons.

The season had much impact on VAM colonisation in *Hevea*. After summer the colonisation increased with the advent of rainy season. The increase in colonisation upon the influence of rain varied considerably in samples collected from different locations. The lowest root colonisation was observed during summer in Punalur, Palakkad and Kanjanhad while it was highest in Mundakayam during the monsoon period.

The appresorium, vesicles and arbuscules of VAM fungi in *Hevea* roots are shown in plates 17-19.

4.3. Effect of soil pH and organic carbon on VAM spore count in soil and root colonisation in *Hevea*

The results of pH analysis of samples from different location in different season are given in Table 8. It is seen that all the soils are acidic. The magnitude of acidity differed in different soils. Among the locations studied, higher value of soil pH (5.8) was recorded in Palakkad soil. This was followed by soil from Kalpatta, Venjaramood, Nedumangad, Vaniampara, Nilackal, Kasaragod and Thiruvananthapuram. Soil pH of Mundakayam and Kottayam was very low (4.6 and 4.5).

Plates 9-12 Spores of different species of *Glomus* sp (x 160)

Plate 13. Spore of *Acaulospora* sp. (x 160)

Plate 14. Spore of *Sclerocystis* sp. (x 160)

Plate 9

Plate 10

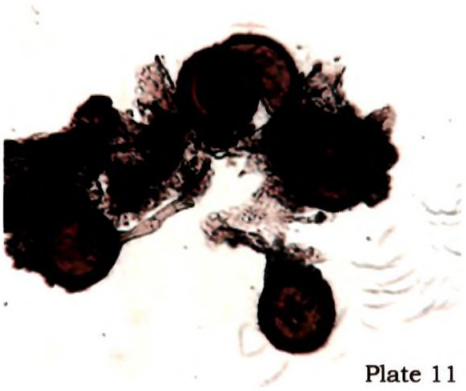


Plate 11

Plate 12

Plate 13

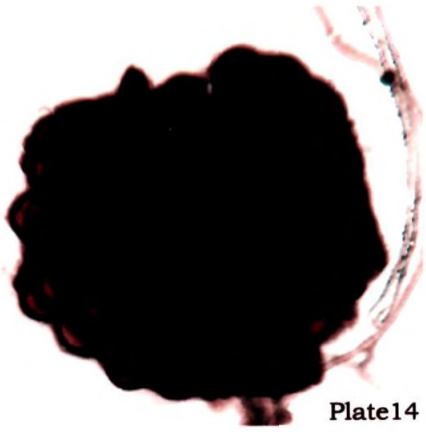


Plate14

Plate 15

Plate 16

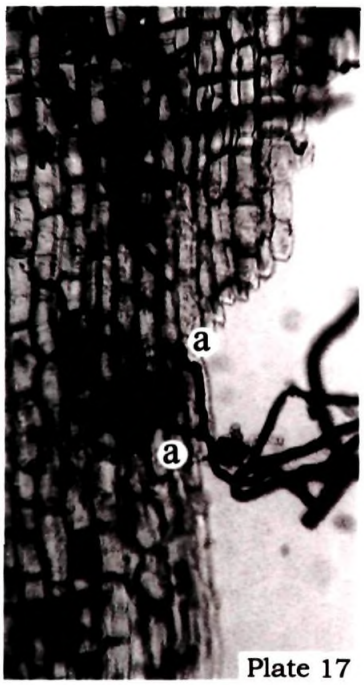


Plate 17

Plate 18

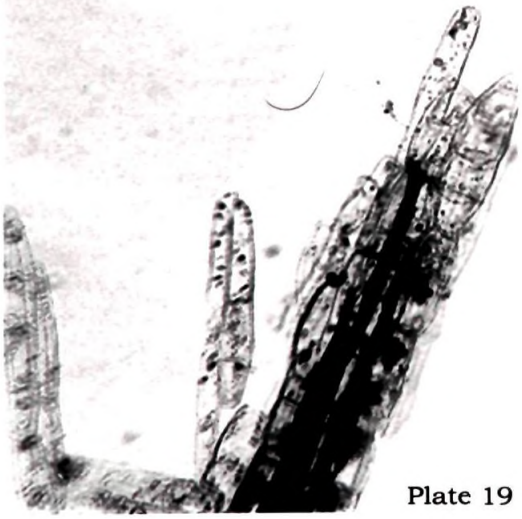


Plate 19

Table 7

Influence of location and season on VAM colonisation in Hevea roots.

Location	Root colonisation (%)				
	Summer	Pre monsoon	Monsoon	Post monsoon	Mean
Thiruvananthapuram	26.33	33.67	35.33	34.33	32.42
Nedumangad	25.67	30.33	35.00	35.67	31.67
Venjaramood	28.67	35.33	39.33	35.67	34.75
Punalur	25.33	31.00	33.33	32.67	30.58
Ranni	31.33	38.33	41.33	36.67	36.92
Nilackal	35.67	41.33	44.67	39.33	40.25
Kottayam	30.33	40.67	43.33	42.00	39.08
Mundakayam	38.33	46.33	51.00	49.00	46.00
Pala	38.67	44.67	48.33	49.00	45.17
Thodupuzha	34.67	46.67	49.67	48.33	44.83
Muvattupuzha	29.33	40.00	43.00	40.00	38.08
Kaladi	32.67	36.33	38.67	37.00	36.17
Palappilly	39.67	43.33	47.67	46.67	44.33
Vaniampara	29.00	36.67	38.33	35.67	34.92
Palakkad	25.33	31.00	34.33	34.33	31.25
Manjeri	27.67	34.67	37.67	35.67	33.92
Kozhikode	30.00	36.67	39.33	38.33	36.08
Kalpatta	27.33	33.67	37.33	37.00	33.83
Kanjanhad	25.33	31.67	35.33	34.33	31.67
Kasaragod	25.67	32.33	35.67	33.33	31.75
Padiyoor	30.33	35.67	39.33	39.33	36.17
Mean	30.32	37.16	40.38	38.78	
CD (P = 0.05) (Season) = 0.63 (Location) = 1.44 (Season x Location) = 2.88					

All the root samples collected from the 21 locations were found infected with VAM fungi irrespective of soil pH. However, the spore count of the soil increased with the increase in pH. Spore count and pH of soil were positively correlated (Tables 8a-d). Kottayam soil having the lowest pH of 4.5 recorded minimum number of spores and the Palakkad soil having pH 5.8 contained maximum number of spores.

Soil pH did not have any relation to the population of different species of VAM spores in most of the soils. However maximum population of *Acaulospora* spp. was recorded in soil from Kottayam, having the lowest pH. Kozhikode soil of pH 5.4 contained maximum *Sclerocystis* spp. spore population in all the four seasons.

Soil organic carbon of all the samples studied varies from 1.18-2.50 per cent (Table 1) and did not show any clear influence on spore population and root colonisation in the rubber growing soils (Tables 8a-d).

4.4. VAM spore population and root colonisation in *Hevea* plantations under different period of cultivation

The result of the study on variation of per cent VAM infection and spore count in rubber growing soils at four different periods of cultivation is given in Figs. 3 and 4. Maximum per cent of VAM colonisation was recorded in plantations

Table 8

Effect of location and season on soil pH

Location	Soil pH			
	Summer	Pre monsoon	Monsoon	Post monsoon
Thiruvananthapuram	5.3	5.3	5.2	5.2
Nedumangad	5.5	5.5	5.4	5.5
Venjaramood	5.6	5.5	5.5	5.6
Punalur	5.3	5.2	5.3	5.3
Ranni	4.9	4.9	4.8	5.0
Nilackal	5.4	5.3	5.4	5.3
Kottayam	4.5	4.5	4.4	4.4
Mundakayam	4.6	4.5	4.6	4.5
Pala	5.2	5.3	5.2	5.2
Thodupuzha	5.0	4.9	5.0	5.1
Muvattupuzha	5.0	5.1	5.1	5.1
Kaladi	5.4	5.4	5.3	5.3
Palappilly	5.2	5.2	5.1	5.3
Vaniampara	5.4	5.3	5.3	5.4
Palakkad	5.8	5.9	5.8	5.6
Manjeri	4.9	5.0	4.9	4.8
Kozhikode	5.0	5.1	5.1	5.2
Kalpatta	5.6	5.6	5.7	5.6
Kanjanhad	5.2	5.3	5.2	5.1
Kasaragod	5.4	5.3	5.3	5.4
Padiyoor	5.0	4.9	5.1	5.1

Table 8a

Correlation matrix in summer season

	Organic C	Soil pH	Spore count	% infection
Organic C	1.0000	0.0203	0.1191	0.3350
Soil pH		0.1049	0.8216**	0.3814
Spore count			1.0000	0.4257
% infection				1.0000

** Indicate significance at P<0.01

Table 8b

Correlation matrix in Pre-monsoon season

	Organic Carbon	Soil pH	Spore count	% infection
Organic Carbon	1.0000	0.0037	0.0738	0.3480
Soil pH		1.0000	0.7914**	0.5688
Spore count			1.0000	0.5208
% Infection				1.0000

** Indicate significance at P<0.01

Table 8c

Correlation matrix in monsoon season

	Organic C	Soil pH	Spore count	% infection
Organic C	1.0000	0.0282	0.0519	0.3466
Soil pH		1.0000	0.8254**	0.5373
Spore count			1.0000	0.5142
% infection				1.0000

** Indicate significance at P<0.01

Table 8d

Correlation matrix in Post-monsoon season

	Organic C	Soil pH	Spore count	% infection
Organic C	1.0000	0.0505	0.0624	0.2657
Soil pH		1.0000	0.7777**	0.5032
Spore count			1.0000	0.3481
% infection				1.0000

** Indicate significance at P<0.01

under five and ten years which were followed by twenty and thirty years of cultivation. Total VAM spore count was significantly more in soils under five years of cultivation and in soils of ten, twenty and thirty years of cultivation, the count was comparatively less.

The per cent of *Glomus* spp. spore population was least in the soils under five years and maximum in twenty years of cultivation. *Gigaspora* spp. and *Acaulospora* spp. were more with five-year-old plants and all other soils showed lesser population. No significant difference was observed in the case of *Sclerocystis* spp. The unidentified spore population was the least in the soils under twenty and thirty years of cultivation and more with five and ten years old plants (Fig. 5).

4.5. Spore population and root colonisation in the soils of three different cycles of *Hevea* cultivation

The results of the study on VAM spore population and *Hevea* root colonisation in soils under three different cycles of cultivation are given in Figs. 6 and 7. During the first and second cycle of rubber cultivation, soils contained more spores and its population got reduced in the third cycle. *Hevea* root colonisation was more in the area under the first cycle of rubber cultivation. Gradual reduction in the per cent of infection was recorded with the increase in the cycle.

Fig 3. **VAM colonisation of *Hevea* roots under different years of cultivation**

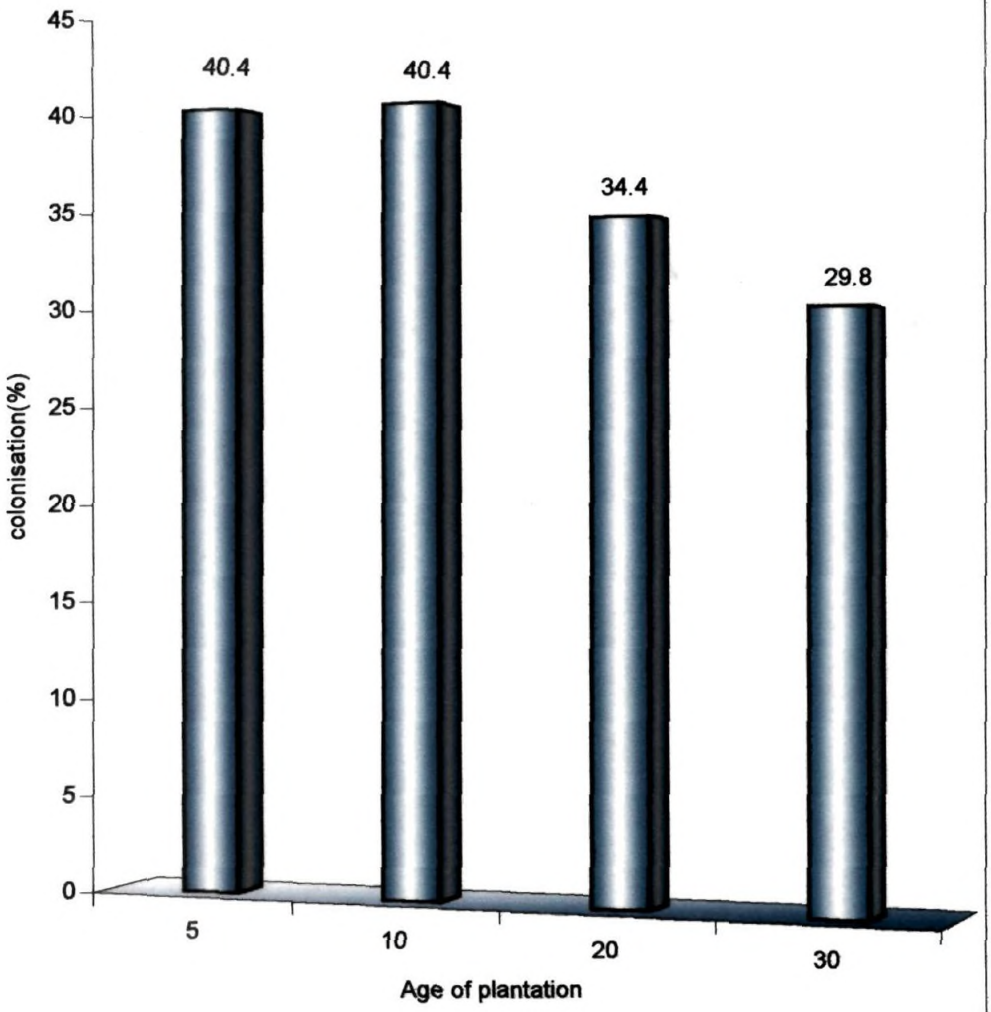


Fig 4. **VAM spore count in rubber plantation soil under different years of cultivation**

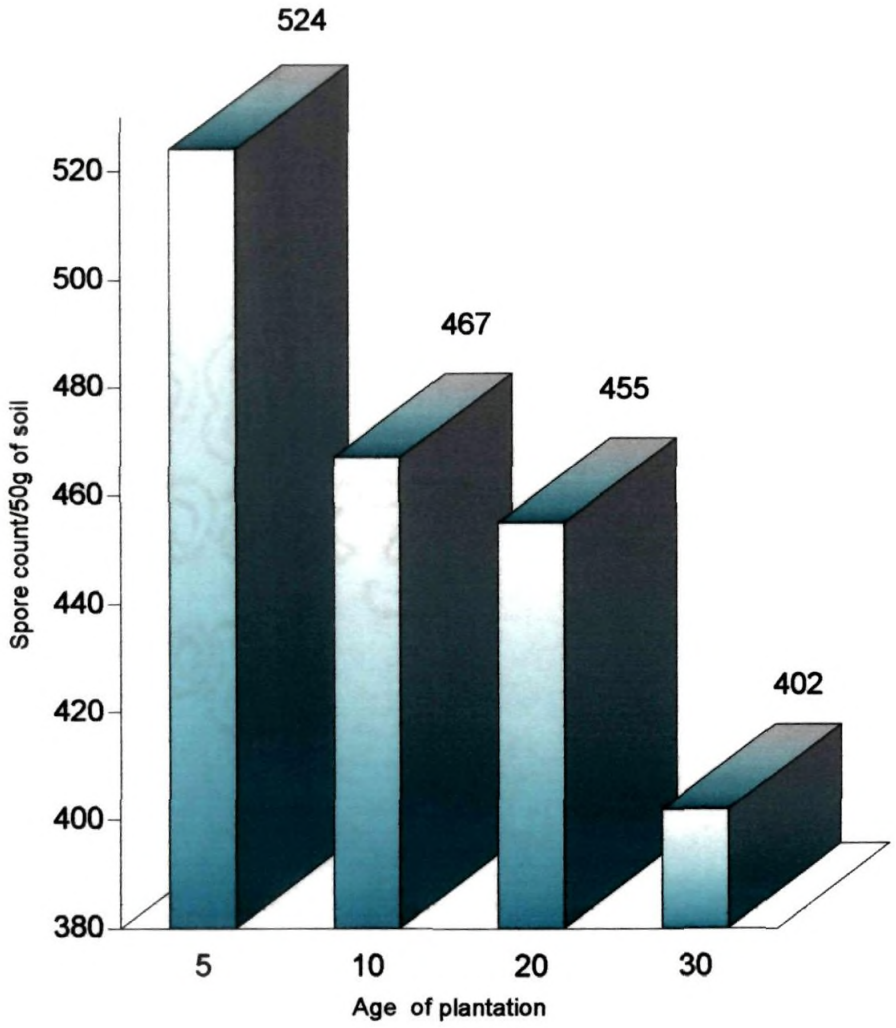


Fig.5. **Per cent occurrence of different genera of VAM spores in *Hevea* plantations under different years of cultivation**

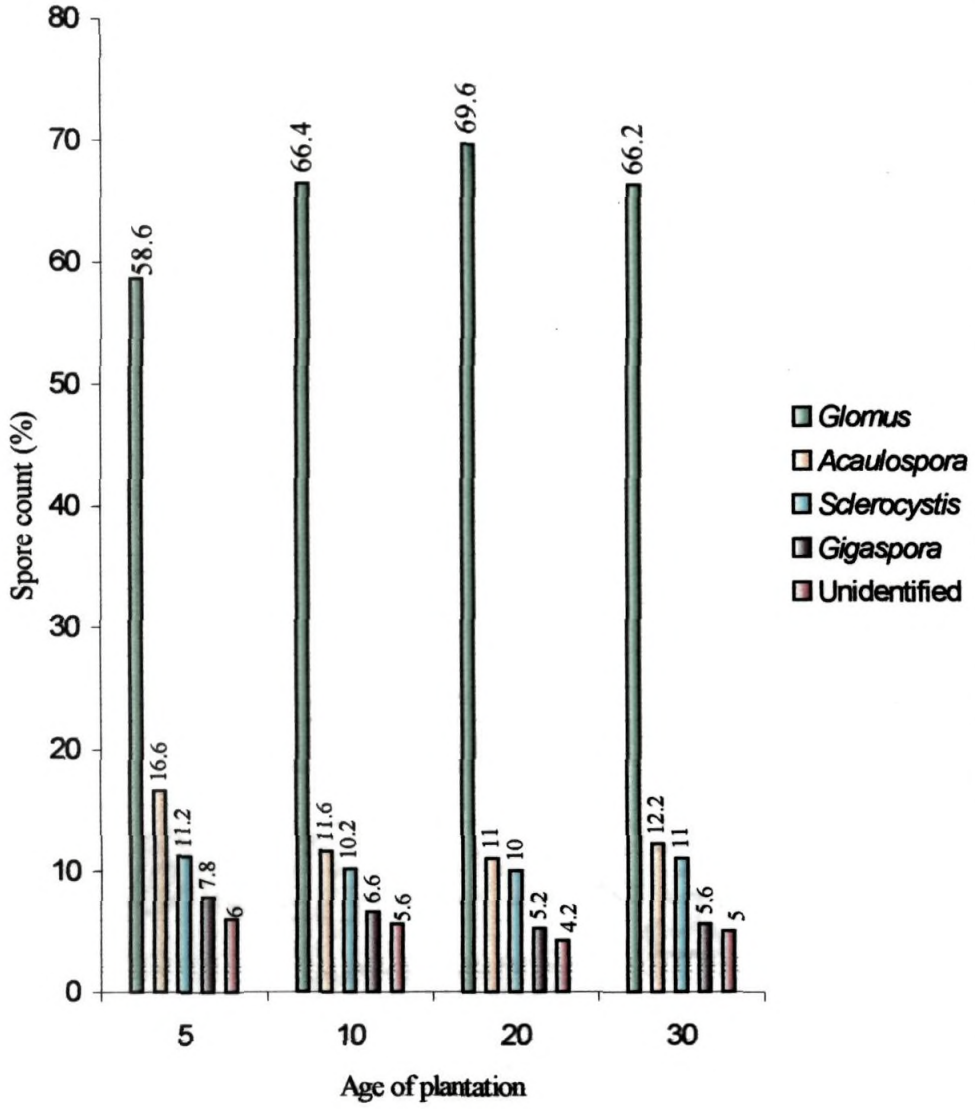


Fig.6. **VAM Spore count in soil of rubber plantation under different cultivation cycle**

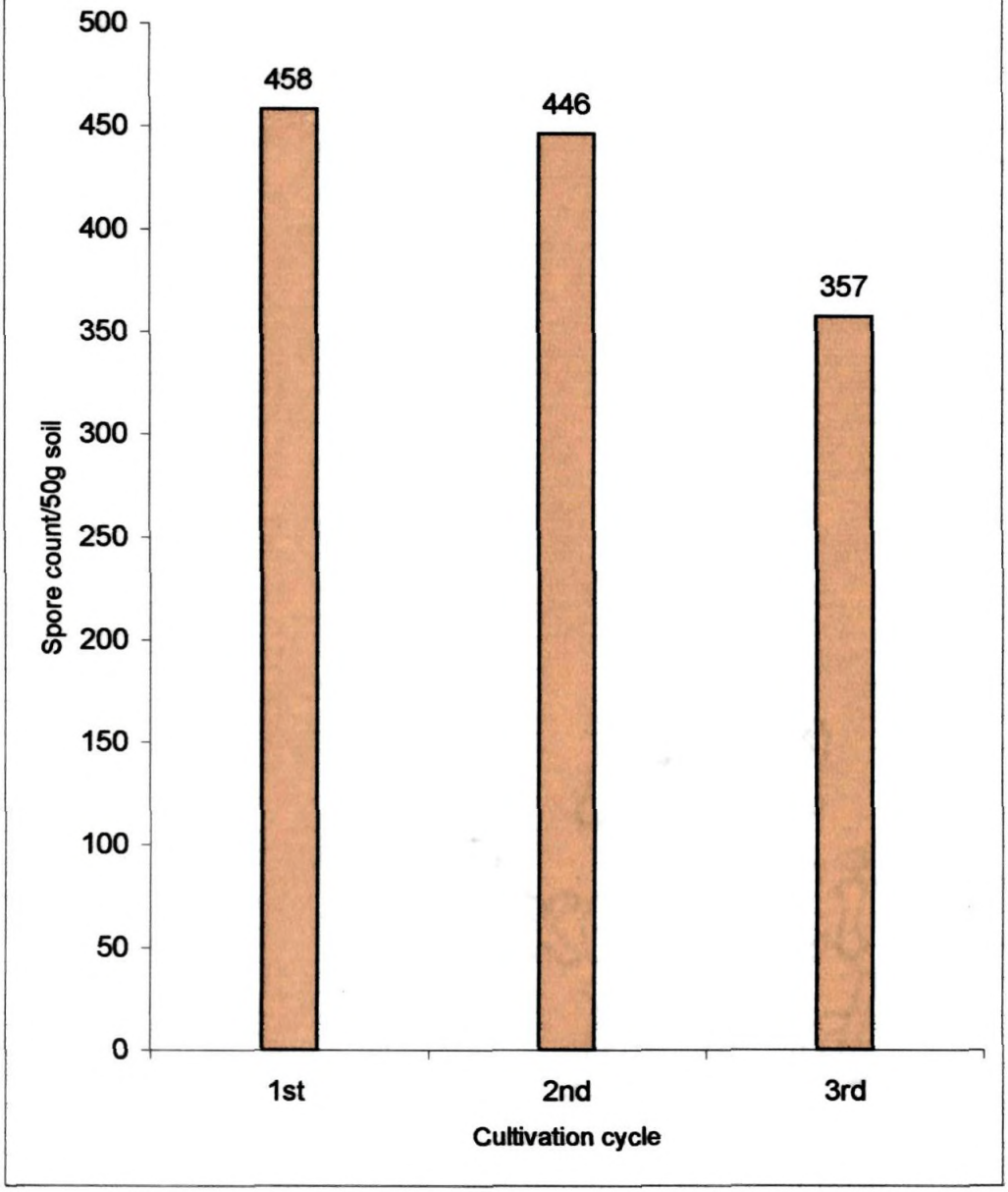
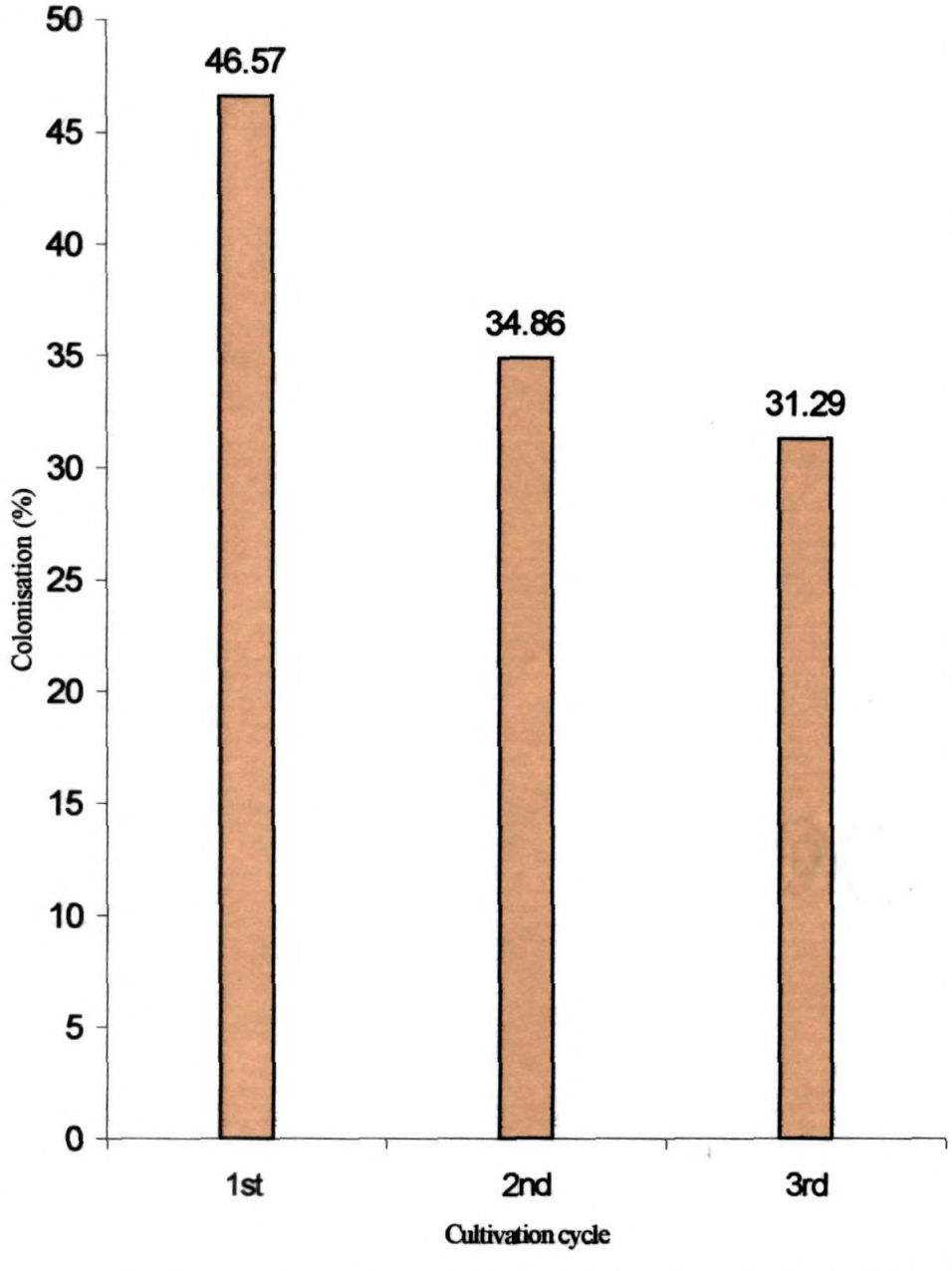


Fig 7. **VAM colonisation in *Hevea* roots under different cultivation cycle.**



4.6. Influence of soil depth on VAM spore population

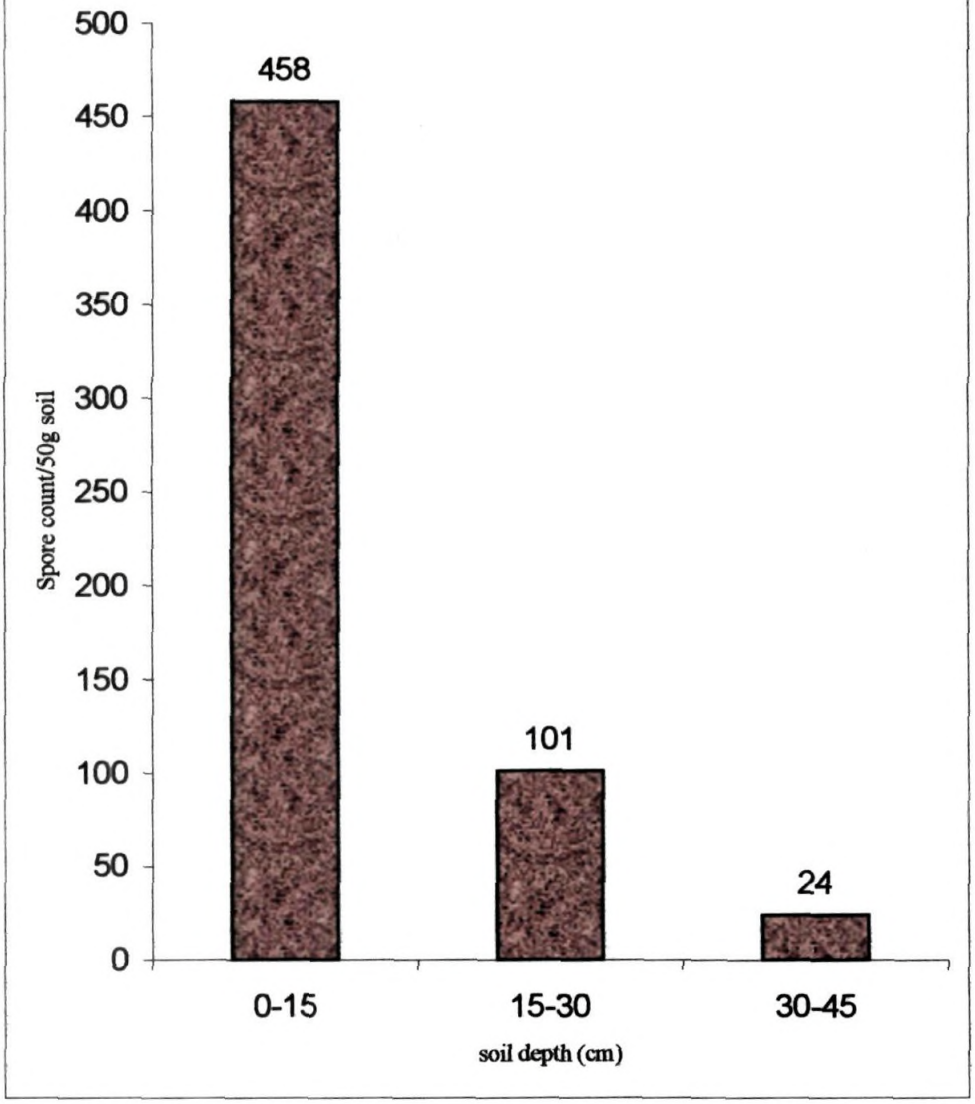
The studies on the effect of soil depth on VAM spore population showed maximum spore population in the top soil of 0-15 cm depth (Fig. 8). 15-30 cm soil recorded a significant reduction of 78% and further reduction was noticed in 30-45 cm soil (75% from 15-30 cm layer).

4.7. Comparative studies on VAM spore count in forest and rubber growing soils

The VAM spore count from four locations where reserve forest and adjacent rubber plantations were selected for the study and the result is given in Table 9. In all the four locations the total VAM spore population was significantly more in soil samples from forests than the rubber plantations (Fig. 9). Among the forest soils, soil from Palakkad registered the highest population followed by Kozhikode, Thrissur and Mundakayam. Almost the same trend was noticed in the samples of rubber plantations.

In both forest and rubber field soils the spore counts of *Glomus* spp. was maximum followed by *Acaulospora* spp., *Sclerocystis* spp. and *Gigaspora* spp. Species of *Glomus* population was significantly higher in forest soils than the

Fig.8. **Influence of soil depth on VAM spore count in rubber plantation**



rubber soils of the given locations (Table 10). Between the locations, spore population of *Glomus* spp. did not show significant variation in rubber soils. In Mundakayam and Kozhikode regions *Acaulospora* spp. showed a significant increase in rubber soils but in Thrissur and Palakkad the increase was not significant (Table 11). The per cent of *Sclerocystis* spp. spore population was more in forest soils of Thrissur and Kozhikode while in Palakkad region rubber growing soils showed an increase. No variation in *Sclerocystis* spp. population was noticed with rubber and forest soils of Mundakayam (Table 12). The *Gigaspora* spp. (Table 13) as well as some unidentified spores were few in numbers and did not show much variation either among the locations or between reserve forest and rubber growing soils except in Thrissur where rubber growing soils showed an increased per cent of unidentified spores (Table 14). The unidentified spores did not show clear morphological characters and other structures.

4.8. Effect of soil factors on VAM

4.8.1. Effect of soil temperature on VAM spore population and root colonisation in sorghum

Results of the study on the effect of soil temperature showed that there was not much variation in spore count based on the changes in temperature from 20 to 40° C. But a significant decrease in viable spore count was showed by an increase

Table 9

Comparative study on VAM spore population in forest and rubber growing soils

Location	Total spore count/50 g soil*		t. value
	Forest soil	Rubber soil	
Mundakayam	403	309	9**
Trissur	445	368	10**
Palakkad	516	410	12**
Kozhikode	476	377	14**

* Mean of 10 observations
** Indicate significance at P<0.01

Fig.9. **Total VAM spore count in forest and rubber soil in different locations**

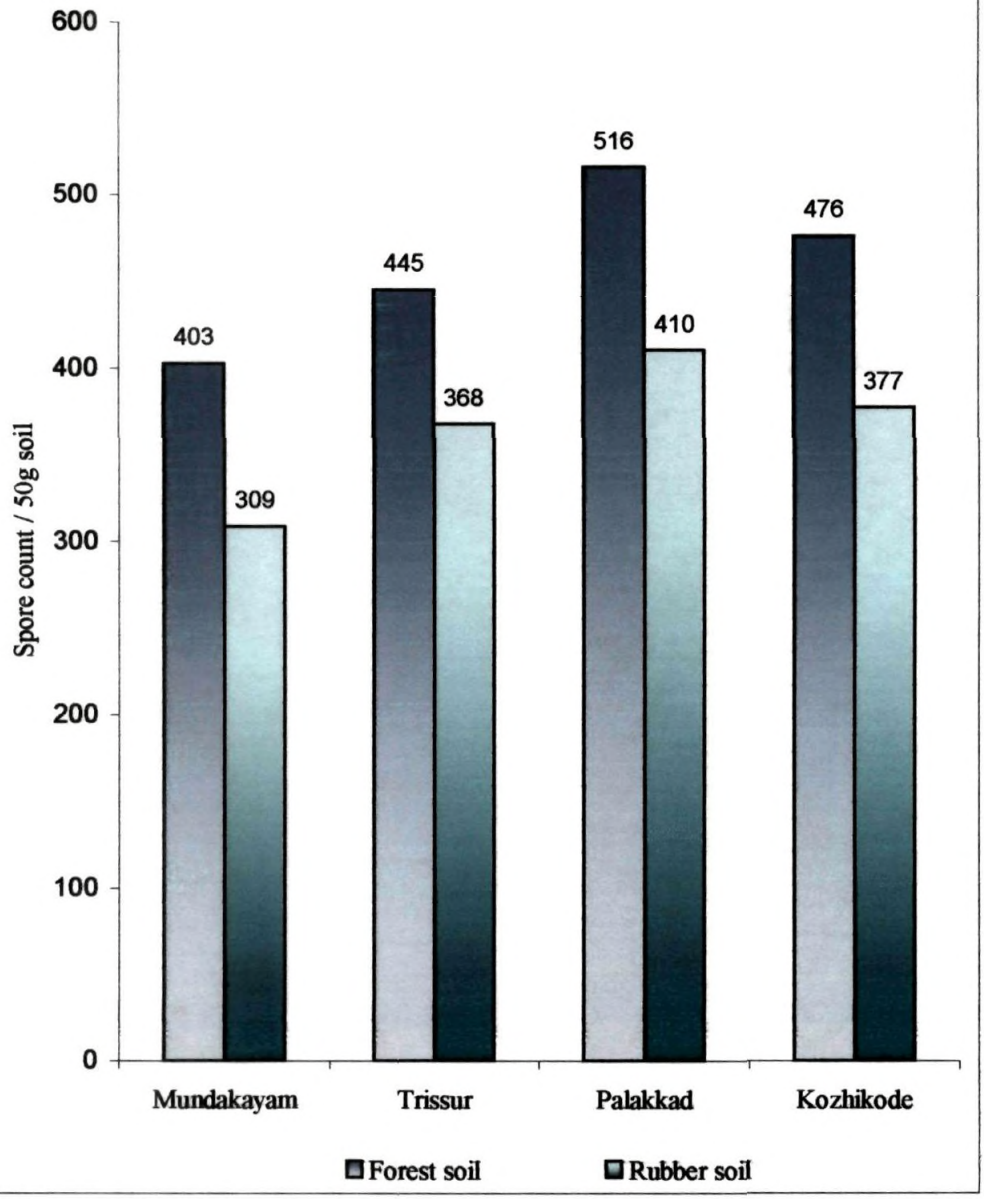


Table 10

Comparative study on Glomus spore population in forest and rubber growing soils

Location	Spore count/50 g soil (%)*		t. value
	Forest soil	Rubber soil	
Mundakayam	68.70	64.40	4.24**
Trissur	71.90	69.30	4.74**
Palakkad	74.10	70.40	3.72**
Kozhikode	70.90	64.10	8.08**

* Mean of 10 observations
** Indicate significance at $P<0.01$

Table 11

Comparative study on Acaulospora spore population in forest and rubber growing soils

Location	Spore count/50 g soil (%)*		t. value
	Forest soil	Rubber soil	
Mundakayam	17.50	18.70	3.51**
Trissur	16.50	16.60	0.20
Palakkad	11.30	12.10	1.74
Kozhikode	10.00	11.80	4.32**

* Mean of 10 observations
** Indicate significance at $P<0.01$

Table 12

Comparative study on Sclerocystis spore population in forest and rubber growing soils

Location	Spore count/50 g soil (%)*		t. value
	Forest soil	Rubber soil	
Mundakayam	5.20	5.70	0.79
Trissur	7.40	5.90	3.62**
Palakkad	3.90	5.10	2.86**
Kozhikode	13.70	12.10	3.31**

* Mean of 10 observations
**Indicate significance at P<0.01

Table 13

Comparative study on Gigaspora spore population in forest and rubber growing soils

Location	Spore count/50 g soil (%)*		t. value
	Forest soil	Rubber soil	
Mundakayam	4.40	3.90	1.21
Trissur	4.70	4.80	0.16
Palakkad	5.20	5.50	0.52
Kozhikode	4.80	5.10	0.70

* Mean of 10 observations

Table 14

Comparative studies on unidentified spore population in forest and rubber growing soils

Location	Spore count/50 g soil (%)*		t. value
	Forest soil	Rubber soil	
Mundakayam	3.00	3.60	1.41
Trissur	3.00	4.10	2.89**
Palakkad	3.70	3.00	1.56
Kozhikode	3.50	3.10	0.97

* Mean of 10 observations
** Indicate significance at P<0.01

in temperature from 40 to 50°C (Fig. 10). The increase in temperature from 20 to 30° C did not show significant change in root colonisation (Fig. 11). But further increase in temperature caused significant reduction in the per cent root colonisation and the least colonisation was noticed in 50° C.

4.8.2. Effect of soil moisture on VAM spore population and root colonisation in sorghum.

The results on the analysis of data on soil moisture influencing VAM colonisation and spore population in soil are given in Figs. 12 and 13. The soil with moisture contents 10, 20, 30 and 40 per cent did not show much variation in the population of viable spores and a significant reduction was observed with further increase in moisture levels. VAM colonisation of sorghum roots was also reduced significantly in soil with 50, 60, 70 and 80 per cent moisture levels.

4.8.3. Effect of soil organic matter on VAM colonisation in sorghum and spore population in soil

None of the organic matter amended to soil showed any influence on the spore population. Sorghum plants grown in soils treated with cow dung and leaf litter showed a significant increase in root colonisation when compared to control. Application of compost did not show any change in root colonisation (Table 15).

Fig. 10 **Effect of temperature on VAM spore count in soil**

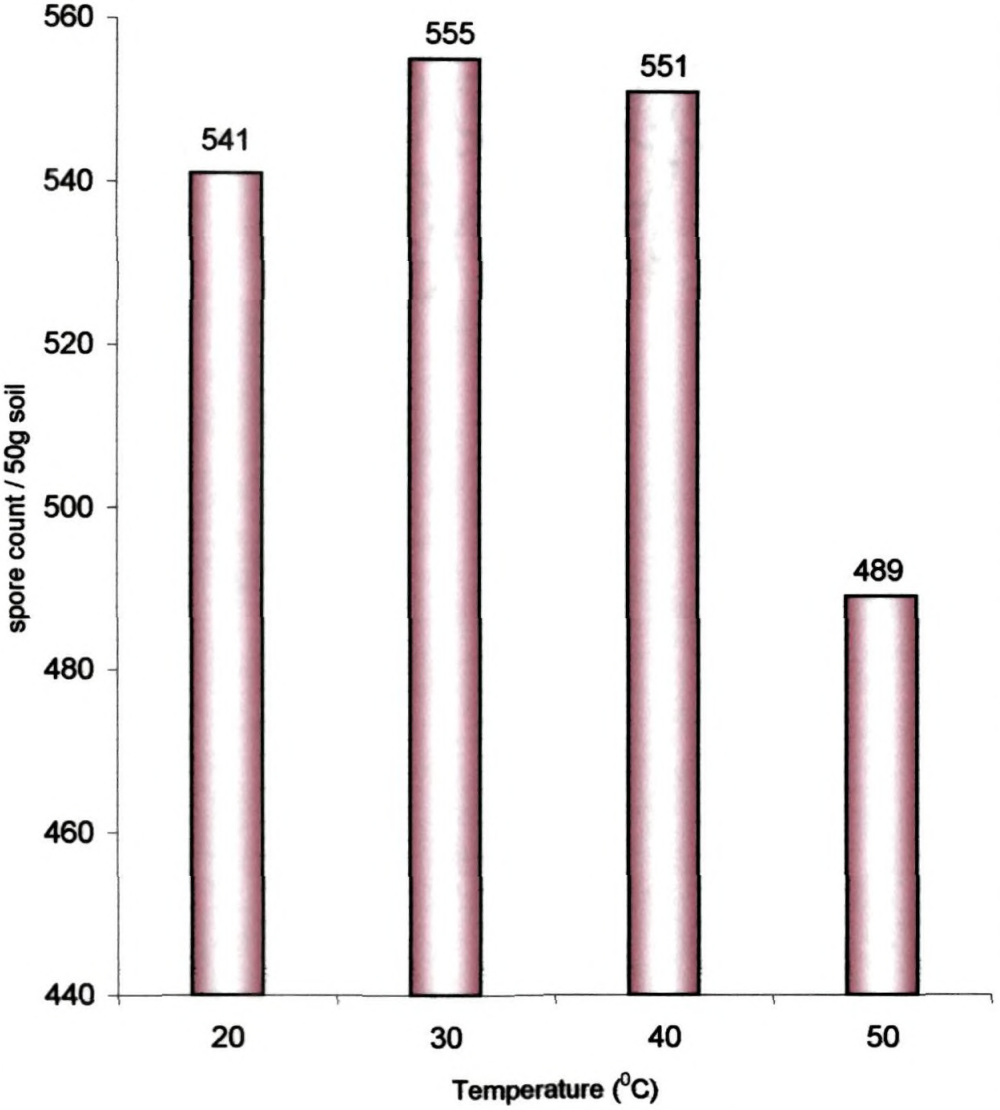


Fig. 11. **Effect of temperature on VAM colonisation in roots of *Hevea***

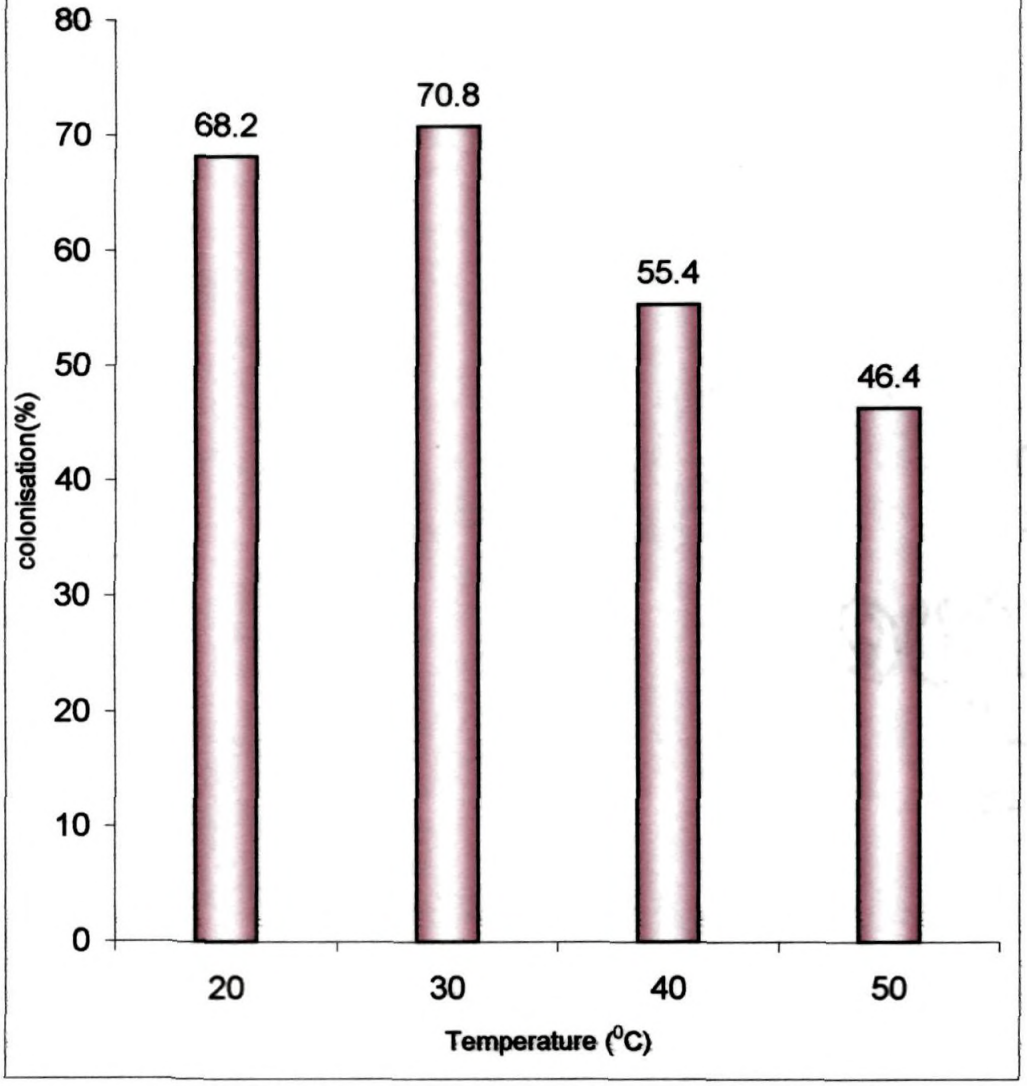


Fig. 12. **Effect of moisture on root colonisation by VAM fungi in sorghum**

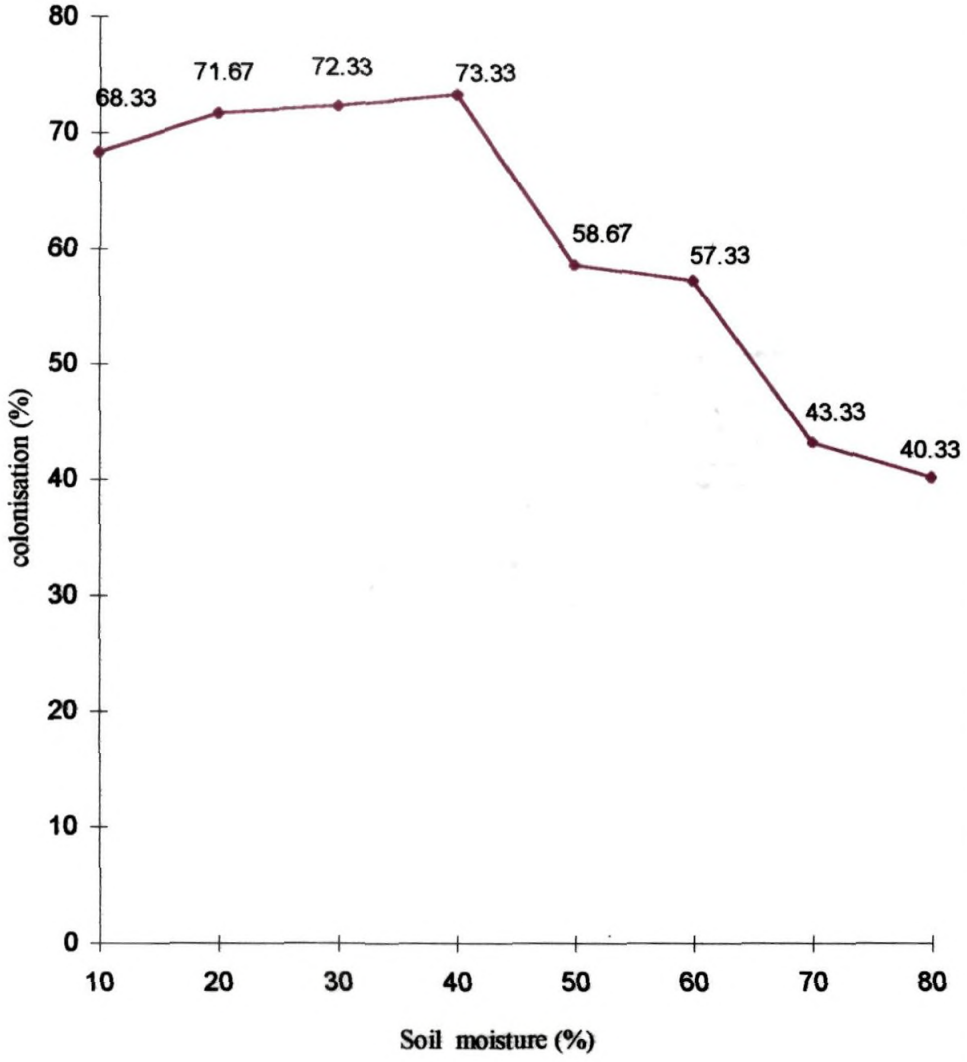


Fig.13. **Effect of moisture on VAM spore count in soil**

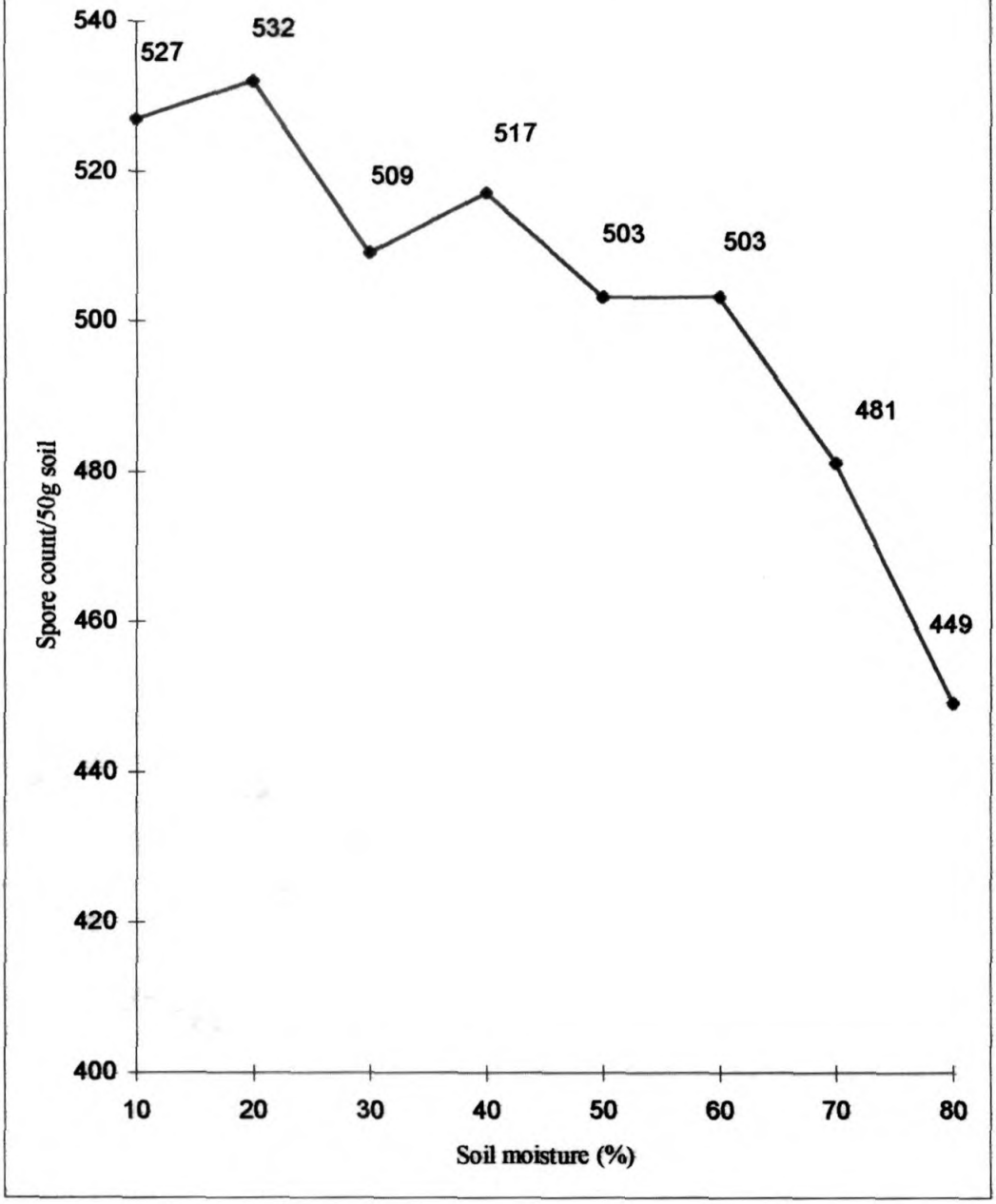


Table 15

Effect of organic matter on VAM colonisation and spore population in sorghum

Organic matter	Infection (%)*	Spore count/50 g soil*
Cow dung	70.40	580.00
Leaf litter	74.40	566.00
Compost	68.20	569.00
Control	67.60	559.00
CD (P = 0.05)	2.03	26.62

* Mean of 5 observations

4.9. Effect of cultural operations on VAM

4.9.1. Effect of fertilizers on per cent root infection and spore population in *Hevea*

Results of studies on the role of synthetic fertilizers on VAM spore population and *Hevea* root colonisation are given in Figs. 14 and 15. Both VAM spore count in soil and per cent root colonisation were found to increase upon the incorporation of synthetic fertilizer at 50 per cent recommended level. Beyond that level significant reduction was noticed with spore count and root colonisation and the reduction was considerably higher at double the recommended dose of fertilizer application.

4.9.2. Effect of plant protection chemicals on VAM

4.9.2.1. Effect of fungicides on VAM root colonisation in *Hevea* roots and spore population in soil

Ten different systemic and non-systemic fungicides were tested for their effect on root colonisation in *Hevea* and spore population in soil and the results are presented in Table 16. The control soil registered 50.33 per cent *Hevea* root colonisation by VAM fungi. Application of wettable sulphur at all the three levels

Fig.14. **Effect of fertilizer on VAM Spore count in soil**

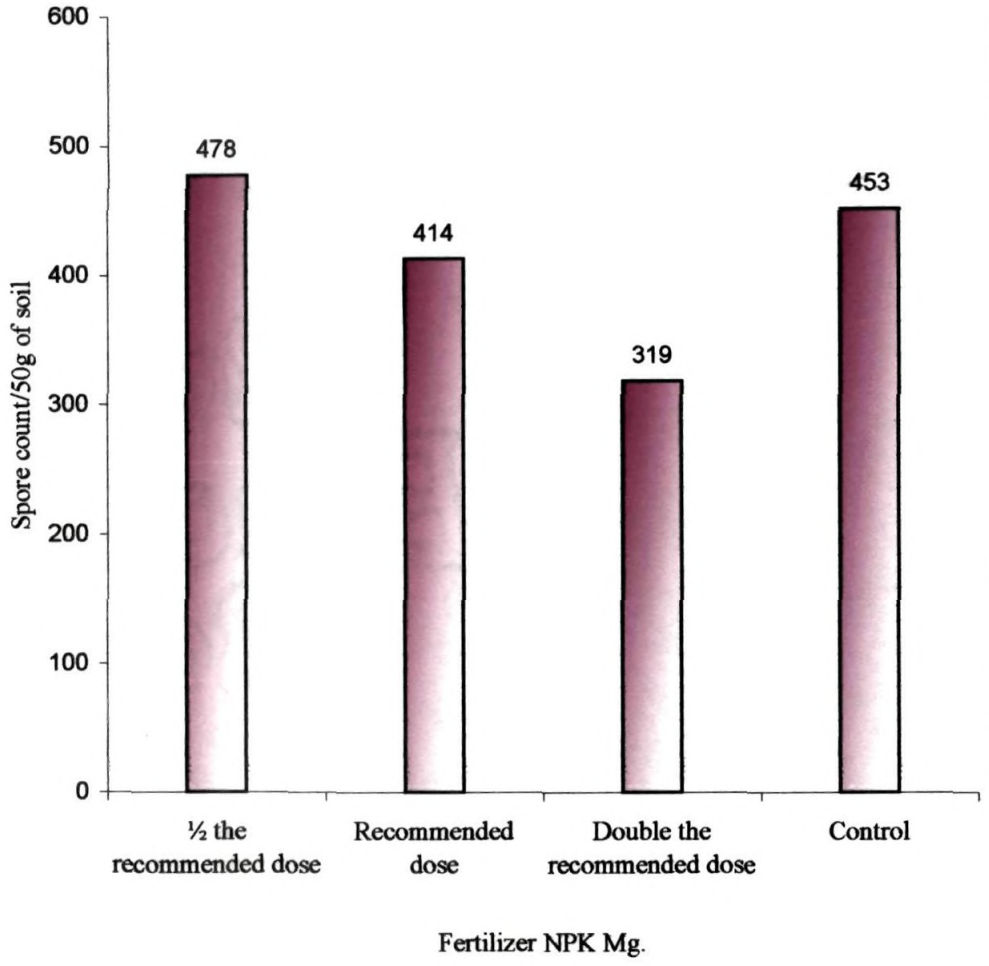
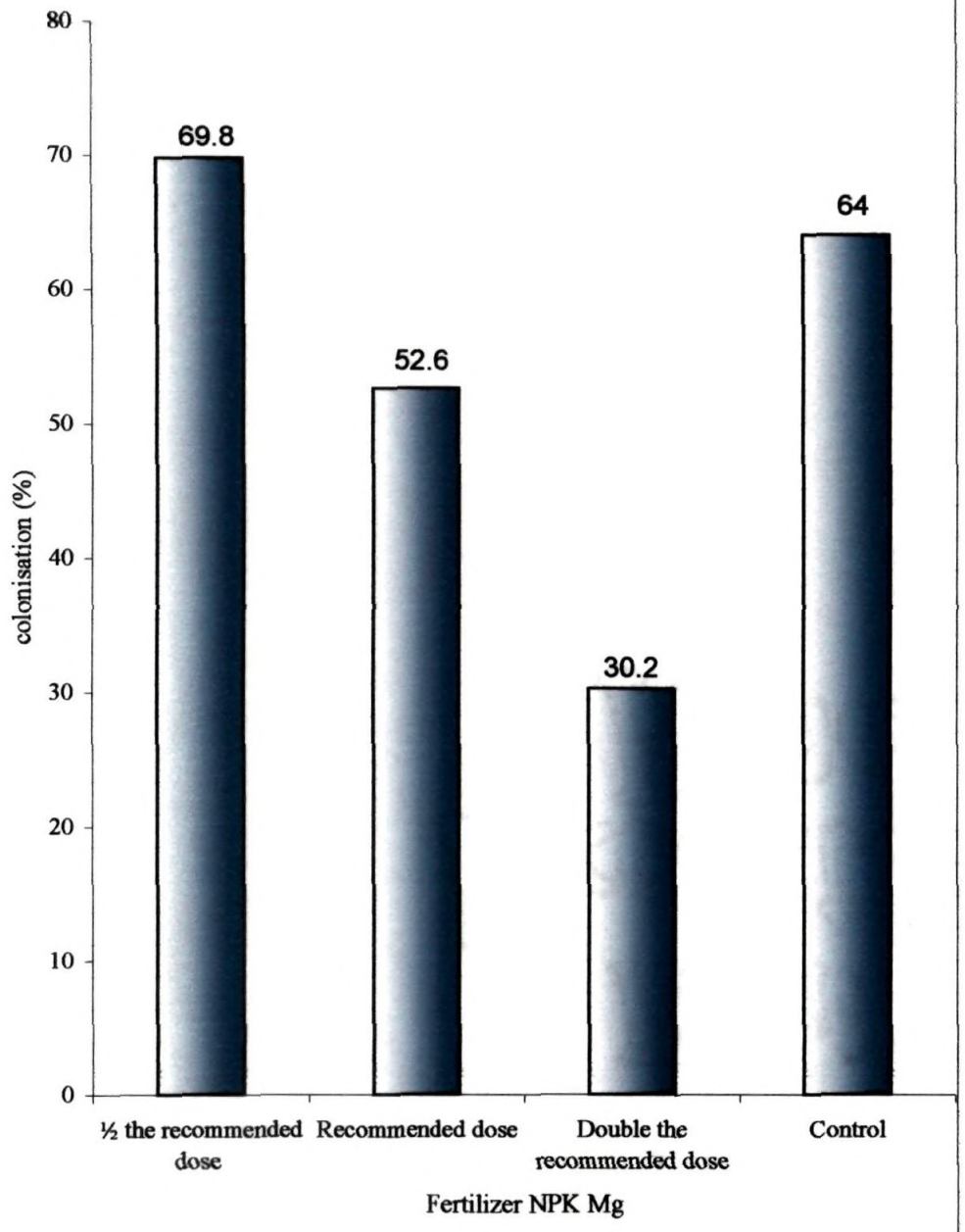


Fig. 15. **Effect of fertilizer on VAM colonisation in *Hevea***



did not influence root colonization by VAM. Bordeaux mixture at 50 ppm and 100 ppm levels did not affect VAM root colonisation whereas at 200 ppm a significant reduction was noticed. Hexaconazole and mancozeb at recommended and double the recommended doses resulted in significant reduction of VAM root colonization in *Hevea*. But at half the recommended dose, colonisation was on par with control. Thiram, tridemorph, carbendazim and benomyl at all the three levels reduced root colonisation by VAM in *Hevea.* Metalaxyl at 100 and 200 ppm level increased root colonisation while 400 ppm dose did not have any effect. All the three levels of phosphorus acid application enhanced the per cent root colonisation.

The application of mancozeb and tridemorph at double the recommended level and carbendazim at recommended and double the recommended doses significantly reduced the population of viable spores in soil, while the other fungicides did not show any adverse effect on VAM spore count.

4.9.2.2. Effect of insecticides on root colonisation in *Hevea* roots and spore population in soil

The results of the experiment on the effect of insecticides on VAM colonisation in *Hevea* roots and spore population in soil are given in Figs. 16 and 17. The insecticides under test differed in their effect on VAM colonisation considerably. Malathion, fenvalerate and endosulphan irrespective of their concentrations significantly reduced the VAM colonisation and living spore population in *Hevea.*

Table 16

Effect of application of fungicides on VAM colonisation and spore population in *Hevea*

Fungicide	Dose (ppm)	Infection (%)	Spore count/50g soil
Bordeux mixture (1%)	50	50.00	432.00
	100	48.33	423.00
	200	44.67	444.00
Mancozeb	250	48.33	426.00
	500	40.33	427.00
	1000	36.00	391.00
Thiram	250	45.67	426.00
	500	39.67	437.00
	1000	34.33	427.00
Tridemorph	100	35.33	425.00
	200	33.67	441.00
	400	31.00	377.00
Phosphorus acid	500	54.33	436.00
	1000	58.00	425.00
	2000	56.33	438.00
Wettable sulphur	500	51.67	423.00
	1000	50.33	434.00
	2000	49.33	425.00
Metalaxyl	100	53.00	418.00
	200	55.00	427.00
	400	50.33	418.00
Carbendazim	100	40.33	438.00
	200	34.67	381.00
	400	33.67	384.00
Benomyl	100	47.00	434.00
	200	42.00	400.00
	400	37.33	407.00
Hexaconazole	100	48.67	430.00
	200	40.00	435.00
	400	35.00	434.00
Control		50.33	435.00
CD (P = 0.05)		02.32	028.96

Chlorpyriphos and monocrotophos at 50 and 100 ppm levels did not affect root colonisation and spore population. On the contrary, considerable reduction in root colonisation was recorded at 200 ppm; no reduction was noticed in the case of spore count in soil. An adverse effect of carbaryl and carbofuran on root colonisation was noticed at 100 and 200 ppm levels while 50 ppm concentration did not affect colonisation by VAM. Carbaryl at 200 ppm significantly reduced the number of viable spores in soil.

4.9.2.3. Effect of weedicides on VAM colonisation in *Hevea* roots and spore population in soil

Results of analysis of data of weedicides influence on VAM colonisation in *Hevea* and spore population in soil (Table 17) indicated that none of the three popular weedicides at the three levels used in rubber plantations recorded any adverse effect on VAM root colonisation and spore count in *Hevea*. Whereas at the recommended dose, diuron significantly increased root colonisation to 64 per cent as against 52.57 per cent in the treatment receiving no weedicides and recorded 25 and 27 per cent increase in root colonisation from half the recommended and double the recommended concentrations respectively.

Fig. 16. **Effect of insecticides on root colonisation by VAM fungi in *Hevea***

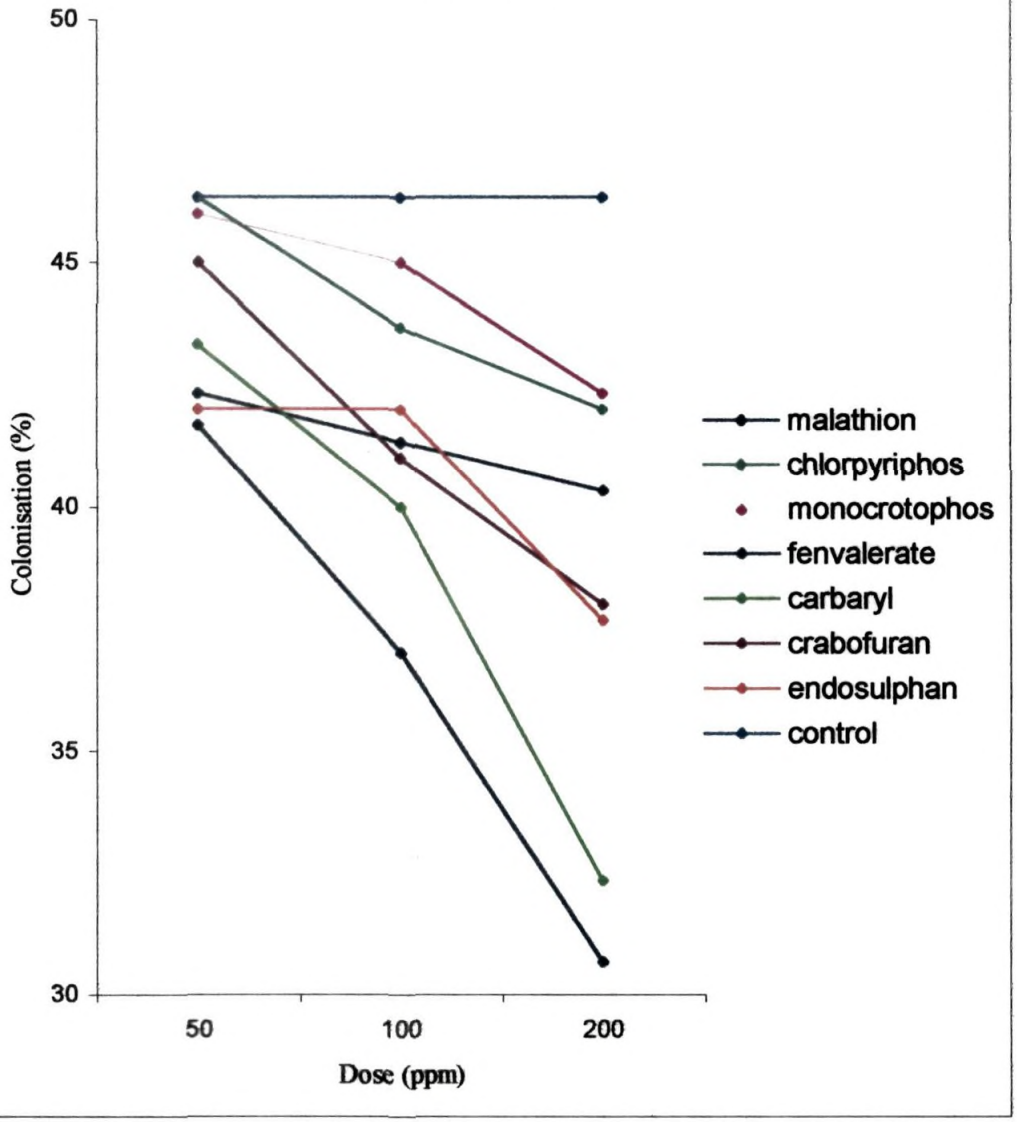


Fig. 17. **Effect of insecticides on VAM spore count in soil**

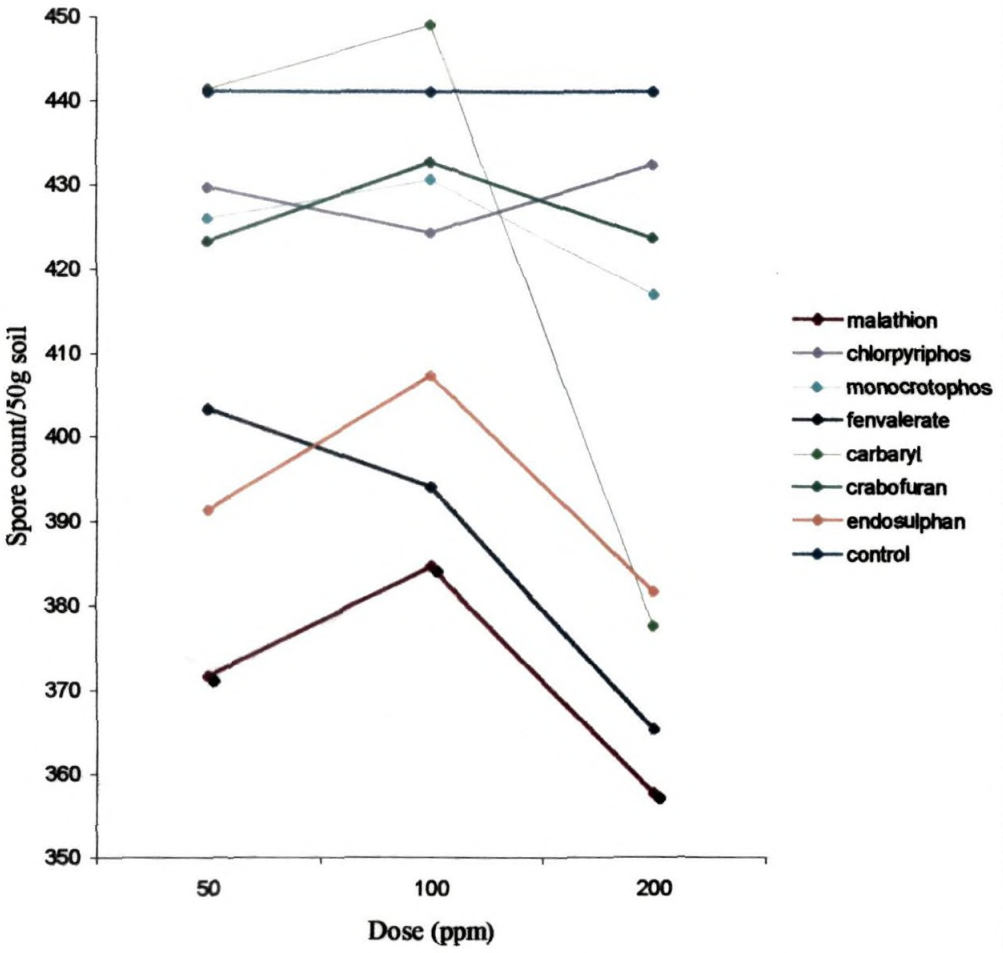


Table 17

Effect of Weedicides on VAM colonisation and spore population in *Hevea*

Weedicide	Dose (ppm)	Infection (%)	Spore count/ 50 g soil
Diuron	175	51.33	442.00
	350	64.00	463.00
	700	50.33	448.00
Paraquat	150	49.33	447.00
	300	49.67	440.00
	600	50.33	433.00
Glyphosate	150	51.00	454.00
	300	54.00	449.00
	600	49.00	439.00
Control		52.67	451.00
CD (P = 0.05)		4.60	36.68

4.9.3. Effect of solarisation on VAM root colonisation and spore population in sorghum

The results of the soil solarisation tests conducted are presented in Table 18. An increase in soil temperature was observed in tarped areas. At 10 cm depth the soil temperature exhibited an increase of 7.2°C and at 20 cm depth 5.5°C increase was noticed. Viable spore count in tarped soil was less than that of nontarped soil. Roots of *Hevea* plants grown in tarped soil showed colonisation to a significantly lesser extent than nontarped soil.

4.10. Influence of various agents on the dissemination of VAM spores

Studies on the dissemination of VAM spores by different biotic agents (Table 19) showed the presence of VAM spores in all the different sources studied. Maximum spore count was observed in earthworm castings followed by soil from termitaries, wasp nests and ant mounds. There was a significant increase in spore count in all these sources of biotic agents, except ant mound, when compared with the field soil. The spore count in ant mound was found same as that of field soil. All the four different genera of VAM fungi were recorded in ant mounds and wasp nests. Earthworm castings did not harbour *Sclerocystis* spp. whereas termitaries were lacking *Gigaspora* spp.

The run-off water from fields contained VAM spores and the spore count was significantly less in samples collected after two hours of onset of rain. The soil collected from the same area just at the time of onset of rain contained more spores than the soil collected after two hours of raining (Table 20). Vaseline coated slides exposed to wind showed the presence of VAM spores, which were very few in number. Out of the 20 spore traps with vaseline coated glass slides, only two slides were found with two spores and three slides with one spore each. Fifteen slides were free from any spores of VAM.

Table 18

Effect of solarisation on VAM spore population and root colonisation

Treatment	Temperature (^{O}C)		Spore count/50 g soil	Root colonisation (%)
	10 cm depth	20 cm depth		
Tarped	47.4	40.0	352.00	45.67
Non-tarped	40.2	34.5	403.00	58.00
CD (P=0.05)			25.85	4.49

Table 19

VAM spore population in different Dissemination agencies

Agency	Spore count/ g soil*	Frequently occurring VAM genera
Earthworm cast	23.00	*Glomus, Acaulospora, Gigaspora*
Termitary	15.00	*Glomus, Acaulospora, Sclerocystis*
Wasp nest	12.00	*Glomus, Acaulospora, Gigaspora, Sclerocystis*
Ant mound	11.00	*Glomus, Acaulospora, Gigaspora, Sclerocystis*
Field soil	11.00	*Glomus, Acaulospora, Gigaspora, Sclerocystis*
CD (P= 0.05)	0.62	

Table 20

VAM spore count in run off water from rubber field at different intervals

Agency	Spore count*			
	Flowing water /litre	SD	Soil/50 g	SD
Just after starting rain	26.00	2.26	438.00	17.46
Two hours after starting rain	8.00	3.55	401.00	14.33

* Mean of 25 observations

5. DISCUSSION

5.1. Distribution of VAM in rubber growing soils

Natural rubber (*Hevea brasiliensis* Muell. Arg.) is an introduced plantation crop, which replaced the conventional cultivated crops and some forests in Kerala. Intensive cultivation of this crop led to disturbances of both macro and micro flora and fauna. Unlike other microorganisms VAM fungi requires the macrosymbiont, the higher plants for their growth and multiplication.

The occurrence and ecological importance of VAM fungi have been extensively studied in certain plant communities such as tropical rain forests (Mosse, 1973a; Diem *et al.*, 1981) and sand dunes (Koske *et al.*, 1975). But information on the ecology of fungal symbiont themselves is lacking. In the present study considerable variation with respect to VAM population in rubber growing soils of Kerala was recorded. The species of VAM fungi also varies to a greater extent. It is well known that microbial population in a given soil is subjected to the influence of edaphic and climatic conditions as well as agro- management practices. Bethlenfalvay *et al.* (1984) studied the distribution pattern of the VAM flora in different localities, and they attributed the site preference by VAM fungal species to the interaction of factors pertaining to the host plants and the edaphic and climatic conditions.

5.2. Influence of location and season on VAM spore population

The present study clearly brought out that the seasons have an impact on the spore population by VA mycorrhizal fungi. VAM spore population in general was maximum in summer in all the locations studied, which decreased in subsequent seasons. Under Kerala conditions, summer months (January to March) with reduced relative humidity (52-58 per cent) and 34-36°C soil temperature seem to favour sporulation by VA mycorrhizal fungi. Kianmehar (1981) working in Iran, another tropical country, observed that endomycorrhizal spore population was high in January and low in July supporting the present study. Hayman (1974) and Furlan and Fortin (1977) have observed similar enhanced spore count in summer and suggested that higher temperature and higher light intensity with longer day length might be responsible for the increased spore count. According to Bergan and Koske (1984) the number of spores in soil and the infection in roots were more in summer than in rainy seasons and they could not explain the reason for maximum root colonisation in summer. However in the present study least root colonisation was recorded in summer. Daniels and Bloom (1983) found high sporulation in summer, which declined during winter and early spring. They explained that soil moisture level (5-8 per cent) influenced mycorrhizal activity. Soil moisture above 6 percent reduced both number of spores and root colonisation. The number of spores decreased during rainy season due to maximum precipitation and water logging (Khan, 1974). According to Slankis (1974) excess soil water caused oxygen deficiency, which limited the

development of symbiotic fungi. Lugo and Cabello (2002) showed highest spore density in dry seasons coincident with the lack of flowering and fruiting of higher plants.

Spores of *Glomus* species were most common and abundant in the study sites. This is followed by *Acaulospora* spp. and *Sclerocysts* spp. Population of *Gigaspora* spp. was very low in all the soils and throughout the year. Rani and Mukerji (1987) while studying the species distribution of VAM fungi found that Indian soils are rich in *Glomus* spp. Kendrick and Berch (1985) reported that species of *Glomus* is linked to acid soils, in conformity with the present study and *Gigaspora* spp. with sand dune soils. Jha *et al.* (1988) also observed that *Glomus* spp. was most abundant in tropical conditions. According to Kannan and Lekshminarasimhan (1988) species of *Glomus* was common in the costal area, Point Calimire. In the salty primary costal dunes (Nicolson and Johntson, 1979) and in arid rangeland (Allen and Boosalis, 1983) also *G. fasciculatum* was the common species. Joseph (1997) studied VAM species distribution in acid soils of South India and found maximum spore count of *Glomus* spp. Mosse (1981) reported lesser spore number of *Acaulospora* in acid soils.

There was a distinct regional preference in species abundance. While spores of *Glomus* spp. were higher in Ranni, Palappilly and Palakkad, *Acaulospora* spp. were more in Kottayam and *Sclerocystis* spp. in Kozhikode. The soils of Nedumangad,

Kottayam, Muvattupuzha and Manjeri harbour more of *Gigaspora* spp. spores. Such location wise predominance of species has been reported earlier by Kumar (2002).

5.3. Influence of location and season on root colonisation by VAM in *Hevea*

Root colonisation is the pre requisite for the successful symbiosis of higher plants and VAM. In the present study root colonisation was observed in all the locations without much variation. There is no correlation of root colonisation with VAM spore population in soil. Mosse and Bowen (1968) studied soils in Australia and reported that spore populations of VAM fungi in natural ecosystems are not regularly correlated with the abundance of VAM root infection. This confirms the findings of the present study. Root colonisation was the lowest in summer in all the 21 soils and started increasing from pre monsoon to monsoon season.

After summer season VAM spore count was reduced in pre monsoon and monsoon seasons whereas there was an increase in root colonisation. New *Hevea* roots develop after the onset of monsoon and thereby increase the percentage of root colonisation. Reduced spore count after summer is due to increased root colonisation and washing of spores by rain as evidenced from the results of the present study. The inverse relationship between the spore count and root colonisation was studied extensively and many authors have emphasized that spores are not important for maintaining infection when colonised roots are present especially in the case of

natural plant communities (Sparling and Tinker, 1978). Mohankumar and Mahadevan (1987) studied VAM spore count and root colonisation in forest plants and could not find any clear-cut interaction between spore count and root colonisation.

The lowest root colonisation observed in Punalur, Palakkad and Kanjanhad during the summer season may be related to the relatively drier weather in these areas, the former two being exposed to dry winds from the peninsular plains across the Western Ghats and the latter due to weak North East monsoon. The very high root colonisation observed in Mundakayam during monsoon season also points to the fact that optimum soil moisture may be a decisive factor in VAM root colonisation of *Hevea*. Admittedly it is not easy to derive a single factor that influence the spore count and root colonisation.

5.4. Effect of soil pH and organic carbon on VAM spore population and root colonisation in *Hevea*

A number of factors are considered to affect the population of VAM fungi in soil (Kruckelmann, 1975; Tarja Lehto, 1994) of which soil pH is believed to be the most important factor (Bethlenfalvay, 1992). Kruckelmann (1975) also found that VAM spore population was influenced more by soil pH than by any other factors as observed in the present study. A positive correlation was observed with respect to soil pH and VAM spore count. Palakkad soil having a pH of 5.8 contained maximum count of VAM spores while Kottayam soil the pH of which is 4.5 had the minimum

spore count. The results of the present study also is in conformity with the findings of Joseph (1997) who studied VAM population in rubber growing soils and their impact on leguminous cover crops of rubber plantations.

Next to soil pH, organic matter content of soil influence VAM population. Sheikh *et al.*, (1975) noticed spores and spore like bodies in organic particles, which were sources of viable inocula of *Endogone*. According to them endogonaceous spore population seems to be closely correlated with the level of organic matter content in plantation soil. Maximum spore numbers were recovered from soil containing 1 – 2 per cent organic matter and spores were sparse in soils with below 0.5 per cent organic matter. Soil organic carbon in the present study varied from 1.18 per cent to 2.50 per cent and has no correlation to VAM population with organic carbon of rubber growing soils in all the four seasons. Irrespective of soil organic carbon normal VAM population was seen in rubber growing soils.

5.5. VAM colonisation and spore population as influenced by duration and cycle of *Hevea* cultivation

Hevea plantations in India are hundred years old and they are in different periods and cycles of cultivation. Soil under rubber is much disturbed and subject to application of synthetic fertilizers and pesticides continuously. The study of enumeration of VAM spores and root colonisation revealed that the total spore count

recorded was maximum in soil under 5 years of *Hevea* cultivation and there after progressively decreased up to 30 years as well as third cycle of cultivation. In immature plantations, the cover crops and weed plants may contribute VAM fungal population. Disturbances associated with cultivation led to reduction in VAM spore population and root colonisation (Douds *et al.*, 1995). NPKMg mixture application is a common agro-management practice in natural rubber cultivation and led to reduction in VAM. Adverse effect of synthetic fertilizer application was well documented (Hayman, 1975). Besides fertilizers, fungicides, pesticides and weedicides may have suppressed root colonisation and VAM spore count in soil as observed by Ocampo and Hayman (1980) and Kruckelmann (1975). On an average 4 kg of copper sprayed as copper oxychloride is reaching soil every year and gradual accumulation contributed to the reduced spore count and root colonisation in *Hevea* as suggested by Mosse (1981) and Gilden and Tinker (1983). Hepper and Smith (1976) also suggested that pesticides containing heavy metals like copper and the presence of such chemicals in soil may be responsible for poor spore count and root colonisation of VAM fungi. Yet another possibility of reduced spore count and root colonisation could be due to reduction in soil organic carbon as suggested by Johnson and Michelini (1974).

5.6. Vertical distribution of VAM spores in soil

VAM fungi are distributed in top soils of all rubber growing areas with varying numbers. Soil depth is an important factor, which governs the degree of distribution of microorganisms. In the present study there is considerable reduction in the VAM spore count as the depth increased. Over 78 per cent of total spores were present in the top 15 cm soil. Readhead (1977) also made similar observation. Spores are normally not found in depths beyond the normal root range of plants (Mosse *et al.*, 1981). *Hevea* being surface feeder the VAM spore count is maximum in the surface soil. In a study conducted in SriLankan soil, Hafeel and Gunatilleke (1988) observed a decreasing VAM population with the increase in soil depth. Another reason assigned to reduced VAM spores in deeper layers is lack of oxygen in the deeper layers of soil (Gerdemann, 1968). Since mycorrhizal spores are aerobic they may perish at deeper layers of soil. Secondly soil layer might often be a barrier against the penetration of spores, which could account for their reduced number. Above all soil organic matter is generally more in topsoil and favoured the augmented spore count as organic carbon encourages VAM spores (Philip *et al.*, 1996).

5.7. Comparative study on VAM population in *Hevea* plantation and nearby forest

Plant communities and soil microorganisms especially VAM exist in equilibrium. Any disturbance lead to change in species and population of

microorganisms. *Hevea* cultivation is mainly taken up in cleared forest lands. Naturally a change in VAM population is expected. In all the four locations studied, forest soils contained more population of VAM spores than plantation soils, especially species of *Glomus*. *Gigaspora* species though less in population did not show significant variation in forest and *Hevea* soils. Other species changes are not in definite pattern.

Prolonged monoculture (Kruckelmann, 1975), fertilizer application, tillage, intercropping, crop rotation, fumigation, irrigation, use of pesticides, breeding for disease resistance *etc*. reduced mycorrhizal populations (Hayman, 1982b; Loree and Williams, 1984). Miller (1987) and Reeves *et al.* (1979) who observed reduced VAM in cultivated land ascribed it to extensive soil disturbance and extraneous inputs like fertilizer and pesticides. They also claimed the influence of crop under cultivation. Jha *et al.* (1988) reported better development of mycorrhizae at less degraded forest site because of the presence of high organic carbon in the soil.

It is widely accepted that maximum root colonisation and sporulation occur in soil of low fertilizer application as seen in forestland. Both phosphorus (Green *et al.*, 1976; Daft and Nicolson, 1969; Ross, 1971; Khan, 1972) and nitrogen (Porter and Beute, 1972 and Readhead, 1975) significantly reduce VAM.

5.8. Effect of environmental factors on VAM spore count and root colonisation

5.8.1. Temperature

Temperature influence VAM spore population and root colonisation directly and indirectly. In the present study, temperature did not show significant influence in the spore count up to 40°C and beyond that a significant reduction was noticed. Soil sample used to study the influence of temperature is same and obviously no variation in spore count was noticed. Temperature treatments with 40°C and 50°C significantly reduced VAM root colonisation. Daniels and Trappe (1980) observed that optimum temperature for the germination of *Glomus* spp. and *Acaulospora* spp. spores is 20-25°C. Furlan and Fortin (1977) found that most rapid infection and greatest spore production by *Gigaspora calospora* occurred at 26°C in onion roots. Studies from temperate countries have shown that higher temperature increased colonisation and sporulation (Hayman, 1974; Daniels and Bloom, 1984). But it does not seem to hold good in tropics as evidenced by the present study. Studying the effect of temperature on VAM establishment Schenck and Schroder (1974) observed that root colonisation was greatest between 28 and 34°C. Temperature above 40°C inhibited germination of *Acaulospora laevis*, *Glomus caledoninus* and *Glomus monosporum* (Tommerup and Kidby, 1980). All these reports confirm the results obtained in this study.

5.8.2. Moisture

Among many environmental factors, moisture plays an important role for survival, spore formation, germination of spore and root colonisation by VAM fungi. Soil moisture up to 40 per cent did not cause any change in VAM spore count as well as root colonisation in test plants. Further increase in moisture is detrimental to both VAM spores and root colonisation. The influence of soil water potential on VAM fungal spores has been studied by Daniels and Trappe (1980) using *Glomus epigaeus* added to silt loam soil of varied moisture content and by Koske (1981) using *Gigaspora gigantea* spores. *Glomus epigaeus* spores germinated best at moisture content between field capacity and soil saturation and the germination of *Gigaspora gigantea* spores was reduced by low water potential. *Glomus* spp. spores are more in soils of Kerala and hence moisture level up to 40 per cent favoured survival of spores and root colonisation. The increased root colonisation during premonsoon and monsoon season is mainly due to optimum moisture in soil. Reduced spore count and root colonisation at high moisture potential might be due to lack of oxygen. This finding is in conformity with that of Nirmala *et al.* (1988) who observed low spore count and root colonisation under flooded condition. Khan (1974) also found that VAM spores were rare in waterlogged soils. According to Slankis (1974) excess soil water caused oxygen deficiency, which limited the development of VAM fungi.

5.8.3. Organic matter

Studies on the effect of three types of organic matter i.e. cow dung, leaf litter and compost on VAM spore population and root colonisation reveal no change of VAM spore count whereas cow dung and leaf litter augmented root colonisation. Soil organic matter will undoubtedly affect incidence of mycorrhizae indirectly through its own structure, water holding capacity, nutrient mineralisation *etc.* (Mosse *et al.*, 1982). Favorable effect of soil organic matter on VA mycorrhiza has been reported earlier by Sheikh *et al.* (1975).

Out of three organic carbon sources only cow dung and leaf litter showed increase in root colonisation over control. This is probably because compost is rich in nutrients compared to cow dung and leaf litter (Sainz *et al.*, 1998) and the soil used in this study is of low fertility status. This is confirming the results of Guttay (1982) who reported least VAM colonisation in soil amended with maize compost. Leaf litter amendment showed more root colonisation than compost amendments. This might be due to narrow C: N ratio of leaf litter, which in turn improved the soil structure and thus facilitating VAM growth (Mariakulandai and Manickam, 1975).

5.9. Cultural practices on VAM spore population and root colonisation

5.9.1. Fertilizer

Application of synthetic fertilizer is considered to play a major role in influencing both the VAM spore count and root colonisation (Daniels, 1984). In the present study addition of 50 per cent of the recommended dose of NPKMg mixture recorded an increase in VAM spore count and root colonisation. Recommended levels of fertilizer application significantly reduced VAM spore count and root colonisation. Doubling the fertilizer dose lead to further reduction. One of the major functions of VAM in plants is the absorption of soil P. Colonisation of plant roots by VAM fungi has been studied in relation to P nutrition (Bagyaraj and Sreeramulu, 1982). Such studies have clearly indicated that there is an inverse relation between root colonisation and P content in soil (Graham *et al.*, 1981; Schwab *et al*, 1983). Baylis (1967), Azcon *et al.* (1978) and Miranda *et al.* (1989) have in fact shown that high P levels decreased and finally eliminated mycorrhizal infection from soil, which are in conformity with the present findings.

There are several reasons for the negative effect of P on VAM spore count and root colonisation. High soil P levels are known to result in root P concentration that may inhibit mycorrhizae formation and reduce external hyphae of VAM in soil

(Sanders, 1975). Menge *et al.* (1981) reported that it is not soil P per cent that regulate mycorrhizal colonisation, but rather the amount of P absorbed by the host plant. Jasper *et al.* (1979) suggested that P influence VAM colonisation by affecting concentration of root carbohydrate or the amount of root exudates. In this study fertilizers at high levels are known to inhibit mycorrhizal fungi (Porter and Beute, 1972 and Strzemska, 1975). Kruckelman (1975) who obtained similar results suggested that this might be due to decreased plant dependence on mycorrhiza when soil is rich in nutrients. Porter *et al.* (1978) observed that long-term application of super phosphate resulted in a population of VAM endophyte that was little affected by subsequent addition of phosphorus. It appeared that P tolerant strains had built up on long-term super phosphate application. It is possible that P tolerant strain might develop in rubber growing soils. It will be interesting to monitor this in future. There is considerable information on the negative effects of nitrogen fertilizer on mycorrhizae. For example in the heavy clay loam soil in the Rothamstead farm monthly sample from wheat plot showed nitrogen markedly reduced both VAM infection and spore number (Hayman, 1970). It is widely accepted that maximum root colonisation and sporulation occur in soils of low fertility. Upon the addition of NPK both phosphorus (Khan, 1972 and Tinker, 1978) and nitrogen (Hayman, 1975) may significantly reduce mycorrhizal root colonisation if present at high levels and a delicate balance between these two elements appear to exist (Hayman, 1975; Strzemska, 1975). The present study indicates that NPK fertilizer application at higher level is detrimental to VAM spores and their root colonisation.

5.9.2. Fungicides

It is well known that fungicides have profound influence on target and non-target fungi. Some first generation fungicides like sulphur and Bordeaux mixture undergo changes before exerting fungicidal activity. Sulphur is to be converted to SO_2 and copper in the Bordeaux mixture is to be reduced to nascent copper for inhibiting fungi. Their action is mainly on the enzyme biosynthesis of the fungi. All the more sulphur is specific for obligate pathogen causing powdery mildew (Nene, 1971) and may not affect VAM colonisation. However, Bordeaux mixture at 200 ppm inhibited VAM colonisation indicating that heavy metals at high dosages may be detrimental (Hepper and Smith, 1976).

Systemic fungicides have different action at varying levels on VAM colonisation. Systemic fungicide action on fungi depends on the nature and concentration. They enter into system of plants and then only act on fungi and hence these fungicides differ in their action on VAM colonisation. The fungicidal effects are due to the interference of these compounds with fungal microtubule formation (Davidse, 1986) and not to phytotoxic effects (Kahiluoto and Vestberg, 2000). Metalaxyl at lower levels encouraged VAM infection while at 400 ppm did not have any such effect. Likewise phosphorus acid at all the levels encouraged VAM colonisation indicating that they are safe to use in rubber plantations. The probable

effect of these fungicides may be preferential exclusion of some other fungi in the rhizosphere thus eliminating competition for the ecological niche.

Fungicides like mancozeb, tridemorph and carbendazim only have adverse effect on VAM spores while other fungicides have no significant adverse effect. Vyas and Vyas (1995) also observed similar action. The difference could be due to the difference in the mode of action of fungicides included in the study.

5.9.3. Insecticides

A variety of pesticides are used in modern agriculture and they have profound influence on plants, insects and microorganisms including VAM fungi (Trappe *et al.*, 1984). Most of the insecticides studied reduced VAM spores and inhibited root colonisation at different levels of application. Chlorpyriphos and monocrotophos did not affect spore population in soil. The adverse effect of carbofuran at recommended and higher doses was reported by Backman and Clark (1977) supporting the present study. The adverse effect of carbaryl and endosulphan also was reported (Parvathi, *et al.* 1985). Vyas and Vyas (1995) observed similar results and they concluded that the adverse effect of insecticides is due to their chemical nature and cautioned approach is necessary on the selection of the insecticides.

5.9.4. Weedicides

Weedicides are chemicals that affect the physiological activity of higher plants leading to their death. Almost all the weedicides are systemic and have little or no effect on fungi like VAM (Dodd and Jeffris, 1989) though a very few reports are available on their favorable effect on VAM (Trappe *et al.*, 1984). Both pre and post-emergence weedicides commonly used in rubber plantation did not have any deleterious effect on VAM. On the other hand diuron the popular pre-emergence weedicide increased root colonisation. Contradictory reports are available on the effects of diuron. Smith *et al.* (1981) observed an increase in VAM population in soil whereas Dodd and Jeffris (1989) and Nemec and Tucker (1983) found no effect either on sporulation or root colonisation. The study on weedicide effect on VAM is very limited and no reason is assigned to the effect of weedicides on VAM spore count as well as root colonisation.

5.9.5. Effect of Solarisation

Mycorrhizal fungi were present in tarped soils and were able to colonise sorghum plant roots, but to a lesser extent than the spores from nontarped soils. Pullaman *et al.* (1981) reported similar findings, which confirms the present study. The thermal death point of *G. fasciculatus* is reported to be 10 min at 51.5 °C (Menge

et al., 1979). This higher thermal tolerance helped the mycorrhizal fungi to survive in the solarised soil.

5.10. Dissemination of VAM

5.10.1. Dissemination by biotic agents

5.10.1. 1.Earthworms

Early investigations on the microbial constitution in the intestinal tracts of earthworms failed to detect the presence of VAM fungi, because of the procedural deficiency (Parle, 1963 and Thornton, 1970). Mc Ilveen and Cole (1976) observed the presence of VAM spores in the intestinal constituents and cast of earthworms for the first time. Their study also brought out that endogonaceous spores remain viable following earthworm ingestion since earthworm casts gave rise to typical VAM colonisation.

In the present study, the air-dried earthworm (*Lumbricus* sp.) cast on examination yielded maximum number of mycorrhizal propagules *i.e.* 19 per g. Harinikumar and Bagyaraj (1988) observed similar results. *Hevea* field soil is rich in *Glomus* spp., and earthworm castings also exhibited more of *Glomus* spp. It is thus

understandable that when one species of mycorrhizal propagules is more in the field soil, the number of propagules in earthworm cast is also more and *vice versa*. It has been estimated that earthworm activity could bring as much as 2 - 5 kg soil to the surface in a decade and the quantity of soil, which passes through the digestive tract of these animal annually, would amount to 0.367 kg/m^2 of dry earth (Lyon *et al.*, 1915). This large amount of soil, which is constantly being mixed through earthworm activity, is possibly an important means of distribution of VAM spores within the soil. The earthworm casts have close contact with the animal fluid and such physiological fluids might have selectively eliminated the *Sclerocystis* spores as observed in this study. The earthworm movement may result in some horizontal dispersion, a few meters at the most. The major contribution of these vectors may be that the soil containing spores are brought to the surface as castings, thus favoring long distance dispersal by other agents like wind, water *etc.*

5.10.1.2.Termites

The soil-inhabiting termites are quite common as mound builders above the ground or as subterranean nest builders. A great majority of termites live in tropical and sub tropical regions (Krishna and Weesner, 1970). Termites feed largely on plant material, soil and humus. While gathering of food for growth (foraging) they travel up to 60 to 70 meters (Siddegowda, 1988).

Termites are active during summer in rubber plantations and they feed on dead organic matter including roots infected with VAM fungi. In addition they use soil to build termitaries. Naturally termitary soils are carried to other area by wind and rainwater.

The present results showed that termitaries are rich in VAM spores especially *Glomus* spp. Soil from termitaries contained 15 spores per g while adjacent soil contained only 11 spores. The increase might be due to excreta of termites, which feed on root containing VAM spores. The lack of *Gigaspora* spores in termitaries may be due to the selective elimination by the physiological fluids in termites.

5.10.1.3.Wasps

Wasps collect soil from fields and use for building nests in protected areas. The density and species of VAM in wasp nests depend on their population in soil from where it was collected. In the present study 12 spores per g of nest soil was recorded which was less than that in the earthworm castings. Spore count of *Glomus* species was more as in field soil. The lesser population could be attributed to probable presence of toxic matter in wasp nests (Deligne *et al.*, 1981). In passive dissemination of VAM propagules wasps are potential vectors because they directly use soil in one way or the other (Mc Ilveen and Cole, 1976). Lakshman and Raghavendra (1990) extracted VAM spores from nests of mud pot wasps and found that it resulted in low

(2.4 per cent) mycorrhizal colonisation. During nest building period, which would last for two days, a female wasp is known to carry up to 40 g of clay and cover a distance of 335 km (Iwata, 1953). *Sceliphron* spp. and *Eumenes* spp., which are the common nest-building wasp in India, can thus act as a potential vector in disseminating VAM fungi.

5.10.1.4.Ants

Ants make mounds in soil by bringing soil from bottom layer to the top. Such soil in the present study contained VAM spores and the number is same as in field soil. Ants are known to eat root tips from sub soils and bring them to the surface and these roots may be colonised by VAM fungi and hence the sub soil VAM count comparable to the field soil. Ants may be less important than earthworms in the dispersal of VAM fungi since ants would transport less soil. Ants have been estimated to bring out soil to the surface annually at the rate of 0.11 kg/m^2 of dry earth (Baxter and Hole, 1966). The soil brought out is available to the further dispersal by wind (Daniels, 1984), water (Powell, 1976), animals (Bagyaraj, 1991), vehicles, agricultural implements and men.

5.10.2. Abiotic factors in the dispersal of VAM fungi

Wind and water are two major abiotic factors that account for dispersal of VAM spores (Ponder, 1980). In the present study both water and wind are found to carry VAM spores of which water carries more spores. As the raining period increased, the VAM spore count got reduced. Powel (1980) observed similar results and concluded that the number of spores carried by water depends on the quantity of water flown from the source. The surface flow of water that would cause mass flow of soil might also move spores from the soil surface. Suggestive of this is Powel's (1980) study dealing with the mycorrhizal infectivity of eroded soils in which spore density decreased with increasing soil erosion.

VAM spores caught in spore traps were very few. Heavy wind in summer carries VAM spores from the surface soil to very distant places. It also carries VAM spores by rain splash to shorter distances. Earlier studies on the role of wind in dispersion of VAM are very few. Marx (1975) suggested that because VAM fungi are large-spored and hypogeous, they are primarily dispersed by animals and wind is not a major dispersal agent as observed in the present study. Warner *et al.* (1984) also demonstrated the wind dispersal of VAM spores using spore traps.

The present investigations on the ecology of VAM in rubber growing soils of Kerala clearly indicate wide variation in VAM population of soil and colonisation in

the roots of *Hevea*. There is a decrease in the VAM population in *Hevea* soils than in forest soils. The agro-management practices showed a detrimental effect on VAM, as evidenced by the decreased VAM population in rubber growing soils. It suggests need for modifying the agro-management practices to sustain the population of VAM. Organic farming, where natural agricultural inputs are used, could be attempted for maintaining the VAM population in rubber growing soils of Kerala.

6. SUMMARY

Natural Rubber cultivation started a century ago in India and is reported to cause considerable changes in the ecosystem. It is likely that VAM, the symbiotic fungi may also have been influenced by this perennial tree crop cultivation and hence a study on the ecology of VAM fungi in rubber plantations of Kerala was carried out. Soils collected from 21 locations in Kerala showed variation in pH ranging from 4.5 to 5.8. All soils tested contained VAM spores in varying numbers. VAM spore population in soil had a positive relation with changes in soil pH.

VAM spore count was more in summer and least in monsoon season. The common genera of VAM fungi recorded in rubber growing soils included *Glomus*, *Acaulospora*, *Sclerocystis* and *Gigaspora*. The frequency of occurrence of *Glomus* spp. was more followed by *Acaulospora* spp. and *Sclerocystis* spp. The count of *Gigaspora* spp. was very less. Species composition of soil and their density varied considerably in all the 21 locations of soil collection.Root colonisation of *Hevea* in samples collected in summer was little which started increasing at the onset of pre monsoon. After pre monsoon season VAM colonisation in *Hevea* roots significantly differed in all the locations.

Forest soils had higher VAM population especially *Glomus* spp. as compared to rubber field soil where also *Glomus* spp. spore number was dominating. Count of

VAM spores decreased with the increase in the duration of rubber cultivation. Repeated cultivation of *Hevea* for three continuous cycles led to a decrease in VAM population. VAM spore population decreased as the depth of soil increased. A temperature range of 20 to 30° C is found to be optimum for root colonisation by VAM fungi in test plants though there was not much change in VAM spore count at various temperature treatments up to 40 ° C and further increase in temperature caused a reduction. Soil moisture above 40 per cent level significantly reduced VAM colonisation and the spore population.

Incorporation of organic manure like cowdung, leaf litter and compost had no influence on VAM spores. However the former two manure encouraged root colonisation in *Hevea*. Synthetic fertilizer comprising NPKMg (10:10:4:1.5) applied at recommended level caused a reduction in VAM population and per cent root colonisation. Both fungicides and pesticides influenced the VAM spore count and root colonisation, which differed with different fungicides and pesticides. Application of sulphur did not cause any change in root colonisation in *Hevea*. Bordeaux mixture also upto recommended dose did not affect root colonisation. Modern synthetic fungicides reduced VAM colonisation. Phosphorous acid application enhanced the per cent root colonisation. Insecticides malathion, fenvalerate and endosulphan irrespective of their concentration significantly reduced living VAM spore count and root colonisation. Other insecticides chlorpyriphos and monocrotophos at recommended and half the recommended level did not affect root colonisation and

VAM spore count. Carbaryl and carbofuran at recommended and double the recommended levels reduced the VAM colonisation in *Hevea*, while carbaryl at double recommended dose significantly reduced the number of viable spores in soil. Weedicides did not cause any damage on VAM spore population and root colonisation whereas root colonisation significantly increased upon diuron application. A significant reduction was noticed in root colonisation and VAM spore population in solarised soil.

VAM spores were observed in the vermicasts, termitaries, ant mounds and wasp nests indicating that they have a role in the passive dissemination of VAM fungi. Water flowing through the field during rainy season contained VAM spores. The number of spores reduced with the increase in duration of rain. VAM spores were collected in the spore traps indicating, wind is also involved in VAM spore dispersal.

The present studies clearly indicate the presence of different species of VAM fungi with the domination of *Glomus* spp. in different rubber growing soils of Kerala. The population of VAM was observed to be reduced by the different cultural operations in rubber fields. Application of fertilizers, some of the fungicides and pesticides caused deterioration of VAM population. Passive dispersal of VAM fungi is found to be taking place by both biotic and abiotic factors.

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