ASSOCIATION OF MICROFLORA WITH RUBBER (*Hevea brasiliensis*) AND THEIR BENEFICIAL ROLES

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Mahatma Gandhi University
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DOCTOR OF PHILOSOPHY
in BOTANY

By

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Dedicated to
my beloved father
(Late) Mr. K. K. Philip

DECLARATION

I hereby declare that the thesis entitled **Association of microflora with rubber (*Hevea brasiliensis*) and their beneficial roles** is an authentic record of the original research work carried out by me under the supervision and guidance of Dr. C. Kuruvilla Jacob, Director (Training) at Rubber Research Institute of India, Rubber Board Kottayam in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Mahatma Gandhi University and that no part of this work has been presented for any degree, diploma or any other similar title of any University.

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CERTIFICATE

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Abstract

The rubber tree (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.), the most important source of natural rubber, is constantly under threat from various pathogens that cause severe diseases like abnormal leaf fall, Corynespora leaf disease, brown root disease, pink disease and Colletotrichum leaf disease.

The diseases are controlled by applying chemical fungicides which may have adverse effects on the environment. Hence the use of biocontrol agents against diseases of rubber needs attention. It is advantageous to search for the biocontrol agents in the vicinity of rubber trees, as the establishment and survival of such organisms would be better due to their adaptation to the environment. Hence study of population dynamics of microorganisms and identification of beneficial ones associated with rubber trees was attempted.

Samples from rubber trees belonging to two different clones (RRII 105 and PB 260) and age groups grown at six major rubber growing zones in south-western India (Nettana, Padiyoor, Palappilly, Chethackal, Lahai and Vaikundam) were collected during three seasons, viz. summer, monsoon and post monsoon, and isolation and enumeration of phylloplane, cauloplane and rhizosphere micro-organisms carried out. Soil samples were also collected from the virgin forests near to the selected rubber plantations to enumerate and compare the soil microflora. The moisture content and pH of soil were recorded to observe their influence on the microflora.

Bacteria and fungi were abundant in all the samples whereas the number of actinomycetes and yeasts were very low or absent in many cases. Phylloplane bacterial population was very low in both mature and immature rubber trees during the summer season, higher during the rainy season and most abundant during the post monsoon season irrespective of the clones RRII 105 and PB 260. Fungi were also most abundant during the post-monsoon season.

The cauloplane bacterial and fungal population were highest during the post monsoon season. Between the two clones of rubber trees, RRII 105 harboured higher population of cauloplane bacteria. Higher cauloplane fungal population was reported in RRII 105 clone than in PB 260 clone. The cauloplane bacterial and fungal population was higher in mature trees.

The rhizosphere bacteria were more abundant during the post monsoon season. Between the two clones PB 260 harboured higher rhizosphere bacteria. The age group of the plantations influenced the

rhizosphere bacteria with mature plantations showing higher population. There was significant seasonal variation in rhizosphere fungi in all the samples from Nettana, Padiyoor, Palappilly, Chethackal and Lahai. Inter-cropping was more favourable to the growth of beneficial microbes, in comparison with sole rubber crop regions. In comparison to the virgin forest soils the rubber plantations harboured higher rhizosphere bacterial and fungal population at all the locations.

Actinomycetes were present in almost all the samples in the post monsoon and rainy season but not in the summer. At all the locations, phosphobacterial colonies on mature RRII 105 and PB 260 clones showed seasonal variation. In general, population of azotobacter was higher in the rhizosphere of clone PB 260.

VAM incidence on the roots of rubber trees showed highly significant seasonal variation at all the six regions in both the clones belonging to the two age groups. The soil moisture and other environmental conditions during the post monsoon season favoured VAM establishment. VAM spore count was consistently high during summer season and low during rainy season. The dry environmental conditions during the summer season, probably led to more production of spores.

Even in the acidic soil, fungi, actinomycetes, azotobacter and VAM growth was not significantly affected. There was no correlation between microbial population in the rubber plantation and soil moisture except that the azotobacter population was lower during rainy season.

The bacterial and fungal antagonists were cultured to study their potential role in biological control of five major pathogens of rubber trees namely, *Corynespora cassiicola, Phytophthora meadii*, *Phellinus noxius*, *Corticium salmonicolor* and *Colletotrichum acutatum*. Several isolates proved to be antagonistic to these pathogens. Bacterial isolates B_{24}, B_{54} and B_{61} and fungal isolates F_{90}, F_{97} and F_{65} showed high antagonism against more than two pathogens *in vitro*.

Production of HCN, siderophore and volatile compounds by the antagonistic bacteria were studied to correlate these properties with the observed antagonism. Assay of enzyme production by the fungal antagonists suggested that the presence of cellulase and Endo β-1, 4 glucanase play a key role in their antagonistic property.

The isolates that showed antagonism *in vitro* were also effective *in vivo* when introduced prophylactically on nursery plants of clone RRII 105. The bacterial isolate B_{54} and fungal isolate F_{65} were superior in affording such protection against *C. cassiicola* and *C. acutatum*.

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1. GENERAL INTRODUCTION

Para rubber tree, *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg., Is the most important commercial source of natural rubber. Rubber occupies a key position among the plantation crops in India. Extension of rubber cultivation beyond the present limits in the traditional rubber tracts is constrained by the unavailability of land. Hence effort should be taken to boost up the production of rubber, to meet the increasing demand of natural rubber. Production of rubber should be increased with minimum ecological impacts. Crop husbandry measures, like application of fertilizers and chemicals for plant protection has become a routine practice. Though this may be beneficial in improving crop production, it can lead to negative influence on the biotic flora of the plantation.

1.1. Rubber tree (*Hevea brasiliensis*)

The Para rubber tree (*Hevea brasiliensis*) often called rubber tree, belongs to the family *Euphorbiaceae* and is the most economically important member of the genus *Hevea* as it yields latex, the primary source of natural rubber.

In *Hevea brasiliensis* latex is present in vessels within the inner bark. Latex is drained from the tree by cutting a shallow groove in the bark that stops short of injuring the cambium. This technique known as tapping is a controlled wounding and hence causes no permanent damage to the tree and allows for a productive life span of about twentyfive years. The rubber tree is prone to many diseases.

1.2. Major diseases of rubber trees

One of the main constraints in sustained production of rubber is the incidence of plant diseases like, abnormal leaf fall of rubber, Corynespora leaf disease, colletotrichum (Gloeosporium) leaf disease, pink disease and brown root disease. Considerable crop loss due to heavy defoliation in abnormal leaf fall disease has been reported by Jacob *et al.* (1989, 2006).

Manju *et al.*, (2001) has given a description of the severity of Corynespora Leaf Fall (CLF). Hilton (1958) and Sripathi Rao (1975), has reported the symptoms and severity of pink disease. Maladies caused by brown root disease have been described by Nandris *et al.* (1987), Geiger *et al.* (1985), Nicole *et al.* (1987) and Rajalakshmi and Jayarathnam(2000). Colletotrichum leaf disease of rubber has been studied in different parts of the world since 1905 (Jayasinghe *et al.*, 1997). Details regarding the symptoms and control measures of Colletotrichum leaf disease have been given by Ramakrishnan and Pillay (1962) and Edathil *et al.* (2000).

1.2.1. Abnormal leaf fall of rubber

The first report of abnormal leaf fall from India was in 1910 from estates near Palappilly in the Trichur district of Kerala state (McRae, 1918). The causative organism of this disease is *Phytophthora meadii* McRae. The disease recurs during south west monsoon period. Prolonged wet weather, coupled with humid atmospheric condition favors the disease. First the fruits rot, later infected leaves fall off prematurely, either green or after turning to copper red. A black lesion may develop on the petiole with a drop of latex in the center which is often coagulated. Lesions develop on the midrib and leaf blades also. The leaf fall is so heavy that the fallen leaves cover the entire ground forming a green carpet. Heavy defoliation leads to considerable crop loss (Jacob *et al.*1989, 2006) and die-back of terminal twigs.

1.2.2. Corynespora leaf fall disease

Corynespora leaf fall disease (CLFD) is a significant threat to the natural rubber industry, especially as outbreaks have been reported from most of the Asian rubber producing countries (Jacob, 2006 a). The disease is caused, by a fungus, *Corynespora cassiicola*. Spots with brown margins and pale centre appear on the leaves. The centre of the disease lesions fall off forming shot holes. The spots coalesce and cause defoliation. During refoliation of mature trees, light green leaves are more susceptible. Several lesions coalesce to form large blighted area. The disease spreads along

the veins leading to a brownish 'railway track'-like appearance. Infection on midrib or base of leaf causes leaf abscission. Defoliation leads to die back of branches (Jacob, 2006 b). If severe, the disease is capable of killing the trees, but in most cases it leads to loss in rubber production.

1.2.3. Colletotrichum (Gloeosporium) leaf disease

Colletotrichum leaf disease occurs mainly during the rainy season. Weak young plants growing under water-logged conditions are more susceptible. In the traditional rubber growing regions of India the disease occurs during April to October. It causes significant damage to plants in nurseries and young plantations (Deka *et al.* 1996; Manju *et al.* 1999). The leaf tips of tender leaves are infected. The leaf margins where numerous spots coalesce dry up and may lead to defoliation. The infected leaves often crinkle and become distorted before shedding (Edathil *et al.*, 2000). On mature trees, secondary leaf fall disease caused by this pathogen leads to crop loss in major rubber producing countries like Malaysia. The persistence of this disease over a long period results in loss of yield upto 50 percent and delay in maturity of rubber trees upto three years(Basuki, 1992).

1.2.4. Pink disease

Pink disease was first recorded from India in 1908. The disease disease occurs during the south west monsoon period from June, but visible effects are noticed from July to November. Plants in the age group of 2 to 12 years get damaged. In young plants upto three years, the infection is noticed on the main stem. But as the plant grows, the infection is mainly in the fork region of the branches (Jacob and Edathil, 1986). White or pink colored cob web mycelial growth on the bark surface is accompanied with streaks of latex oozing out from the lesions. This is followed by rotting, drying up and cracking of the affected bark. Sprouts develop from below the affected portion. The distal portion of branches dry and dried leaves remain intact on the dead branches (Hilton, 1958).The entire crown or mostly leading branches may have to be cut and removed

due to infection, adversely affecting establishment of young trees and thereby the tree stand. Poor stand leads to low land productivity.

1.2.5. Brown root disease

Phellinus noxius (Corner) G. H. Cunn. is the fungal pathogen causing this disease. The first symptom is a general yellowish discoloration of the foliage and unhealthy condition. The affected roots show encrustation of soil, sand and fungal hyphae cemented to the root and brown lines in the affected roots (Rajalakshmi and Jayarathnam, 2000). In advanced cases the plants dry up.

1.3. Major plant pathogens affecting rubber tree

Plant pathogens include a variety of microorganisms like bacteria, fungi, viruses, viroids and phytoplasma that cause diseases in plants. Only fungi are known to cause diseases of rubber trees. Plant-pathogenic fungi produce an array of extra cellular hydrolytic enzymes that enable them to penetrate and infect the host tissue; these enzymes are collectively called cell wall-degrading enzymes (CWDE). They may contribute to pathogenesis by degrading wax, cuticle and cell walls, thus aiding tissue invasion and pathogen dissemination. They may also act as elicitors of host defense reaction.

The rubber trees are infected by several plant pathogenic fungi causing diseases that lead to loss in latex production. These include *Phytophthora* spp., *Corynespora cassiicola, Colletotrichum acutatum, Corticium salmonicolor and Phellinus noxius.*

1.3.1. *Phytophthora* spp.

Different species of *Phytophthora* cause shoot rot, pod rot, bark rot, patch canker and leaf fall disease of rubber in various rubber growing regions. Liyanage and Wheeler (1989) examined eighty-nine *Phytophthora* isolates from rubber. Five species were distinguished namely, *Phytophthora palmivora* morphological form 1 (MF1), *P. meadii, P. botryose, P. citricola, P. citrophthora* and *P. palmivora* (MF4). Variation

among thirty nine isolates of *Phytophthora* of six morphological species (*P. citrophthora*, *P. parasitica, P. capsici, P. palmivora* and *P. meadii* from rubber and citrus trees and *P. colocasiae* from taro were studied by Zheng and Ward (1998).

Thirty three isolates of *Phytophthora meadii* obtained from different *Hevea brasiliensis* clones grown in different climatic regions in Sri Lanka were studied for pathogenicity by Jayasuriya *et al.* (1999). Growth and pathogenicity levels varied among the isolates. Some isolates obtained from moderately susceptible rubber clones were highly pathogenic, compared to isolates from resistant clones. Highly pathogenic isolates produced a higher number of sporangia on agar at 27± 2^0 C

Favorable temperature also is a contributing factor to the severity of Phytophthora diseases because of its effect on growth and sporulation of the pathogen. *Phytophthora palmivora* has an optimum temperature for growth of 30^0C, a maximum of 36^0C and a minimum of 12^0C. The pathogen produces more sporangia at 25^0C but no sporangia are produced at temperatures higher than 35^0C or lower than 15^0C.

1.3.2. *Corynespora cassiicola*

Corynespora cassiicola is another important pathogen affecting *H. brasiliensis.* The distinctive features of the genus *Corynespora* has been described by Wei, (1950); Ellis (1957); Morgan-Jones (1988) and Sulton and Pascoe (1988).

Corynespora cassiicola has been described to cause a root-rot disease of soybeans (*Glycine max* (L.) Merr.) (Rinzo and Kenji, 1980). The fungus causing disease on rubber, *Corynespora cassiicola* (Berk. & Curt.) Wei has been described in detail and is similar to that of Ellis (1957). Conidia of this fungus are brown, curved, and broad at the base, formed on conidiophores singly or in chain, and varied in morphology with 2 to 28 septa and a hilum at the base. Conidiophores are brown and erect formed singly or in groups with 1 to 7 septa and a pore at the end. No stromata are

observed. The basal cells of conidiophores are swollen. Young mycelia are white and fluffy and turn grey with age. Growth on PDA occur from 5 to 30°C with an optimum temperature range from 20 to 25°C. The optimum pH for growth is 5.0, and the range is from 4.0 to 8.0.

Toxin production by *Corynespora cassiicola* has been proven (Onesirosan *et al.* 1975). Isolates of *C. cassiicola* highly pathogenic to tomato produce a toxin in synthetic medium which induce symptoms in susceptible, but not in resistant tomato. Toxin production was maximal after 12 days' incubation between 27 and 28° C at a pH between 6 and 7. Toxin production was correlated with virulence of isolate.

Similar observations were made using rubber leaves by Breton *et al.* (2000) and rubber clones were screened using this technique (Joseph, 2006).

Changes in chemical constituents of tobacco leaves infected with *Corynespora cassicola* has been studied by Oke (1988). In infected leaves, the sugar and phenol contents were greatly reduced while an increase in nitrogen content was observed when compared with healthy leaves.

Incidence and severity of Corynespora Leaf Fall (CLF) disease of rubber in Coastal Karnataka and North Malabar region of Kerala has been studied by Manju *et al.*, (2001). The intensity varied with location and clone. The clone RRII 105 was severely infected.

Sporulation, pathogenecity and epidemiology of *Corynespora cassiicola* on rubber has been reported by Chee, (1988). Isolates varied in their ability to produce spores and sporulation was best in potato sucrose agar. Conidial sporulation was highest when cultures were incubated in the dark for three days followed by a daily 2h exposure to ultraviolet light or continuous light for three to six days. Leaves are most susceptible to infection for up to four weeks. There was no clear-cut seasonal pattern of spore release in relation to rainfall.

1.3.3. *Colletotrichum acutatum*

The causal agent of Colletotrichum leaf disease of rubber in Sri Lanka and other parts of the world has been described as *Colletotrichum gloeosporioides,* since 1905. A study on the vegetative and reproductive characteristics of fiftytwo isolates of *Colletotrichum* spp. from leaf disease lesions on *Hevea brasiliensis* in Sri Lanka showed that only eighteen isolates belonged to *C. gloeosporioides.* The remaining thirtyfour isolates represented Colletotrichum *acutatum* indicating that *C. acutatum* is the main cause of the disease in Sri Lanka (Jayasinghe *et al.*, 1997).

Fernando *et al.* (2000) studied the factors affecting spore production, germination and viability of *C. acutatum* isolates from *H. brasiliensis.* They sporulated on PDA at 10-40^{0}C with peaks at around 15^{0}C and 25^0 C. Ultraviolet radiation inactivated the spores. Spores could withstand temperatures upto 35^{0}C.

The first record of *C. acutatum* on *Hevea* in India was by Saha *et al.* (2002). Isolates from three different disease symptoms of Colletotrichum leaf disease such as raised spots, anthracnose and papery lesions were studied using molecular markers. It suggested that two species of *Colletotrichum* associated with *Hevea* incited the development of the three different symptoms. *Colletotrichum acutatum* caused raised spot symptom and *Colletotrichum gloeosporioides* caused both anthracnose and papery lesions.

1.3.4. *Corticium salmonicolor*

Corticium salmonicolor (Berk. & Br.) causes pink disease of rubber (Hilton, 1958). It thrives best and occurs with greater frequency in the tropics and subtropics. Under warm, moist climatic conditions, the pink fungus mat produces spores which are carried by rain splash or wind to other hosts. This is the most serious stem disease affecting mainly young rubber trees. The disease incidence varies in different regions of India and

is reported to be low in the absence of prolonged wet weather (Ramakrishnan and Pillay, 1962).

Outbreaks of pink disease, caused by *Corticium salmonicolor* (Berk. & Br), in *Eucalyptus grandis* plantations in Kerala State have been reported (Sharma *et al.*, 1984). The fungus grows well when cultured on media but sporulation is rare (Rajalakshmi and Pillay, 1975).

1.3.5. *Phellinus noxius*

Phellinus noxius (Corner) G. H. Cunn was first described as *Fomes noxius* by Corner in 1932. This pathogen has a wide host range, which include mahogany, teak, rubber, oil palm, tea, coffee and cacao as well as a variety of fruit, nut and ornamental trees. Brown root disease caused by *Phellinus noxius* is a major root disease of rubber in India. It occurs in most of the rubber growing countries.

The mycelium of *P. noxius* produces enzymes that break down the middle lamella and cell walls of the plant into simple sugars. This supplies nutrients to the mycelium and allows it to move deeper into the wood. It degrades lignin, a complex molecule that gives wood much of its strength and brown colour.

The survival of *Phellinus noxius* as arthroconidia, basidiospores and mycelia on colonized wood was measured under different soil matrix potentials by Chang (1996). It was found that in treatments with lower soil moisture, *P. noxius* survival ranged from 80 to 90% over two years. Woody debris in soils harbouring *P. noxius* played an important role in the long - term survival of the fungus. He suggested that flooding infested fields may help in disease control.

Phellinus noxius survives in soils of pH 4 to 9 and most frequently of pH 5 to 8 (Chang and Yang, 1998).

Pathogenicity of isolates of *Phellinus noxius* was tested in rubber seedlings by artificial inoculation under controlled conditions (Nandris *et al.* 1983). The isolates exhibited variation in virulence. The studies revealed

that root penetration and bark colonization were rapid, while xylem invasion was rather slow.

1.4. *Hevea brasiliensis* clones under study

Two popular clones of rubber (*Hevea brasiliensis*), namely RRII 105 and PB 260 were selected for the present investigation.

1.4.1. RRII 105

This is currently the most popular clone cultivated by both large as well as small scale farmers. It is developed by the Rubber Research Institute of India by cross breeding the clones Tjir1 and GL1. It was released for planting in 1980.It has an average annual yield of 2400 kg/ha. The clone is fairly tolerant to abnormal leaf fall disease (*Phytophthora* spp.) but highly susceptible to pink disease and Corynespora leaf disease.

1.4.2. PB 260

This clone was developed at the Prang Besar Estate, Malaysia by cross breeding PB 5/51 and PB 49. This is a very vigorous clone with dense canopy and balanced branching. This is also a high yielding clone with an average annual yield of over 2000 kg/ha. This clone is susceptible to diseases caused by *Phytophthora* spp., *Corticium salmonicolor* and *Pythium* spp.

1.5. Lay out and sampling

Samples were collected from major rubber growing zones in south western India, namely South Tamil Nadu, South Kerala, Central Kerala, North Kerala, South West Karnataka and Tropical High Altitudes, in six large scale rubber plantations, one each at Nettana (Karnataka), Padiyoor (North Kerala), Palappilly (Central Kerala), Chethackal (South Kerala), Lahai (Tropical High Altitude, Kerala) and Vaikundam (South Tamil Nadu).

Rubber trees belonging to two age groups, mature (>10 years) and immature were selected. Plots (1 ha) were marked in each location in the fields planted with the clones under study for these age groups. Five

healthy trees were selected randomly avoiding border areas of the plantation and marked. Samples were collected from the marked trees during the summer, monsoon and post-monsoon seasons.

Leaves bark and root samples were collected, in order to conduct studies on phylloplane, cauloplane and rhizosphere microflora. Soil samples were collected from near the trees by digging fifteen cm deep from the ground level. Soil samples were also collected from nearby virgin forest land.

A summary of the different samples of leaf, bark, roots and soil of the two rubber clones (RRII 105 and PB 260) belonging to the two age groups, (mature and immature) from six locations as well as virgin forest soil is presented in Table 1.5.1

Summary of Sampling

Location	Clone / Virgin	Age	No. of Trees	No. of Seasons	Phylloplane Samples	Cauloplane Samples	Rhizosphere samples	Root for VAM	Soil Samples for pH / SM
Nettana	RRII 105	Mature	5	3	15	15	15	15	5
		Immature	5	3	15	15	15	15	5
	PB 260	Mature	5	3	15	15	15	15	5
	Virgin								4
Padiyoor	RRII 105	Mature	5	3	15	15	15	15	5
		Immature	5	3	15	15	15	15	5
	Virgin								4
Palappilly	RRII 105	Mature	5	3	15	15	15	15	5
		Immature	5	3	15	15	15	15	5
	PB 260	Mature	5	3	15	15	15	15	5
		Immature	5	3	15	15	15	15	5
	Virgin								4
Chethackal	RRII 105	Mature	5	3	15	15	15	15	5
		Immature	5	3	15	15	15	15	5
	PB 260	Mature	5	3	15	15	15	15	5
		Immature	5	3	15	15	15	15	5
	Virgin								4
Lahai	RRII 105	Mature	5	3	15	15	15	15	5
		Immature	5	3	15	15	15	15	5
	PB 260	Mature	5	3	15	15	15	15	5
	Virgin								4
Vaikundam	RRII 105	Mature	5	3	15	15	15	15	5
		Immature	5	3	15	15	15	15	5
	PB 260	Mature	5	3	15	15	15	15	5
		Immature	5	3	15	15	15	15	5
	Virgin								4
				Total	300	300	300	300	124

Total Number of Samples collected	1324

Table 1.5.1: Lay out and sampling

1.6. Importance of the present study

Biological control is a potent strategy for reducing the damage caused by plant pathogens. Commercialized systems for the biological control of plant diseases are few. Although intensive activity is currently being geared towards the introduction of an increasing number of biocontrol agents into the market, the performance of biocontrol agents cannot always be expected to equal that of fungicides. However, some biocontrol agents have been reported to be as effective as fungicides.

The major constraint in the efficient use of biocontrol agents is the capability of these organisms to survive in the infection court to fight against the invading pathogen. In that context it is prudent to scout for such organisms in the host vicinity for better survival. The survival may also be influenced by the environment and age of the host plant.

Seasonal variation of microbes residing on rubber trees has not attracted detailed investigation. The aim of the research was, therefore, to provide insight into the variation in population of fungi, bacteria and other microbes inhabiting rubber trees, during the different seasons and to check whether the natural microflora residing in the phylloplane, cauloplane and rhizosphere can act as biocontrol agents against the major pathogens attacking rubber trees.

The present study aims at enumerating the population of phylloplane, cauloplane and rhizosphere microflora associated with two clones of *Hevea brasiliensis* and studying their spatial distribution in rubber plantations in the major rubber growing zones of South India during the different seasons and to check whether the natural microflora can act as biocontrol agents against the major pathogens, causing diseases of rubber trees.

2. SEASONAL VARIATION IN MICROBIAL POPULATION

2.1. Introduction

The various microbes associated with rubber plants can be grouped into phylloplane, cauloplane and rhizosphere organisms. A detailed study on the microflora associated with the phylloplane, cauloplane and rhizosphere of different clones of rubber has not so far been attempted. It is also likely that rubber cultivation might have altered the native microflora to a great extent.

2.1.1. Phylloplane microbes

The phylloplane, the surface of living leaves, harbours a variety of microflora. There exists an association between the leaves and the microflora present on it. The leaf surface provides an ecological niche for these microorganisms. The leaf leachates serve as nutrients for such microbes.

Phylloplane organisms may be residents including bacteria, fungi, actinomycetes and yeasts (Ruinen, 1956; Leben, 1969; Kothandaraman, 1984). Rao and Mullaiah (1988) listed a number of bacteria from the leaf surface of higher plants. These include *Azotobacter, Beijerinkia, Pseudomonas, Pseudobacterium* and *Sarcina*. Presence of fungi like *Aspergillus, Rhizopus, Trichoderma, Mucor, Fusarium* and *Alternaria* on rubber leaves were reported by Kothandaraman (1984), and George (1999).

2.1.2. Cauloplane microbes

The cauloplane or the surface of the stem also harbours numerous microflora.

Cauloplane organisms are mainly influenced by environmental factors and vegetation around the trees (Mukerji and Rao, 1982).

Some of the cauloplane microfloras are antagonistic to parasites (Bier, 1963). Fungi have been isolated from the bark of hardwoods, often to investigate the antagonism of these fungi to bark pathogens. Bier and Rowat (1962) found species of *Auriobasidium and Epicoccum purpurascens* to be the most common fungi in the caulosphere of both blackwood (*Populus tricocarpa*) and willow (*Salix* spp.).

Cotter and Blanchard, (1982) cultured1910 fungal isolates from bark of American beech trees (*Fagus grandifolia*), nearly two-thirds of which belonged to deuteromycetes.

Trichoderma was isolated from decayed hazelnut wood in northern Italy. Its antagonistic activity against *Armillaria mellea*, the causal agent of root rot in agricultural crops and forests, was observed by *in vitro* tests (Longa *et al.* 2008).

Mathew and Jacob (2004) reported that cauloplane of rubber trees harboured lower number of microorganisms when compared to phylloplane and rhizosphere.

2.1.3. Rhizosphere microflora

Rhizosphere, the region of the soil immediately surrounding the roots of a plant favours the growth of many microorganisms. The microbial population of the soil is influenced by the chemical activities of the plant, particularly the root exudates

The rhizosphere is rich in microbial colonization. Enumeration of rhizosphere microorganisms associated with rubber has been attempted (Kothandaraman *et al.* 1989; Ikram, 1989; Deka *et al.*,1992; Joseph *et ai.*, 1998; Ikram and Yusoff, 1999; Mathew and Jacob, 2004).

2.2. Review of Literature

2.2.1 Seasonal variation in microflora

Seasonal variations in microbial population and relationship of microbes with some soil parameters were studied in tea ecosystem of Assam by Gogol *et al.*(2003). Populations of bacteria, fungi, phosphate solubilizers, ammonifiers and nitrifiers decreased significantly with depth. Except for nitrifiers, other microbes attained population peak in monsoon season. Effect of depth and seasonal interaction on soil pH was found to be statistically insignificant.

More or less, a similar pattern of distribution of soil microbial population was observed in different aged fallows by Deka and Mishra, (1984). In general, the population decreased with the increase in soil depth. The total number of bacteria and actinomycetes was higher when compared to fungal population. Seasonal variation in bacterial and actinomycetes populations was found to be correlated with moisture regimes and pH of the soil. Qualitatively, the composition of fungal species was identical in all the fallows. Only a few species were isolated quite frequently with a high percentage of relative abundance. *Trichoderma viride, Penicillium chrysogenum, Aspergillus niger , Cladosporium herbarum, Cephalosporium coremioides, Fusarium solani* and *Pythium* sp. were the most dominant forms. The effect of season and soil depth did not seem to have any influence on the composition of fungal species. Apparently, the age of the fallows also did not exert any direct effect either on the distribution of soil microbial populations or on the fungal species composition.

Seasonal effect on the bacterial and fungal populations of an oilfield wastewater - polluted soil was investigated for a period of 12 months. (Obire and Wemedo, 2002). Many bacterial and fungal species were present in the soil and the wastewater of the area investigated. Some of the organisms occurred in both the soil and oilfield wastewater while others occurred only in the soil. Eighteen organisms were isolated, 4 species

(2 bacteria and 2 fungi) occurred in all the seasons whereas the other organisms occurred in at least one season. This showed that different seasons selectively favour the growth of certain microbial types. Seasonal variations showed that the drier seasons supported large active microbial populations and the wet season had smaller populations. However, seasonal influence was more pronounced on the fungi than on the bacteria. Studies on soil microbial biomass by Wardle *et al.*, 1999 in annual (maize) and perennial (asparagus) cropping systems for over seven years showed that microbial biomass was positively correlated with weed biomass and negatively with crop plant biomass.

To investigate the role of microorganisms in the ecology and the nutrient transformation of forest soil, soil property, microbial population, biomass and organic acid content of Spruce soil in Tatachia Mountain were determined during January 1997 to November 1999 (Yang *et al.*, 2003). Although rhizosphere of Spruce had higher total organic carbon and total nitrogen content than non-rhizosphere and dwarf bamboo areas, the microbial population had no significant difference among them.

Variation in diversity of fungi isolated from soil was studied for seven years by Persiani *et al.*, 1998, in an area of the Tai National Park (S W Ivory Coast) which had a tropical climate. The disturbances and the change in soil moisture due to variation of rainfall act as extrinsic factors to the community, influencing the level of diversity.

2.2.2. Spatial distribution of soil microflora

A large number of different microorganisms including bacteria, fungi, protozoa and algae interact in soil. The distribution pattern of soil microflora was studied in a five year old rubber plantation in Tripura by placing fertilizers at varying soil depths. (Deka *et al.*, 1998) The fungal, bacterial and mycorrhizal population was found to decrease significantly in deeper soil layers. Horizontal distribution of bacteria showed maximum activity in the region of higher root concentration.

Among microorganisms inhabiting soils, fungi commonly rank as the most abundant in terms of bio-mass and physiological activity (Kjoller and Struwe, 1982). Fungi comprise an estimated 78-90% of the total decomposer bio-mass in grassland soils and in a British deciduous forest, fungal mycelia contributing approximately 89% of the total living microbial bio-mass (Frankland, 1982)

2.3. Materials and Methods

2.3.1. Collection of phylloplane samples

Five trees were selected from each plot, representing the four quadrants and centre of the plot. They were labelled using paint in order to collect samples from the same trees in the succeeding seasons.

Mature and healthy undamaged leaves from different branches representing those growing towards north, east, south and west, of the same tree were collected and pooled together and considered as one sample. These leaflets were used for isolation.

Preparation of phylloplane samples

Four leaflets were selected at random from each sample. They were gently washed and the lamina was cut (5 x 5 cm) from each leaflet under sterile conditions. Four such pieces (100 sq. cm) were introduced into conical flasks containing 100ml sterile water and was shaken in a rotary shaker for 30 minutes and the leaf bits were removed. The leaf washing containing the inoculum was serially diluted upto 10^{-5}. 1ml aliquots were plated with respective media for isolation of bacteria, fungi and yeasts.

2.3.2. Collection of cauloplane inoculum

Bark scrapings were collected from the selected trees. The scrapings were collected from an area of 25 cm^2 (5 cm X 5 cm) at four regions from each plant and were pooled together to get one sample. Five such samples were collected from each plot in polythene bags, labelled and stored in the refrigerator till used for culturing and isolation. To avoid

contamination from soil microbes, and to avoid human influence during cultural operation, scrapings were taken only from stem regions 150 cm above ground level.

Preparation of cauloplane samples

The bark scrapings collected from approximately $100cm^2$ was introduced into 100ml of sterile water in a conical flask and was shaken on a rotary shaker for 30 minutes. This was used as the stock inoculum for serial dilution upto 10^{-5}. 1ml aliquots were plated with respective media for isolation of bacteria, fungi and yeasts.

2.3.3. Collection of rhizosphere samples

Removed the humus from the base of 5 trees, from each plot from which samples were to be collected. Dug out sub soil from four sides of each tree and pooled 500g of soil along with feeder roots. These were collected in polythene bags and labelled properly. The samples were stored in a refrigerator till used for culturing.

Soil samples were also collected from the nearby forest area of each plot representing virgin soil for a comparative study of microbes inhabiting cultivated and virgin lands.

Preparation of rhizosphere samples

Feeder roots of *Hevea* along with attached soil particles were added into 100ml of sterile water in a 250ml conical flask. The quantity of roots added was such that after dissolving and drying, the final dry weight of the soil was approximately 1g. The conical flasks with the roots and soil, were shaken well in a rotary shaker for 30 minutes, and were allowed to settle slightly. From this rhizosphere inoculum, 1ml was removed and used for serial dilutions up to 10^{-7}.

After the inoculum was used for serial dilution, the rest of the inoculum in the conical flask was transferred into a clean petridish whose initial weight was determined previously. All the roots were removed and

the water along with the soil was allowed to dry on a water bath. After drying the final weight was determined to confirm the approximate weight of the dry soil.

2.3.4. Serial dilution technique

Nine ml of water was pipetted out into test tubes which were plugged and then sterilized. One ml of the inoculum (stock inoculum from the leaf, bark and rhizosphere samples) was pipetted into the 9ml sterile water in the test tube under sterile conditions. These were shaken thoroughly to get the first level dilution (10^{-1}). From this another 1ml was transfered into a test tube with 9ml sterile water and shaken well to get the second level of dilution (10^{-2}). This process was repeated until the desired dilutions were obtained. Dilutions upto 10^{-7} were prepared in the present study, to get distinct countable colonies. The counts were adjusted to and expressed at convenient dilutions for comparison.

2.3.5. Media for different micro-organisms

Medium for phylloplane and cauloplane bacteria

Leben's medium was modified in various ways and used in the isolation of bacteria, fungi and yeasts. Phylloplane bacteria were assayed by modification of the basal medium using cycloheximide and tetrazolium chloride. Cycloheximide acts as a fungicide whereas tetrazolium chloride provides color to bacterial colonies.

The composition of the basal medium is described in Appendix 13.1

Medium for phylloplane and cauloplane fungi and actinomycetes

Five hundred milligrams of the broad spectrum antibiotic tetracycline hydrochloride was added to one litre of the cooled sterilized Leben's medium and mixed well just before pouring into the plates.

Medium for phylloplane and cauloplane yeasts

To 250ml of the basal medium amended with 125mg of tetracycline hydrochloride, 5.5ml of 0.1 normal sulphuric acid was added (to adjust the pH between 4.4 and 4.8) just prior to pouring the medium into the petriplates.

Media for rhizosphere microflora

The different media used for rhizosphere microflora were:

- Soil extract agar (SEA) – [Appendix 13.2]
- Rose Bengal agar (RBA) – [Appendix 13.3]
- Apetite agar (AA) – [Appendix 13.4]
- Ken Knight agar (KA) – [Appendix 13.5]
- Jensen's agar(JA) – [Appendix 13.6]

2.3.6. Isolation and enumeration of natural microflora

Phylloplane and cauloplane bacteria

The same dilution levels of the inoculum, medium and isolation techniques were used for both phylloplane and cauloplane bacteria. Sterile petriplates were labelled so as to contain the place, type of sample, dilution and date of inoculation. One ml of different dilutions of inoculum was pipetted out into sterile petriplates under sterile conditions. About 20ml of the medium for either phylloplane or cauloplane bacteria were added into the petridish and were mixed well by rotating the petridishes in both directions. This was repeated in duplicate for all samples. The plates were incubated at 28 $\pm$ 1^0C for 4 to 5 days.

After 4 to 5 days incubation the petriplates were examined for bacterial growth. Based on the colour and shape of the colonies, bacteria were differentiated into different groups. Representative isolations were made, labelled and stored at 5^0 C. The bacteria were counted separately

using a colony counter. The total bacterial count was calculated from this, considering the level of serial dilution in each case.

Phylloplane and cauloplane fungi

One ml of different dilutions of inoculum was poured into sterile petriplates under sterile conditions. Into this about 20ml of the medium was added and mixed well by rotating the petridish. This was repeated with all the samples in duplicate. The plates were incubated at 28 + 1^0C for 4 to 5 days.

After the incubation period the petriplates were examined for fungal colonies. Since each fungus had a characteristic growth habit and colour, fungi could be easily differentiated. Representative isolations were made, labelled and stored at 5^0C.The number of each fungal colony was recorded. The total number of fungi per unit area (1cm^2) was calculated by considering the dilution.

Phylloplane and cauloplane yeasts

One ml of different dilutions of inoculum were added into sterile petridishes and 20ml of the Leben's medium modified for yeasts (pH 4.4 to 4.8) was added and mixed well prior to solidification. The plates were incubated at 20^0C for 7 days. Colonies appeared as small creamy white spots. These were counted and the numbers of yeast cells present per unit area (1cm^2) of the leaf and bark surfaces were calculated considering the dilution factor.

Phylloplane and cauloplane actinomycetes

The procedure adopted for isolation of phylloplane and cauloplane fungi was used in the case of separating actinomycete population from the leaf and bark surfaces. Since actinomycetes grow well in the fungal medium to produce smaller thick colony masses which could be easily distinguished from the rapidly spreading fungal colonies, incubation period was extended upto 7 days at 28 $\pm$ 1^0C as the growth rate was slow. Actinomycete colonies were identified and counted from the dilution plates.

Rhizosphere bacteria

Soil extract agar (SEA) was used for isolating and culturing soil bacteria. Inoculum was diluted to 10^{-5} and was used for isolation. The plates were incubated at 28 ±1°C for 4 to 5 days and bacterial count was recorded. The total number of bacteria per gram of soil was calculated.

Rhizosphere fungi

To 100 ml of molten and cooled RBA, 3 ml of streptomycin was added. It was mixed well and about 20 ml of this was poured into a petriplate with 1 ml of 10^{-5} diluted inoculum. The plate was rotated both ways so that the inoculum mixed well with the medium. This was repeated in duplicate with all inoculum samples. Plates were incubated for 4 to 5 days at 28 ±1°C. Total count and specific count of individual strains were then recorded with a colony counter, from which their number per gram of soil was calculated.

Rhizosphere actinomycetes

The rhizosphere inoculum (10^{-5} dilution) was plated thinly with Kenknight's agar (KA). Since actinomycetes grow very slowly, plates were incubated for about two weeks for the colonies to start sporulation making them much denser. Based on the number of colonies formed, their number per gram of soil was determined.

Rhizosphere phosphobacteria

Apetite agar (AA) was the specific medium used for isolating phosphobacteria. To every 100 ml of the molten medium, 6 ml of 10% K_2HPO4 and 4 ml of 10% $CaCl_2$ (both sterilized separately) were added so that the phosphate is precipitated in the medium. Phosphate solubilizing bacteria helps to dissolve this precipitate, indicated by a clear halo around such colonies.

One ml of 10^{-5} dilution inoculum was taken in a Petriplate and about 12-15 ml of the precipitated medium was poured on to it and mixed well to

form a thin layer. This was repeated with all samples in duplicate. The plates were incubated at 28 ±1°C for about four days. Colonies showing the halo were counted as phophobacteria. Different strains could be identified by their colony colour and shape. The total number per gram of soil was calculated.

Rhizosphere azotobacter

Azotobacter was isolated from soil by culturing concentrated inoculum (10^{-5}) on Jensen's Agar (JA). Due to the absence of any nitrogen source in the medium only nitrogen fixers can grow well on this medium.

2.3.7. Purification of colonies

The isolated colonies were purified using the following steps. A small bit of each colony was transferred carefully onto thin clear potato dextrose agar plates in the case of fungi, actinomycetes and yeasts. NA plates in the case of bacteria. Bacteria and yeasts were streaked on the plates and isolated colonies growing from single cells were picked. Actively growing and isolated single hyphal tip of fungi or actinomycetes were carefully removed and introduced at the center of plates with PDA and allowed to grow. The transfer was done very carefully under aseptic conditions to avoid further contamination while transferring. These were allowed to grow for 4 to 5 days.

Once the organisms establish themselves in the culture plates in pure form, they were subcultured on PDA, NA or King's B medium [Appendix 13.7] slants on which they were maintained for longer duration without any contamination or deterioration of vigour, by periodic subculturing.

2.3.8. Maintenance of pure cultures

The isolated cultures were maintained on potato dextrose agar medium (PDA) for fungi, actinomycetes and yeasts. Bacteria (including phosphobacteria) were maintained on nutrient agar (NA) medium. The

composition and the method of preparation of these media are given in Appendices 13.8 and 13.9

2.4. Observation and results

The isolated organisms included 144 fungal isolates, 118 bacterial, 7actinomycetes and 4 yeast isolates from phylloplane and cauloplane sources and 12 phosphobacterial and other bacterial isolates from rhizosphere sources.

The observation and results of the various experiments described in section 2.3 are presented under the respective headings.

2.4.1. Seasonal variation in phylloplane bacteria

In the present study, it was observed that all the 300 phylloplane samples collected from 6 regions namely Nettana, Padiyoor, Palappilly, Chethakkal, Lahai and Vaikundam harboured a variety of microorganisms. Bacteria and fungi were present in all the samples whereas the number of actinomycetes and yeasts were very low or absent in many cases. Table 2.4.1.1 shows the populations of bacteria present in the phylloplane samples collected from the different regions during summer, rainy and post-monsoon seasons.

Sample Name	Mature / Immature	Type	Location	Mean Population [cfu x 10^2]		
				Summer	Monsoon	Post-monsoon
NET/PHY-B/105/M	Mature	RRII 105	Nettana	5.40	19.0	48.6
NET/PHY-B/105/IM	Immature	RRII 105	Nettana	11.0	26.0	55.8
NET/PHY-B/260/M	Mature	PB 260	Nettana	13.6	20.4	32.2
PAD/PHY-B/105/M	Mature	RRII 105	Padiyoor	18.0	28.0	51.4
PAD/PHY-B/105/IM	Immature	RRII 105	Padiyoor	9.8	22.4	42.6
PAL/PHY-B/105/M	Mature	RRII 105	Palappilly	3.6	15.2	17.0
PAL/PHY-B/105/IM	Immature	RRII 105	Palappilly	1.8	8.6	13.4
PAL/PHY-B/260/M	Mature	PB 260	Palappilly	3.4	10.6	14.2
PAL/PHY-B/260/IM	Immature	PB 260	Palappilly	3.6	5.4	11.8
CHE/PHY-B/105/M	Mature	RRII 105	Chethackal	2.6	4.0	5.0
CHE/PHY-B/105/IM	Immature	RRII 105	Chethackal	1.8	2.8	6.2
CHE/PHY-B/260/M	Mature	PB 260	Chethackal	2.0	3.8	6.0
CHE/PHY-B/260/IM	Immature	PB 260	Chethackal	13.4	19.0	24.2
LAH/PHY-B/105/M	Mature	RRII 105	Lahai	4.4	12.4	16.0
LAH/PHY-B/105/IM	Immature	RRII 105	Lahai	13.4	24.4	26.6
LAH/PHY-B/260/M	Mature	PB 260	Lahai	11.4	14.8	16.2
VAI/PHY-B/105/M	Mature	RRII 105	Vaikundam	10.0	18.6	47.0
VAI/PHY-B/105/IM	Immature	RRII 105	Vaikundam	8.6	14.4	26.2
VAI/PHY-B/260/M	Mature	PB 260	Vaikundam	8.0	11.6	16.2
VAI/PHY-B/260/IM	Immature	PB 260	Vaikundam	8.8	17.2	29.6

Table 2.4.1.1: Occurrence of phylloplane bacteria

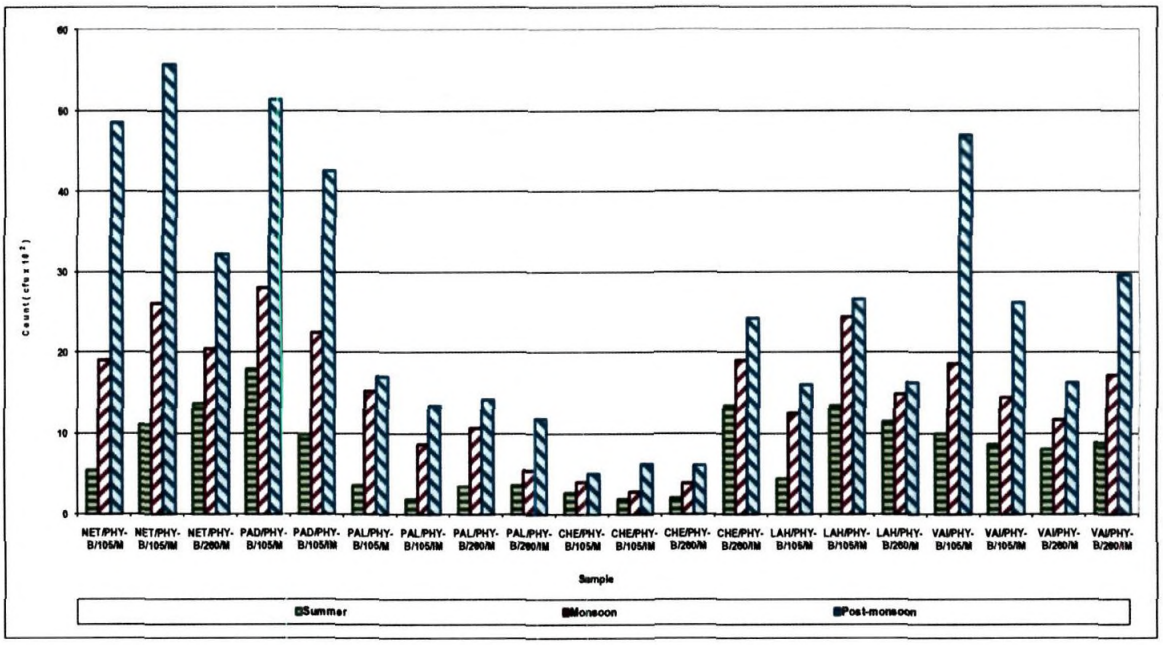

Fig.2.4.1.1 – Seasonal variation of phylloplane bacteria in rubber trees

During the summer season, microbial population was very low in the phylloplane of both mature and immature rubber plants. It was higher during the rainy season. The population was found to be most abundant during the post-monsoon period as can be observed from Fig 2.4.1.1.

Maximum count of bacteria (56 X 10^2) was recorded in immature RRII 105 plants at Nettana. When all the three seasons are considered, mature trees of RRII 105 recorded higher bacterial population at Padiyoor compared to other locations. However, post-monsoon population was high also at Nettana, Padiyoor and Vaikundam. There was very little variation in phylloplane bacteria on mature trees at Chethackal [Fig.2.4.1.2].

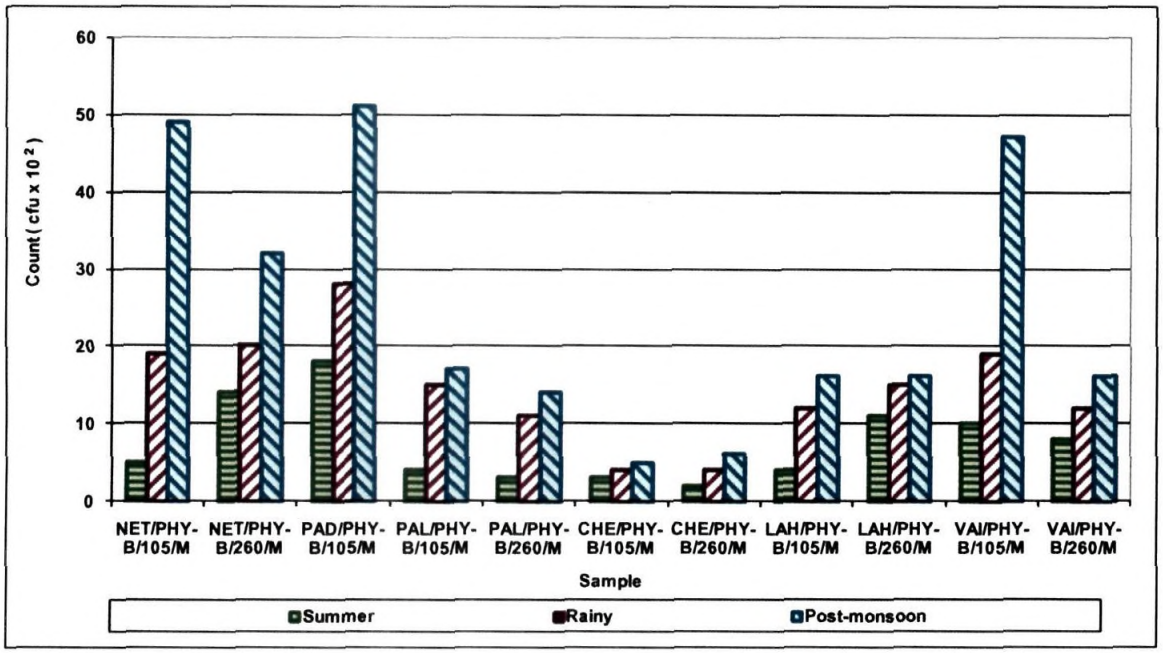

Fig.2.4.1.2 - Seasonal variation of phylloplane bacteria in mature rubber trees

Mature trees of RRII 105 recorded higher bacterial population at Padiyoor compared to other locations in all the three seasons. However, post-monsoon results were high at Nettana, Padiyoor and Vaikundam [Fig. 2.4.1.2]

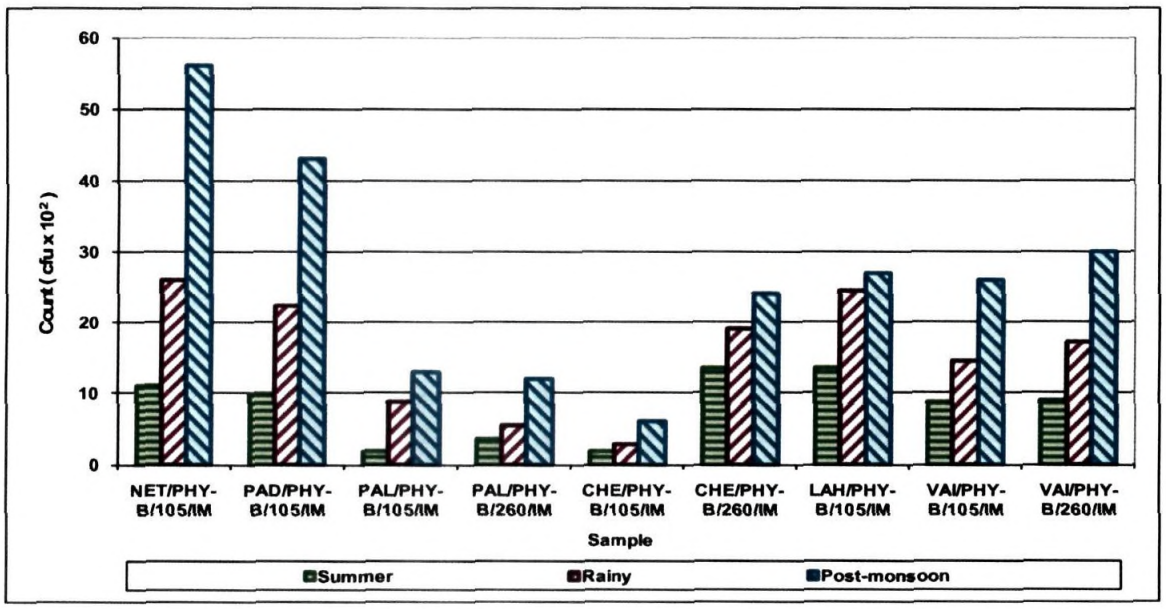

Fig.2.4.1.3 - Seasonal variation of phylloplane bacteria in immature rubber trees

In the immature trees, phylloplane bacterial count was higher at Nettana and Padiyoor across the seasons (Fig.2.4.1.3). In general, immature trees harbour higher population of phylloplane bacteria.

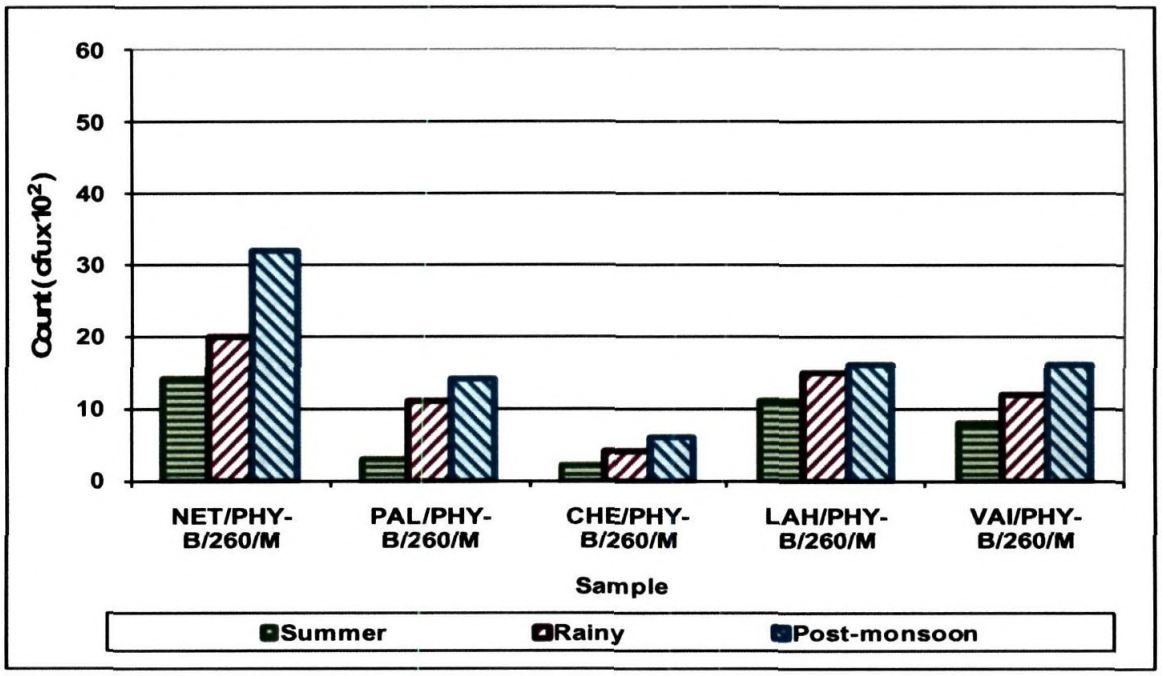

Fig.2.4.1.4 - Seasonal variation of phylloplane bacteria in PB 260 mature rubber trees

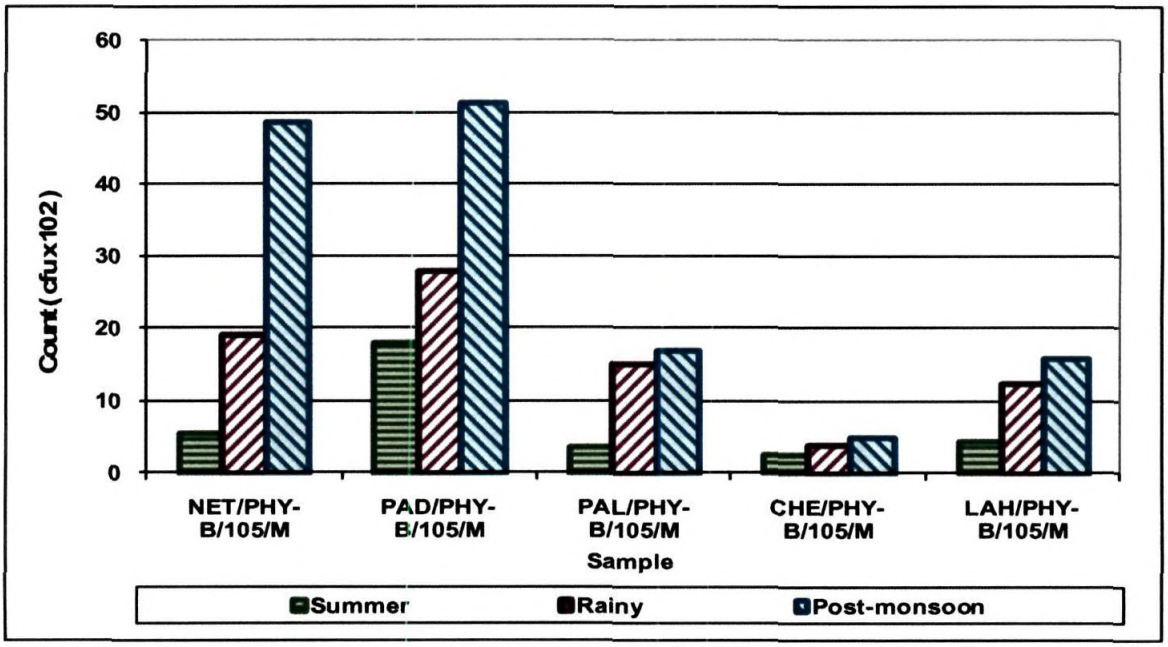

Fig.2.4.1.5 - Seasonal variation of phylloplane bacteria in RRII 105 mature rubber trees

Between the two clones, higher population counts were observed for RRII 105 at Padiyoor and Vaikundam while, for PB 260 it was at Nettana (Fig. 2.4.1.4 and 2.4.1.5). The trees of clone RRII 105 supported higher phylloplane population than that of PB 260.

Analysis of seasonal variation:

Locationwise analysis of seasonal variation was attempted for each clone in relation to their age (Tables 2.4.1.2 to 2.4.1.7). It was found that there was significant (F =118.80) variation in phylloplane bacteria between seasons in RRII 105 mature trees at Nettana. Immature RRII105 trees at Nettana also had significant (F =11.85) seasonal variation. But seasonal variation was not significant (F =4.38) in the case of PB 260 mature trees.

Seasonal variation of phylloplane bacteria at Padiyoor, for mature as well as immature trees of RRII 105 was significant (F values 15.72 and 28.55 respectively).

At Palappilly RRII 105 mature and immature and PB 260 mature and immature trees had significant seasonal variation of phylloplane bacteria (F values 7.8, 8.81, 15.75 and 6.28 respectively).

Seasonal variation of phylloplane bacteria at Chethackal showed no significant variation in mature RRII105 trees while it was significant in immature trees of RRII 105(F value 8.76). Highly significant variation was observed in PB 260 mature trees (F value 22.29). Significant variation was observed in the case of immature PB 260 trees (F value 5.17) also.

Phylloplane bacteria at Lahai had highly significant seasonal variation in RRII 105 mature trees (F value 22.89) while no significance was observed in immature trees of RRII 105 trees (F value 1.8) and mature PB 260 trees (F value 2.33).

Highly significant variation was present in phylloplane bacteria at Vaikundam (F value 198.72) in the case of RRII 105 mature rubber trees while it was moderate (F= 49.65) in RRII 105 immature trees. PB 260

ature trees showed less significance (*F*=5.27) while in immature trees ore significant variation was present (*F*=16.47).

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	5.4	13.60	11
Rainy	**19**	20.4	26
Post Monsoon	48.6	32.2	55.8
F(Variance ratio)	118.8	4.39	11.85
CD(P=0.05)	6.24	13.85	20.41

Table 2.4.1.2: Mean seasonal variation in phylloplane bacteria at Nettana

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	18	9.8
Rainy	**28**	22.4
Post Monsoon	51.4	42.6
F(Variance ratio)	15.72	28.55
CD(P=0.05)	13.32	9.54

Table 2.4.1.3: Mean seasonal variation in phylloplane bacteria at Padiyoor

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	3.6	3.40	1.8	3.60
Rainy	15.2	10.6	8.6	5.4
Post Monsoon	17	14.2	13.4	11.8
F(Variance ratio)	7.82	15.75	8.82	6.29
CD(P=0.05)	8.02	4.27	6.05	5.3

Table 2.4.1.4: Mean seasonal variation in phylloplane bacteria at Palappilly

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	2.6	2.00	1.8	13.40
Rainy	4	3.8	2.8	19
Post Monsoon	5	6	6.2	24.2
F(Variance ratio)	3.46	22.29	8.77	5.18
CD(P=0.05)	N.S	1.31	2.4	7.31

Table 2.4.1.5: Mean seasonal variation in phylloplane bacteria at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	4.4	11.40	13.4
Rainy	12.4	14.8	24.4
Post Monsoon	16	16.2	26.6
F(Variance ratio)	22.89	2.33	1.8
CD(P=0.05)	3.82	NS	NS

Table 2.4.1.6: Mean seasonal variation in phylloplane bacteria at Lahai

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	10	8.00	8.6	8.80
Rainy	18.6	11.6	14.4	17.2
Post Monsoon	47	16.2	26.2	29.6
F(Variance ratio)	198.72	5.28	49.65	16.47
CD(P=0.05)	4.23	5.51	3.92	7.94

Table 2.4.1.7: Mean seasonal variation in phylloplane bacteria at Vaikundam

Analysis on variation in relation to rubber clones:

The variation in population in relation to rubber clones for the three seasons are presented in Tables 2.4.1.8 to 2.4.1.13

In phylloplane bacteria, the variation in population was significant in RRII 105 and PB 260 mature trees in Nettana and Lahai (*t*-value -2.02 and -5.68 respectively) during the summer season. But clonal variation

was not significant in phylloplane bacterial population in the regions, Palappilly, Chethackal and Vaikundam during the summer season.

There was significant clonal variation in phylloplane bacterial population on mature rubber trees in Nettana (*t*-value -0.33) and Lahai (*t*-value -0.96) during the monsoon season while no significant clonal variation of bacterial population was observed during monsoon in Palappilly, Chethackal and Vaikundam.

Mature trees of Chethackal and Lahai showed significant clonal variation in bacterial population during the post monsoon season (*t*-value of -1.29 and -0.09 respectively) while no significant variation of the same was observed in Nettana, Palappilly and Vaikundam.

Bacterial population in immature rubber trees showed significant clonal variation at Palappilly (*t*-value -1.27), Chethackal (*t*-value -3.96) and Vaikundam (*t*-value -0.07) during the summer season. During the monsoon season and post monsoon season there was no significant clonal variation at Palappilly in phylloplane bacterial population. Significant variation of bacterial population was observed in the two clones at Chethackal and Vaikundam during the monsoon (*t*-values -7.15 and -0.76 respectively) and post monsoon (*t*-values -8.07 and -1.84 respectively).

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	5.4	7.8	13.6	74.3	-2.02
Palappilly	3.6	1.3	3.4	10.3	0.13
Chethackal	2.6	1.3	2	1	0.88
Lahai	4.4	2.3	11.4	5.3	-5.68
Vaikundam	10	10	8	6.5	1.1

Table 2.4.1.8: Clonal variation in phylloplane bacteria during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	19	1	20.4	87.8	-0.33
Palappilly	15.2	54.7	10.6	14.8	1.23
Chethackal	4	2.5	3.8	1.2	0.23
Lahai	12.4	16.3	14.8	14.7	-0.96
Vaikundam	18.6	15.8	11.6	10.8	3.03

Table 2.4.1.9: Clonal variation in phylloplane bacteria during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	45.6	52.8	32.2	140.7	2.64
Palappilly	17	45.5	14.2	3.7	0.89
Chethackal	5	2.5	6	0.5	-1.29
Lahai	16	4.5	16.2	19.2	-0.09
Vaikundam	47	2.5	16.2	30.7	11.95

Table 2.4.1.10: Clonal variation in phylloplane bacteria during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	1.8	0.7	3.6	9.3	-1.27
Chethackal	1.8	1.7	13.4	41.3	-3.96
Vaikundam	8.6	12.3	8.8	27.7	-0.07

Table 2.4.1.11: Clonal variation in phylloplane bacteria during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	8.6	15.8	5.4	8.8	1.44
Chethackal	2.8	1.7	19	24	-7.15
Vaikundam	14.4	2.3	17.2	64.7	-0.76

Table 2.4.1.12: Clonal variation in phylloplane bacteria during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	13.4	41.3	11.8	26.2	0.44
Chethackal	6.2	5.7	24.2	19.2	-8.07
Vaikundam	26.2	9.7	29.6	7.3	-1.84

Table 2.4.1.13: Clonal variation in phylloplane bacteria during post-monsoon season on mature trees

2.4.2. Seasonal variation in phylloplane fungi

The variation in phylloplane fungi during the summer, rainy and post-monsoon seasons are presented in Table 2.4.2.1. The fungal population was observed to be higher during the post-monsoon season.

Although there was a general trend towards increase of fungal population from summer to rainy and finally post-monsoon, the difference was more at Nettana and Chethackal. The overall seasonal variation of phylloplane fungi in rubber plants is shown in Figure 2.4.2.1.

In general, mature trees harboured higher population of phylloplane fungi than immature trees (Figure 2.4.2.2 and 2.4.2.3).

Fungal population on the leaves of immature rubber trees of clone RRII 105 was high at Lahai during all the three seasons when compared to

the other regions (Figure 2.4.2.3). In Vaikundam immature trees of clone PB 260 showed higher population during post monsoon season.

Of all the mature trees of both clones, maximum number of phylloplane fungi (13 X 10^2) was observed in mature trees, at Nettana for RRII 105 and at Chethackal for PB 260 during post monsoon season as seen in Fig 2.4.2.4 and 2.4.2.5.

Sample Name	Mature / Immature	Type	Location	Mean Population [c f u x 10^2]		
				Summer	Monsoon	Post-monsoon
NET/PHY-F/105/M	Mature	RRII 105	Nettana	3.00	8.00	13.00
NET/PHY-F/105/IM	Immature	RRII 105	Nettana	2.04	4.00	5.00
NET/PHY-F/260/M	Mature	PB 260	Nettana	3.00	5.00	10.98
PAD/PHY-F/105/M	Mature	RRII 105	Padiyoor	3.00	6.00	7.98
PAD/PHY-F/105/IM	Immature	RRII 105	Padiyoor	3.00	7.02	8.02
PAL/PHY-F/105/M	Mature	RRII 105	Palappilly	4.00	7.04	9.00
PAL/PHY-F/105/IM	Immature	RRII 105	Palappilly	0.34	4.04	5.00
PAL/PHY-F/260/M	Mature	PB 260	Palappilly	3.00	6.02	7.00
PAL/PHY-F/260/IM	Immature	PB 260	Palappilly	3.00	5.00	6.02
CHE/PHY-F/105/M	Mature	RRII 105	Chethackal	2.00	3.00	4.00
CHE/PHY-F/105/IM	Immature	RRII 105	Chethackal	1.06	2.00	3.02
CHE/PHY-F/260/M	Mature	PB 260	Chethackal	3.02	7.02	13.00
CHE/PHY-F/260/IM	Immature	PB 260	Chethackal	1.04	2.00	4.00
LAH/PHY-F/105/M	Mature	RRII 105	Lahai	5.00	8.00	9.00
LAH/PHY-F/105/IM	Immature	RRII 105	Lahai	5.00	12.02	15.00
LAH/PHY-F/260/M	Mature	PB 260	Lahai	3.02	4.00	5.00
VAI/PHY-F/105/M	Mature	RRII 105	Vaikundam	3.02	6.04	7.00
VAI/PHY-F/105/IM	Immature	RRII 105	Vaikundam	1.02	5.00	9.00
VAI/PHY-F/260/M	Mature	PB 260	Vaikundam	3.00	6.00	7.00
VAI/PHY-F/260/IM	Immature	PB 260	Vaikundam	3.04	7.00	14.00

Table 2.4.2.1: Occurrence of phylloplane fungi

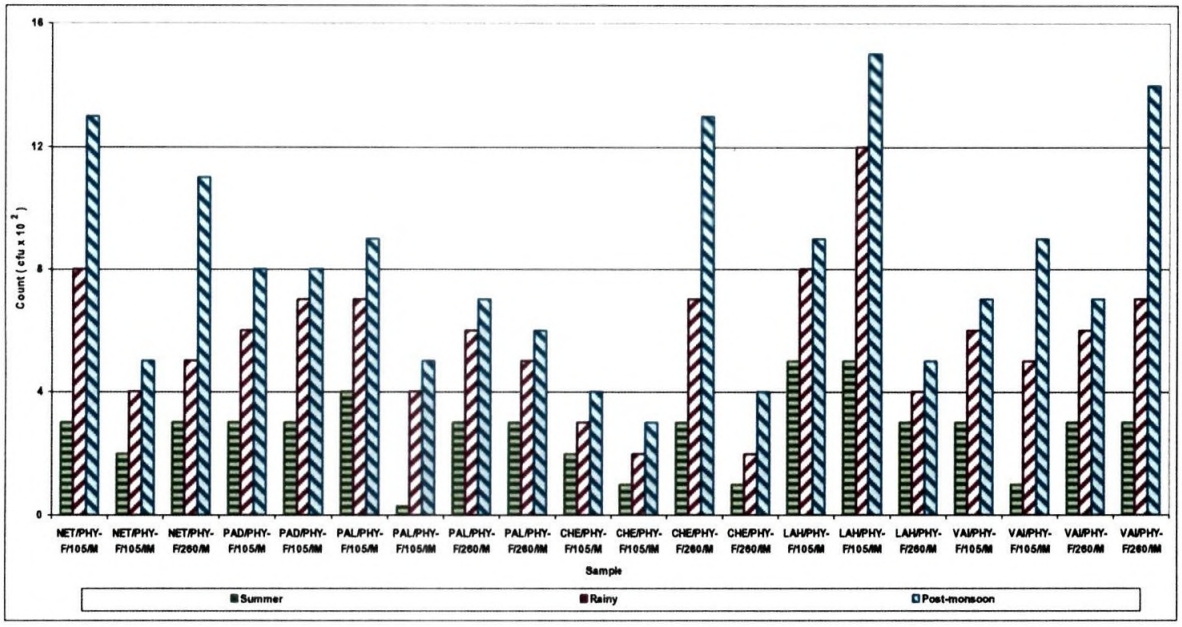

Fig.2.4.2.1 - Seasonal variation of phylloplane fungi in rubber trees

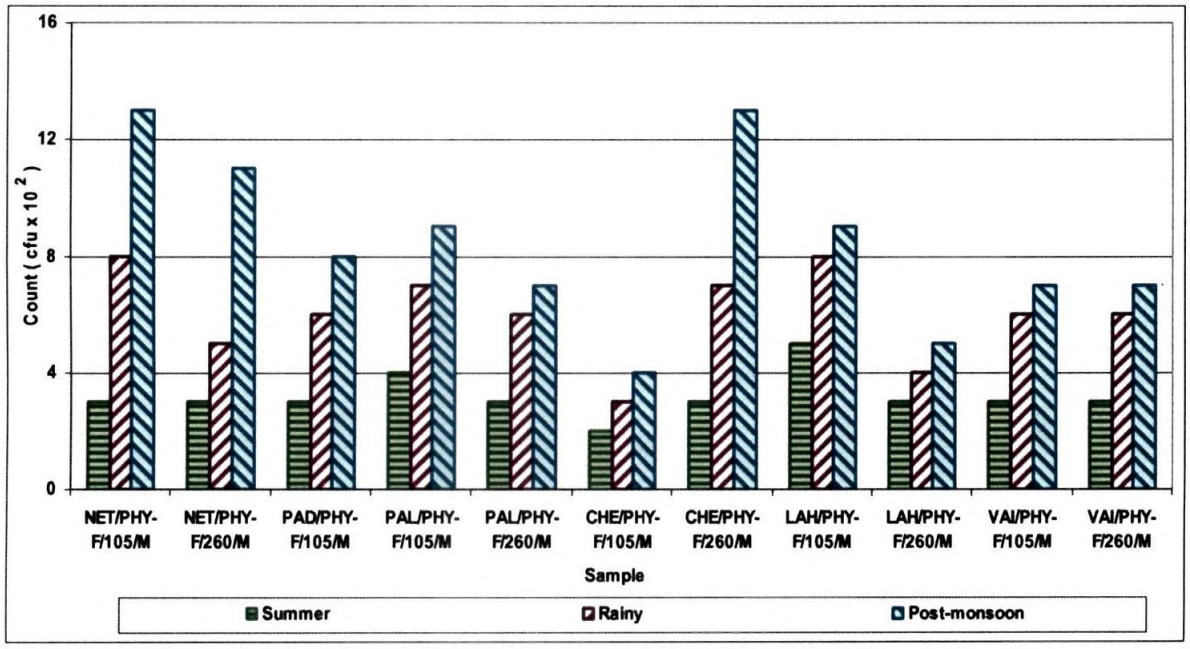

Fig.2.4.2.2 - Seasonal variation of phylloplane fungi in mature rubber trees

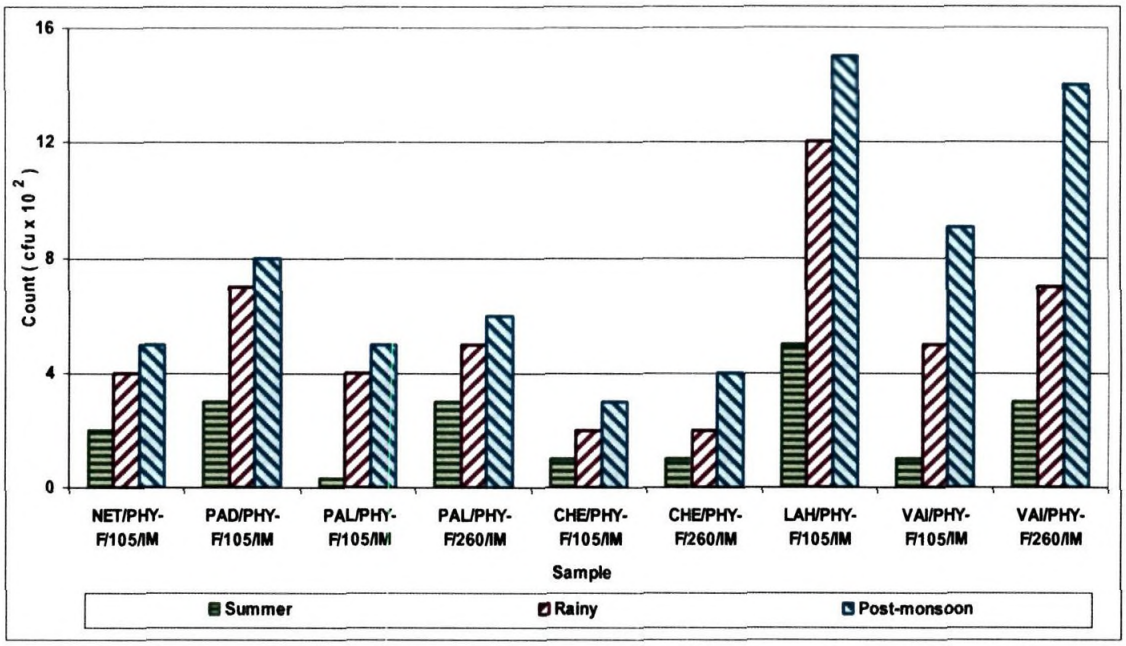

Fig.2.4.2.3 - Seasonal variation of phylloplane fungi in immature rubber trees

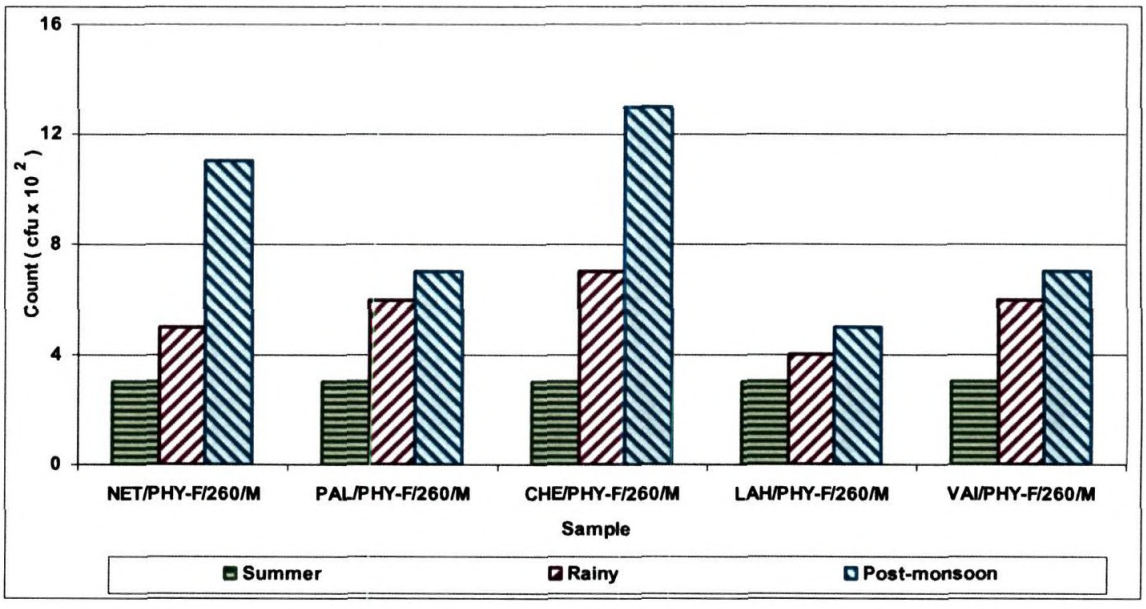

Fig.2.4.2.4 - Seasonal variation of phylloplane fungi in PB 260 mature rubber trees

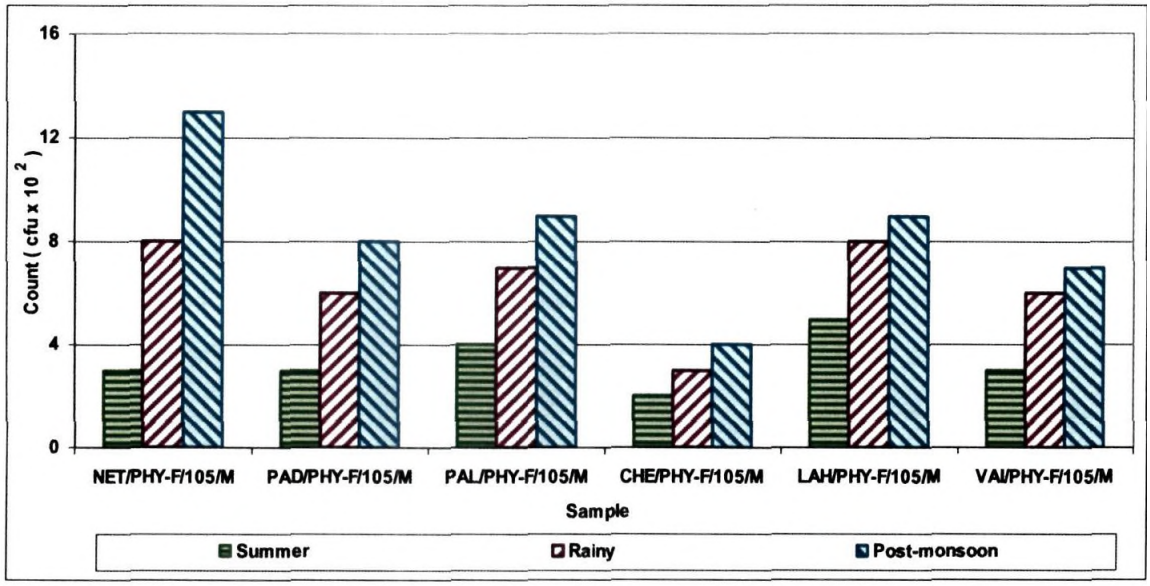

Fig.2.4.2.5 - Seasonal variation of phylloplane fungi in RRII 105 mature rubber trees

Fig 2.4.2.4 clearly indicates that the occurrence of phylloplane fungi in PB 260 mature rubber trees was uniform in all the regions, during the summer season. But there was variation between locations in all the three seasons, in the case of RRII 105 mature trees (Fig. 2.4.2.5).

Analysis of seasonal variation:

The seasonal variation in population of phylloplane fungi are presented in Tables 2.4.2.2 to 2.4.2.7

It was observed that population of phylloplane fungi at Nettana showed significant variation for RRII 105(mature and immature and PB 260 (mature) trees (*F* value 8.65, 16.13 and 20.2 respectively). At Padiyoor there was significant variation in RRII 105 mature and immature trees (*F* = 8.09 and 7.83 respectively).

Variation in fungal population at Palappilly was significant in RRII 105 mature and immature and PB 260 immature trees (*F* value 26.38, 104.26 and 34.63 respectively) while no significance was observed in mature PB 260 trees (*F* value 3.11).

Significant seasonal variation was observed in samples from Chethackal RRII 105 mature, immature and PB 260 mature trees (*F* value 6, 14.27 and 84.01 respectively). But immature PB 260 trees showed no significant seasonal variation.

Slightly significant variation was observed in mature and immature RRII 105 trees (*F* value 5 and 6.47) at Lahai while no significant variation was observed in mature PB 260 trees.

All the phylloplane fungi samples from Vaikundam namely RRII 105 mature and immature as well as PB 260 mature and immature had significant seasonal variation (*F* value 8.86, 11.93, 19.57 and 18.71 respectively).

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	3	3.00	2.04
Rainy	7.2	5	4
Post Monsoon	13	10.98	5
F(Variance ratio)	8.65	20.2	16.13
CD(P=0.05)	5.26	2.85	1.16

Table 2.4.2.2: Mean seasonal variation in phylloplane fungi at Nettana

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	3	3
Rainy	6	7.02
Post Monsoon	7.98	8.02
F(Variance ratio)	8.09	7.84
CD(P=0.05)	2.72	2.92

Table 2.4.2.3: Mean seasonal variation in phylloplane fungi at Padiyoor

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	4	3	0.34	3.00
Rainy	7.04	6.02	4.04	5
Post Monsoon	9	7	5	6.02
F(Variance ratio)	26.39	3.12	104.27	34.64
CD(P=0.05)	1.51	3.64	0.74	0.8

Table 2.4.2.4: Mean seasonal variation in phylloplane fungi at Palappilly

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	2	3.02	1.06	1.04
Rainy	3	7.02	2	2
Post Monsoon	4	13	3.02	4
F(Variance ratio)	6	84.01	14.27	3.8
CD(P=0.05)	1.26	1.69	0.79	2.39

Table 2.4.2.5: Mean seasonal variation in phylloplane fungi at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	5	3.02	5
Rainy	8	4	12.02
Post Monsoon	9	5	15
F(Variance ratio)	5	3.67	6.47
CD(P=0.05)	2.89	1.59	6.22

Table 2.4.2.6: Mean seasonal variation in phylloplane fungi at Lahai

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	3.02	3.00	1.02	3.04
Rainy	6.04	6	5	7
Post Monsoon	7	7	9	14
F(Variance ratio)	8.86	19.58	11.94	18.71
CD(P=0.05)	2.15	1.45	3.56	3.95

Table 2.4.2.7: Mean seasonal variation in phylloplane fungi at Vaikundam

Analysis of variation in relation to rubber clones:

The variation in population of phylloplane fungi in relation to rubber clones for the three seasons are presented in Tables 2.4.2.8 to 2.4.2.13

It was found that phylloplane fungal population in the mature trees of the two clones in summer showed significant variation in Chethackal (*t* value -2.28) and Vaikundam (*t* value 0.03) and Nettana while no significant variation was observed in Palappilly and Lahai. During the rainy season variation was significant in Chethackal and Vaikundam (*t* value -6.35 and 0.04 respectively) while at Nettana, Palappilly and Lahai it was not significant. In the post monsoon season also, variation was significant at Chethackal and Vaikundam (*t* value -10.06 and 0 respectively).

With regard to the phylloplane fungi on the immature of rubber trees during the summer season, variation was signlflcant at Palappilly and Vaikundam (*t* value -8.22 and -45.17 respectively). No significant variation was observed at Chethackal. But in the rainy as well as post monsoon seasons, variation was significant at Chethackal and Vaikundam.

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	3	4	3	0.5	0
Palappilly	4	1.5	3	2.5	1.12
Chethackal	2	0.5	3.02	0.502	-2.28
Lahai	5	1.5	3.02	0.502	3.13
Vaikundam	3.02	1.052	3	1.5	0.03

Table 2.4.2.8: Clonal variation in phylloplane fungi during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	8	17.5	5	1	1.56
Palappilly	7.04	1.108	6.02	5.402	0.89
Chethackal	3	0.5	7.02	1.502	-6.35
Lahai	8	1.5	4	2	4.78
Vaikundam	6.04	4.748	6	0.82	0.04

Table 2.4.2.9: Clonal variation in phylloplane fungi during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	13	15	10.98	11.302	0.88
Palappilly	9	1	7	13	1.20
Chethackal	4	1.5	13	2.5	-10.06
Lahai	9	10	5	1.5	2.64
Vaikundam	7	1.5	7	1	0

Table 2.4.2.10: Clonal variation in phylloplane fungi during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	0.34	0.023	3	0.5	-8.22
Chethackal	1.06	0.008	1.04	0.003	0.43
Vaikundam	1.02	0.002	3.04	0.008	-45.17

Table 2.4.2.11: Clonal variation in phylloplane fungi during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	4.04	0.348	5	0.5	-2.33
Chethackal	2	0.5	2	1	0
Vaikundam	5	5.5	7	0.68	-1.80

Table 2.4.2.12: Clonal variation in phylloplane fungi during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	5	0.5	6.02	0.022	-3.16
Chethackal	3.02	0.502	4	8	-0.75
Vaikundam	9	14.5	14	24	-1.80

Table 2.4.2.13: Clonal variation in phylloplane fungi during post-monsoon season on immature trees

2.4.3. Seasonal variation in cauloplane bacteria

Seasonal variation of cauloplane bacteria can be clearly observed in Table 2-4-3-1. Highest count of cauloplane bacteria was reported from Padiyoor, RRII 105 mature tree samples ($235.2x10^2$) in the post monsoon season while least count was in samples from immature trees of RRII 105 (4.0×10^2) from Vaikundam during the summer season.

Sample Name	Mature / Immature	Type	Location	Mean Population [c f u x 10^2]		
				Summer	Monsoon	Post-monsoon
NET/CAU-B/105/M	Mature	RRII 105	Nettana	161.6	184.0	218.4
NET/CAU-B/105/IM	Immature	RRII 105	Nettana	70.4	89.6	104.0
NET/CAU-B/260/M	Mature	PB 260	Nettana	51.2	101.6	185.6
PAD/CAU-B/105/M	Mature	RRII 105	Padiyoor	40.8	100.0	235.2
PAD/CAU-B/105/IM	Immature	RRII 105	Padiyoor	16.8	51.2	75.2
PAL/CAU-B/105/M	Mature	RRII 105	Palappilly	56.8	76.0	139.2
PAL/CAU-B/105/IM	Immature	RRII 105	Palappilly	8.0	27.2	43.2
PAL/CAU-B/260/M	Mature	PB 260	Palappilly	40.8	55.2	91.0
PAL/CAU-B/260/IM	Immature	PB 260	Palappilly	14.4	24.8	42.4
CHE/CAU-B/105/M	Mature	RRII 105	Chethackal	53.6	96.8	129.6
CHE/CAU-B/105/IM	Immature	RRII 105	Chethackal	23.2	38.4	69.2
CHE/CAU-B/260/M	Mature	PB 260	Chethackal	29.6	72.0	112.0
CHE/CAU-B/260/IM	Immature	PB 260	Chethackal	24.0	35.2	64.8
LAH/CAU-B/105/M	Mature	RRII 105	Lahai	47.2	164.8	182.4
LAH/CAU-B/105/IM	Immature	RRII 105	Lahai	20.8	57.2	96.0
LAH/CAU-B/260/M	Mature	PB 260	Lahai	41.6	99.6	119.2
VAI/CAU-B/105/M	Mature	RRII 105	Vaikundam	12.8	25.2	171.2
VAI/CAU-B/105/IM	Immature	RRII 105	Vaikundam	4.0	16.8	24.0
VAI/CAU-B/260/M	Mature	PB 260	Vaikundam	8.0	27.2	73.6
VAI/CAU-B/260/IM	Immature	PB 260	Vaikundam	8.4	14.4	20.8

Table 2.4.3.1: Occurrence of cauloplane bacteria

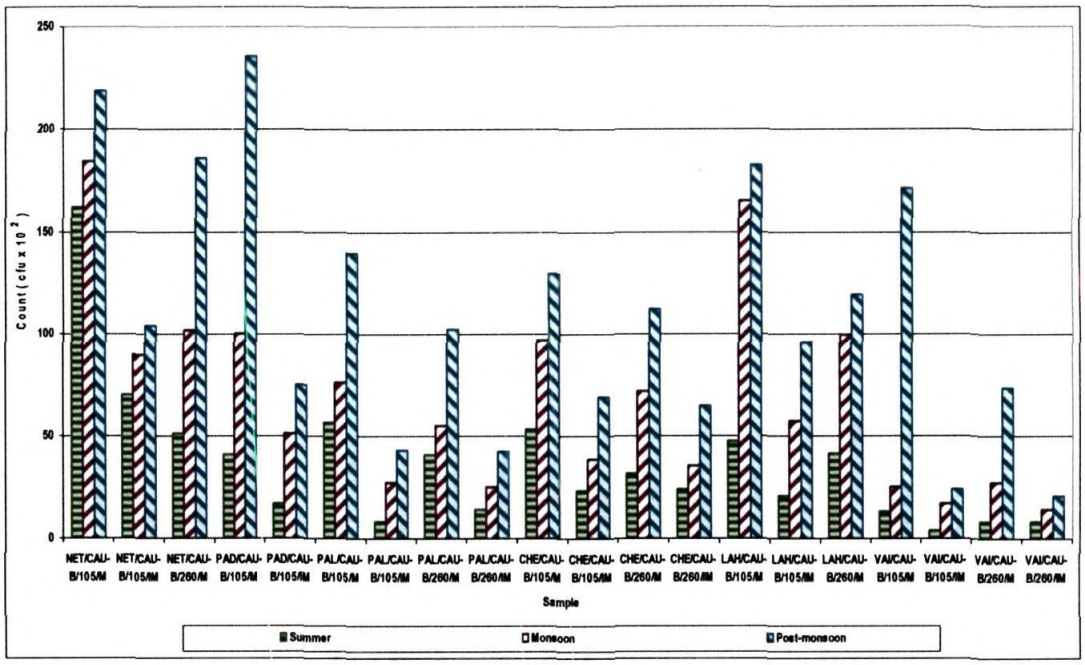

Fig.2.4.3.1 - Seasonal variation of cauloplane bacteria in rubber trees

Cauloplane bacterial population was highest in the post-monsoon period, followed by rainy seaon and least during the summer. Cauloplane bacterial count obtained from all the samples from the six regions under study is presented in Fig. 2.4.3.1.

In both clones of mature and immature trees there was uniformity in variation of cauloplane bacteria. There was less number of cauloplane bacteria during the summer which increased in the rainy season and was highest during the post monsoon period. This can be observed in Fig. 2.4.3.2 and Fig. 2.4.3.3. In general, cauloplane bacterial population was higher in mature trees than immature, across the seasons.

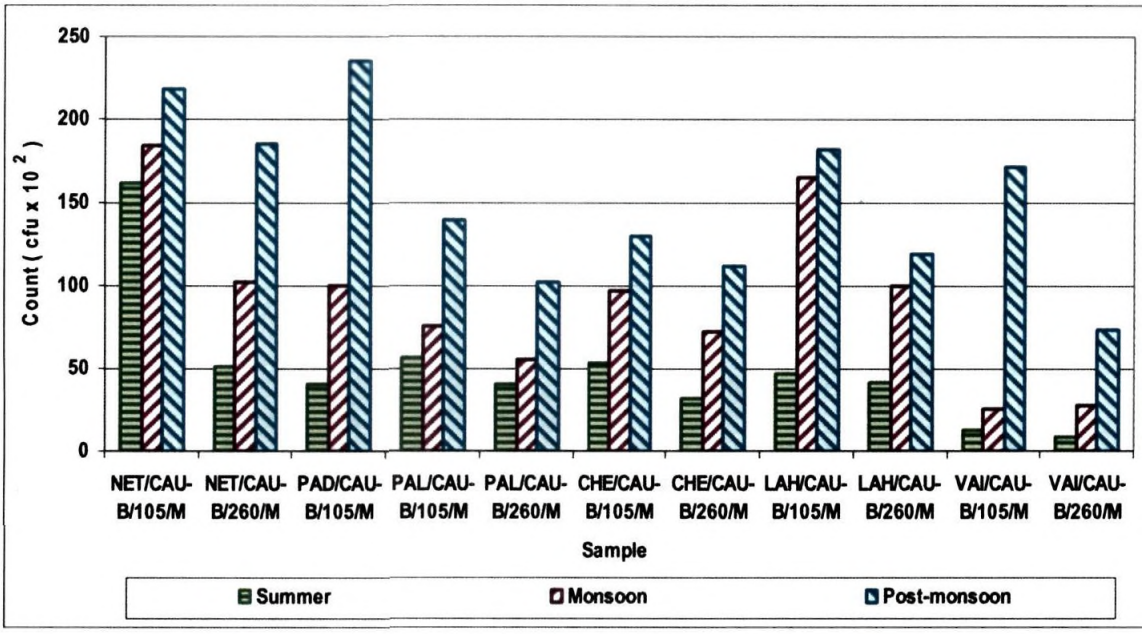

Fig.2.4.3.2 - Seasonal variation of cauloplane bacteria in mature rubber trees

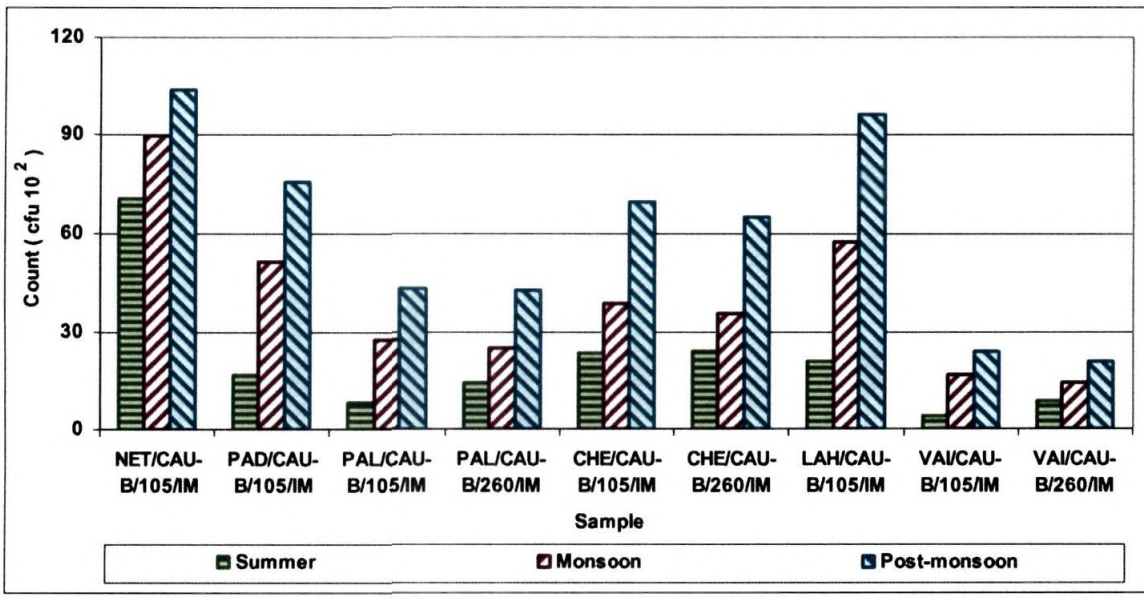

Fig.2.4.3.3 - Seasonal variation of cauloplane bacteria in immature rubber trees

Fig. 2.4.3.4 and Fig. 2.4.3.5 show the difference in the pattern of seasonal variation in RRII 105 and PB 260 clones of rubber. The population of cauloplane bacteria at Lahai during rainy season was close to that of post monsoon season in both the clones. In general, RRII 105 harboured higher population of cauloplane bacteria.

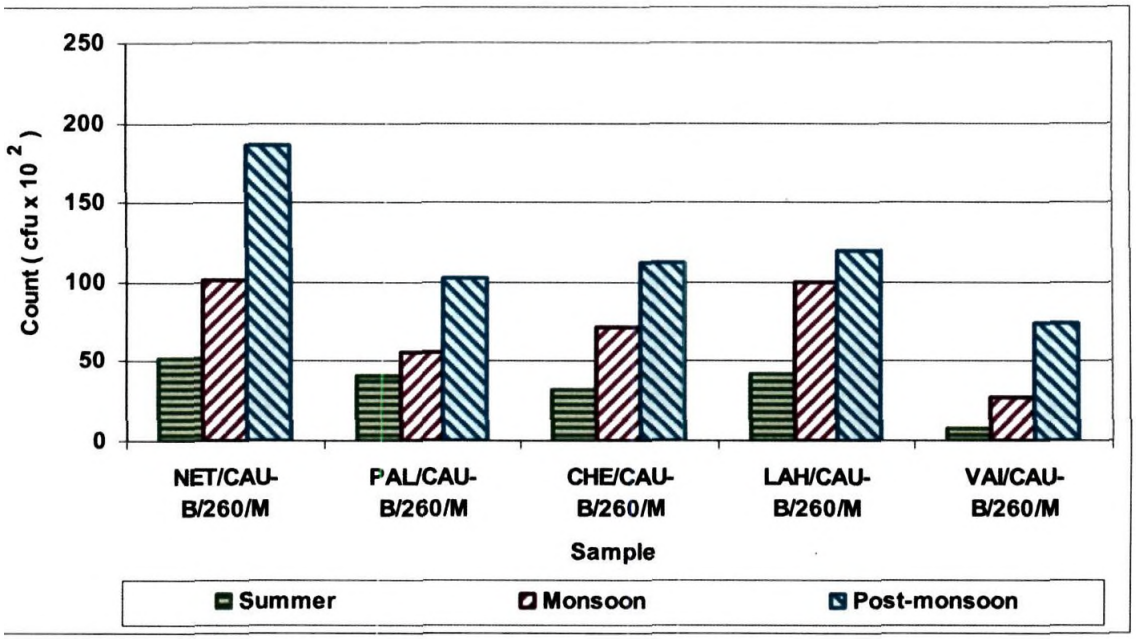

Fig.2.4.3.4 - Seasonal variation of cauloplane bacteria in PB 260 rubber trees

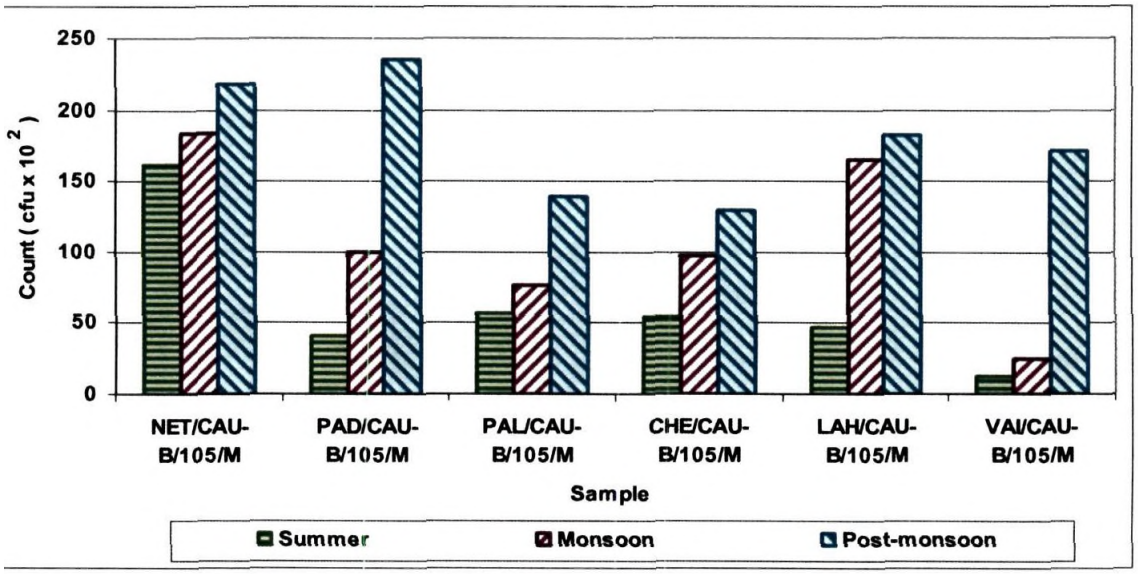

Fig.2.4.3.5 - Seasonal variation of cauloplane bacteria in RRII 105 rubber trees

Analysis of seasonal variation:

Analysis of the seasonal variation of cauloplane bacteria with regard to each clone in relation to their age are shown in Tables 2.4.3.2 to 2.4.3.7

At Nettana, seasonal variation of cauloplane bacteria was significant only in the case of PB 260 mature trees (*F* value 10.46). Both RRII 105 mature and immature trees had insignificant variation.

RRII 105 mature trees at Padiyoor showed slight significance in variation of cauloplane bacteria (*F* value 5.44) while in immature trees of the same clone the variation was more (*F* value 14.52).

At Palappilly, RRII 105 mature and immature as well as PB 260 mature and immature trees showed significant variation (*F* value 42.60, 17.23, 17.01 and 12.68 respectively).

Significant seasonal variation in cauloplane bacterial population was observed in Chethackal for RRII 105 mature, immature and mature PB 260 trees (*F* value 12.23, 7.66 and 10. 91 respectively).

PB 260 immature trees showed no significant variation (*F* value 3.70).

Mature and immature RRII 105 trees as well as PB 260 mature trees at Lahai had significant seasonal variation (*F* value 7.17, 44.71 and 27.11 respectively).

Seasonal variation of cauloplane bacteria at Vaikundam was significant in mature and immature trees of RRII 105 and PB 260 (*F* value 577.38, 22.40, 213.28 and 12.87 respectively).

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	161.6	51.20	70.4
Rainy	184	101.6	89.6
Post Monsoon	218.4	185.6	104
F(Variance ratio)	2.99	10.47	2.24
CD(P=0.05)	50.93	64.66	34.66

Table 2.4.3.2: Mean seasonal variation in caulopiane bacteria at Nettana

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	40.8	16.8
Rainy	100	51.2
Post Monsoon	235.2	75.2
F(Variance ratio)	5.45	14.53
CD(P=0.05)	131.59	23.73

Table 2.4.3.3: Mean seasonal variation in cauloplane bacteria at Padiyoor

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	56.8	40.8	8.00	14.40
Rainy	76	55.2	27.2	24.8
Post Monsoon	139.2	102.4	43.2	42.4
F(Variance ratio)	42.61	17.02	17.23	12.69
CD(P=0.05)	20.35	24.07	13.08	12.24

Table 2.4.3.4: Mean seasonal variation in cauloplane bacteria at Palappilly

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	53.6	29.60	23.2	24.00
Rainy	96.8	72	38.4	35.2
Post Monsoon	129.6	112	69.2	64.8
F(Variance ratio)	12.24	10.92	7.66	3.71
CD(P=0.05)	33.58	38.43	26.09	33.74

Table 2.4.3.5: Mean seasonal variation in cauloplane bacteria at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	47.2	41.60	20.8
Rainy	164.8	99.6	57.2
Post Monsoon	182.4	119.2	96
F(Variance ratio)	7.18	27.11	44.72
CD(P=0.05)	84.55	23.88	17.33

Table 2.4.3.6: Mean seasonal variation in cauloplane bacteria at Lahai

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	12.8	8.00	0.8	8.40
Rainy	25.2	27.2	16.8	14.4
Post Monsoon	171.2	73.6	24	20.8
F(Variance ratio)	577.38	213.28	22.4	12.88
CD(P=0.05)	11.3	7.12	7.73	5.33

Table 2.4.3.7: Mean seasonal variation in cauloplane bacteria at Vaikundam

Analysis of variation in relation to rubber clones:

The variation in cauloplane bacterial population in relation to rubber clones for the three seasons are presenred in Tables 2.4.3.8 to 2.4.3.13

It was observed that mature rubber trees had no significant variation in bacterial population in the cauloplane during the summer season at Nettana, Palappilly, Chethackal, Lahai and Vaikundam.

During the monsoon season cauloplane bacteria in the two clones showed significant variation only at Vaikundam (*t* value, -0.39) while at Nettana, Palappilly, Chethackal and Lahai the variation was not significant.

No significant variation in cauloplane bacteria was observed in the two mature clones of RRII 105 and PB 260 during the post monsoon season at Nettana, Palappilly, Chethackal, Lahai and Vaikundam.

There was significant variation between the immature trees of the two clones during the summer season at Palappilly (*t* value -2.67) and Chethackal (*t* value -0.07). But during the monsoon and post monsoon season there was no significant variation.

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	161.6	3212.8	51.2	395.2	4.11
Palappilly	56.8	43.2	40.8	27.2	4.26
Chethackal	53.6	244.8	29.6	164.8	2.65
Lahai	47.2	155.2	41.6	92.8	0.80
Vaikundam	12.8	19.2	8	16	1.81

Table 2.4.3.8: Clonal variation in cauloplane bacteria during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	184	456	101.6	2220.8	3.56
Palappilly	76	144	55.2	35.2	3.47
Chethackal	96.2	363.2	72	368	2.05
Lahai	164.8	6443.2	99.6	260.8	1.78
Vaikundam	25.2	123.2	27.2	11.2	-0.39

Table 2.4.3.9: Clonal variation in cauloplane bacteria during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	218.4	428.8	185.6	3988.8	1.10
Palappilly	139.2	467.2	102.4	852.8	2.265
Chethackal	129.6	1172.8	112	1800	0.72
Lahai	182.4	4692.8	119.2	547.2	1.95
Vaikundam	171.2	59.2	73.6	52.8	20.62

Table 2.4.3.10: Clonal variation in cauloplane bacteria during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	8	8	14.4	20.8	-2.67
Chethackal	23.2	203.2	24	440	-0.07

Table 2.4.3.11: Clonal variation in cauloplane bacteria during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	27.2	75.2	24.8	35.2	0.51
Chethackal	38.4	84.8	35.2	411.2	0.32
Vaikundam	16.8	51.2	14.4	20.8	0.63

Table 2.4.3.12: Clonal variation in cauloplane bacteria during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	43.2	187.2	42.4	180.8	0.09
Chethackal	69.2	787.2	4.8	947.2	0.74
Vaikundam	24	40	20.8	19.2	0.93

Table 2.4.3.13: Clonal variation in cauloplane bacteria during post-monsoon season on immature trees

2.4.4. Seasonal variation in cauloplane fungi

Cauloplane fungi were present in all the 300 bark samples taken during three seasons (Table 2.4.4.1). The master data table of cauloplane fungal studies indicates the seasonal variation in the 2 clones of two age groups belonging to six different locations in summer, rainy and post monsoon seasons.

Pronounced seasonal variation was exhibited in the samples from Nettana, Palappilly and Vaikundam in both the clones of rubber as can be seen in Figure 2.4.4.1, but such wide variation was not observed in the samples from Chethackal. Samples from immature trees of RRII 105 from Lahai also did not show much variation ($9.6X10^2$ in summer, $10.4 X 10^2$ in rainy and $12 X10^2$ in post monsoon seasons).

Sample Name	Mature / Immature	Type	Location	Mean Population [c f u x 10^2]		
				Summer	Monsoon	Post-monsoon
NET/CAU-F/105/M	Mature	RRII 105	Nettana	9.6	67.6	98.4
NET/CAU-F/105/IM	Immature	RRII 105	Nettana	8.8	51.2	111.2
NET/CAU-F/260/M	Mature	PB 260	Nettana	16.0	56.8	96.0
PAD/CAU-F/105/M	Mature	RRII 105	Padiyoor	12.4	32.0	56.0
PAD/CAU-F/105/IM	Immature	RRII 105	Padiyoor	8.4	25.2	49.6
PAL/CAU-F/105/M	Mature	RRII 105	Palappilly	14.4	30.4	36.0
PAL/CAU-F/105/IM	Immature	RRII 105	Palappilly	7.2	18.4	32.8
PAL/CAU-F/260/M	Mature	PB 260	Palappilly	14.4	35.2	46.4
PAL/CAU-F/260/IM	Immature	PB 260	Palappilly	15.2	28.0	95.2
CHE/CAU-F/105/M	Mature	RRII 105	Chethackal	8.8	12.0	16.8
CHE/CAU-F/105/IM	Immature	RRII 105	Chethackal	8.8	10.4	14.4
CHE/CAU-F/260/M	Mature	PB 260	Chethackal	6.8	7.2	8.0
CHE/CAU-F/260/IM	Immature	PB 260	Chethackal	2.4	5.6	8.0
LAH/CAU-F/105/M	Mature	RRII 105	Lahai	8.0	32.0	40.0
LAH/CAU-F/105/IM	Immature	RRII 105	Lahai	9.6	10.4	12.0
LAH/CAU-F/260/M	Mature	PB 260	Lahai	12.8	23.2	55.2
VAI/CAU-F/105/M	Mature	RRII 105	Vaikundam	9.6	25.6	49.6
VAI/CAU-F/105/IM	Immature	RRII 105	Vaikundam	4.0	14.4	20.4
VAI/CAU-F/260/M	Mature	PB 260	Vaikundam	12.8	23.2	31.6
VAI/CAU-F/260/IM	Immature	PB 260	Vaikundam	9.2	32.8	42.4

Table 2.4.4.1: Occurrence of cauloplane fungi

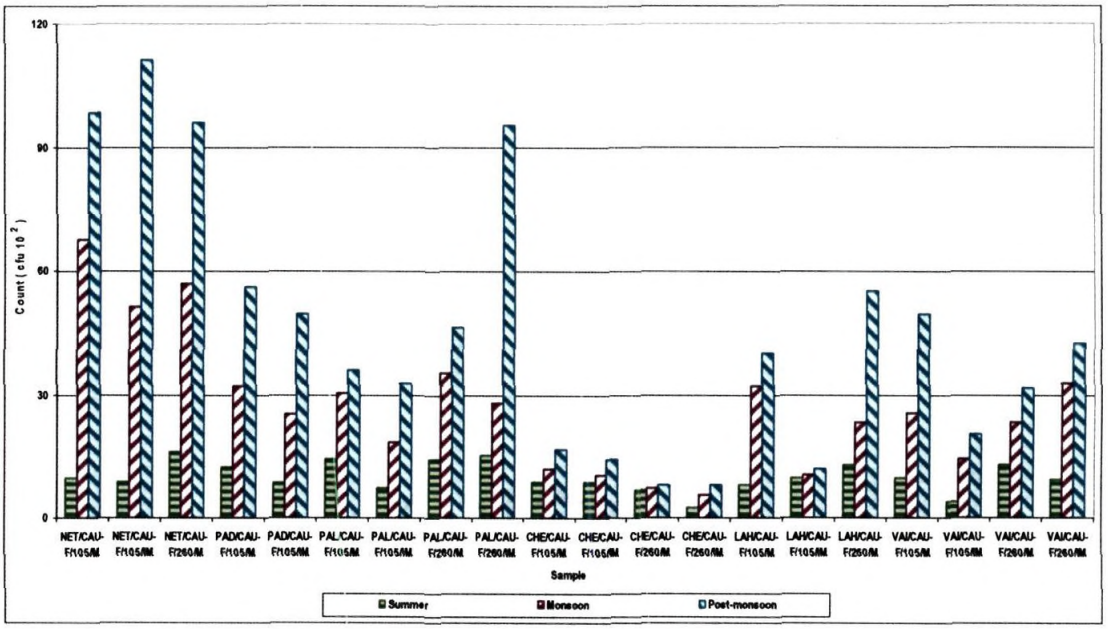

Fig.2.4.4.1 - Seasonal variation of cauloplane fungi in rubber trees

Analysis of the overall data obtained for cauloplane fungi has been shown in Fig.2.4.4.1. Increased cauloplane fungi count was recorded in all samples collected during the post-monsoon season. Samples from Nettana plantation recorded the highest growth in cauloplane fungi among all samples followed by Palappilly, Padiyoor and Lahai. Chethackal recorded the lowest cauloplane fungi count. Wider variation were observed for immature trees of RRII 105 at Nettana and PB 260 at Palappilly during the post monsoon period, while a similar trend in population was uniformly observed for all mature tree samples (Fig. 2.4.4.2 and 2.4.4.3).

A comparative account of mature and immature trees can be obtained from Figures 2.4.4.2 and 2.4.4.3. The results of analysis of cauloplane fungi can be observed in Figure 2.4.4.4 and 2.4.4.5

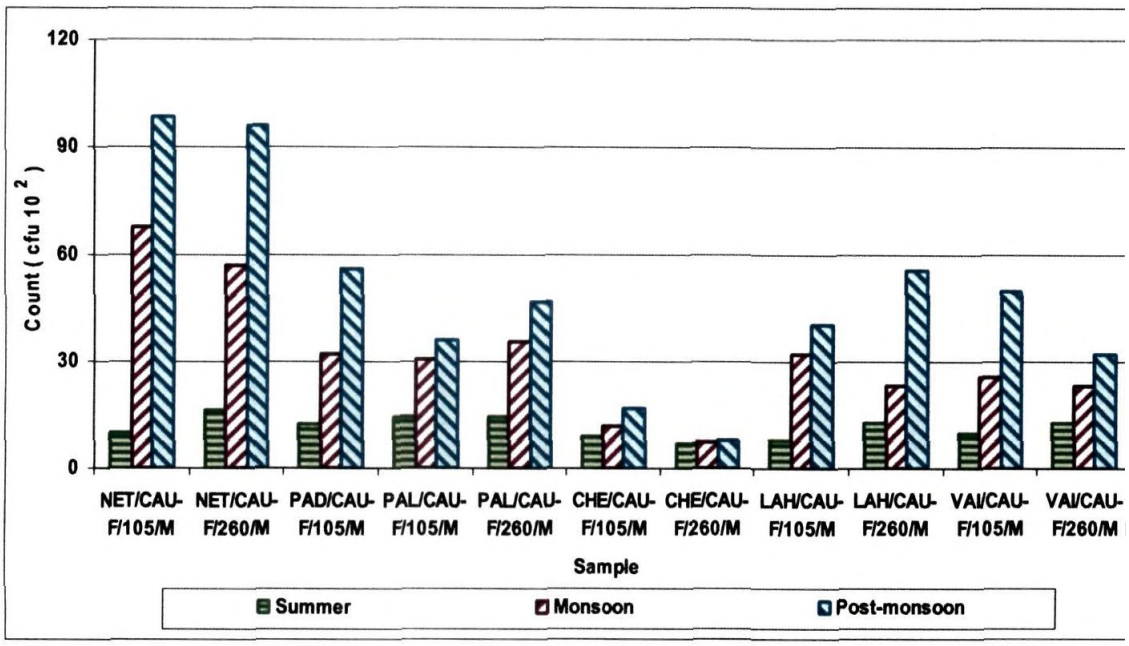

Fig.2.4.4.2 - Seasonal variation of cauloplane fungi mature in rubber trees

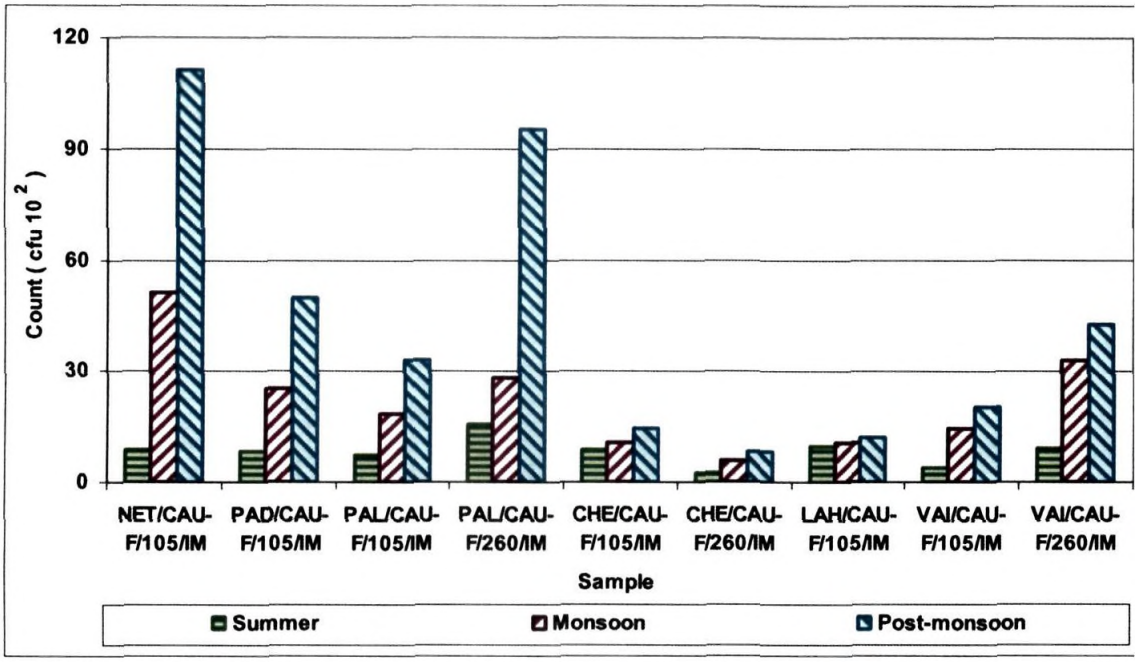

Fig.2.4.4.3 - Seasonal variation of cauloplane fungi in immature rubber trees

The seasonal variation of cauloplane fungi was consistent across both mature and immature rubber trees, as is evident from Fig.2.4.4.2 and Fig.2.4.4.3

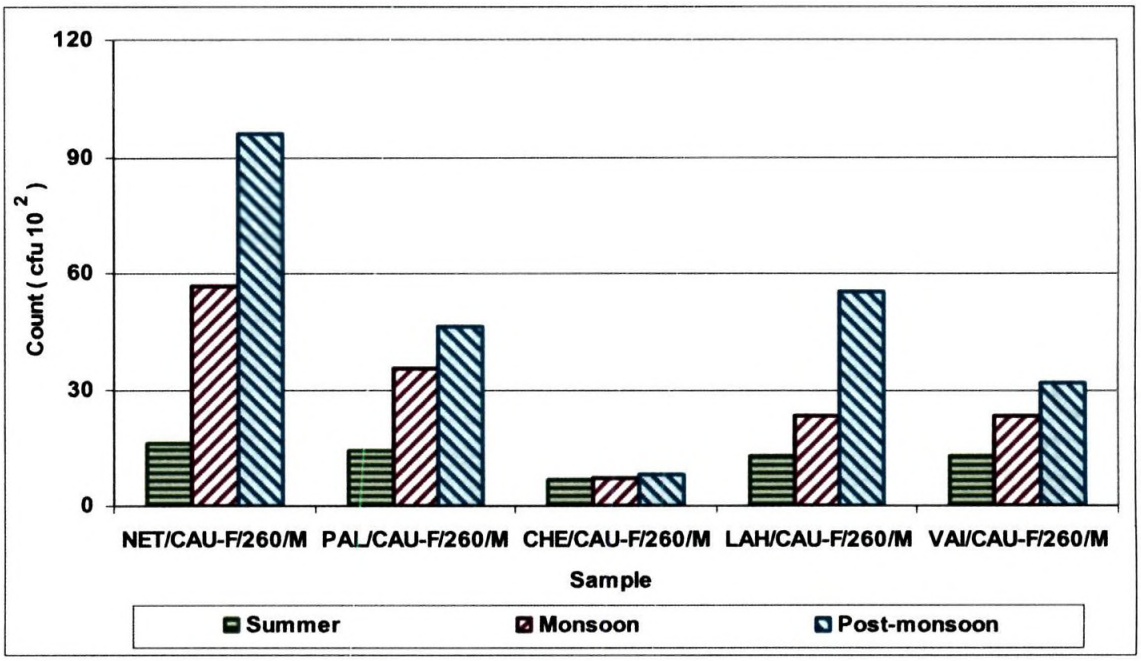

Fig.2.4.4.4 - Seasonal variation of cauloplane fungi in PB 260 rubber trees

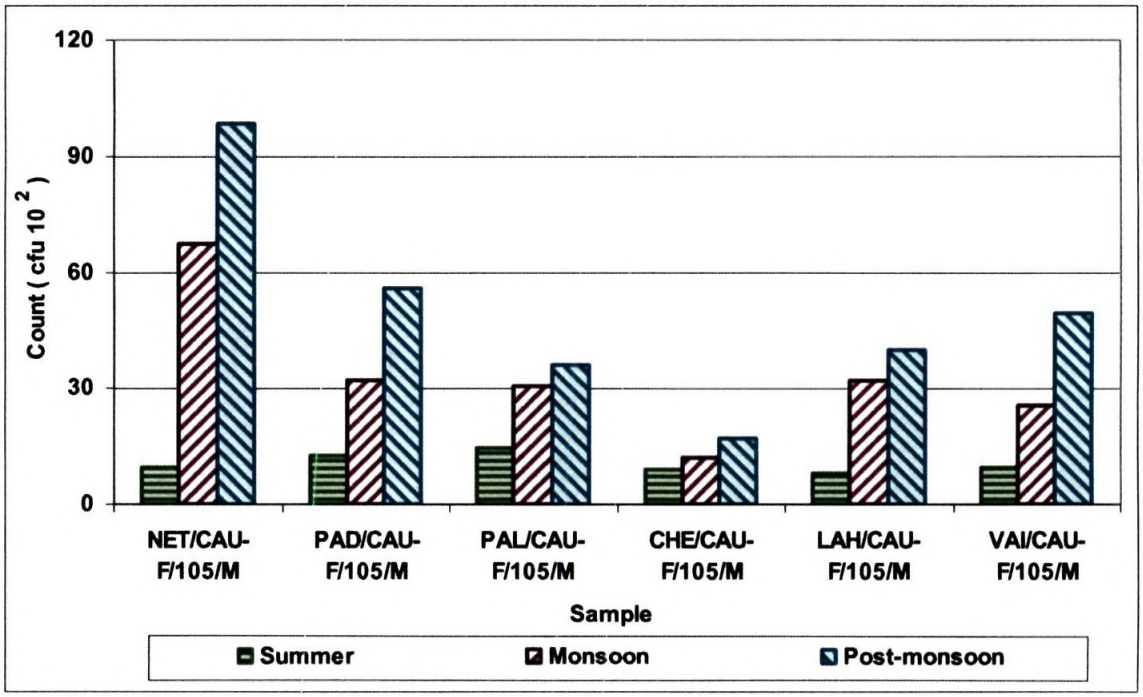

Fig.2.4.4.5 - Seasonal variation of cauloplane fungi in RRII 105 rubber trees

In mature trees at Chethackal, for both the clones, RRII 105 and PB 260, much variation in cauloplane fungal population was not observed during all the three seasons, as is evident from Fig.2.4.4.4 and Fig.2.4.4.5

Analysis of seasonal variation:

Locationwise analysis of seasonal variation in the case of cauloplane fungi was attempted for each clone in relation to their age (Tables 2.4.4.2 to 2.4.4.7).

Cauloplane fungal population showed significant seasonal variation for RRII 105 mature trees at Nettana (*F* value 14.27) while much high significance was seen in RRII 105 immature trees (*F* value 122.7). Population of cauloplane fungi in PB 260 mature trees also showed significant variation (*F* = 41.58).

Both RRII 105 mature and immature tree samples from Padiyoor had significant seasonal variation in cauloplane fungal population (*F* value 15.25 and 10.85 respectively).

Palappilly cauloplane fungi samples had significant variation in the case of mature RRII 105 trees and immature trees (*F* values 7.09 and 10.87 respectively). While no significance was shown in the case of mature PB 260 trees (F value 4.39), high significance was present in immature PB 260 trees (F value 92.98).

There was no significant variation of cauloplane fungi in RRII 105 mature, immature and PB 260 mature trees at Chethackal. But immature PB 260 trees had significant variation (F value 9.28).

At Lahai, cauloplane fungal population showed slightly significant variation on RRII 105 mature trees while no significant variation was present in the immature trees of the same clone. But mature PB 260 trees had more significant seasonal variation (*F* value 25.29).

Cauloplane fungi samples of RRII 105 mature trees from Vaikundam had significant variation (*F* value 22.48). Mature trees of PB 260 clone had

only slight significance (*F* value 6.67). Immature PB 260 trees had no significant variation (F value 4.38).

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	9.6	8.80	16
Rainy	67.6	51.2	56.8
Post Monsoon	98.4	111.2	96
F(Variance ratio)	14.27	122.71	41.59
CD(P=0.05)	36.78	14.31	19.12

Table 2.4.4.2: Mean seasonal variation in cauloplane fungi at Nettana

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	12.4	8.4
Rainy	32	25.2
Post Monsoon	56	49.6
F(Variance ratio)	15.26	10.86
CD(P=0.05)	17.23	19.37

Table 2.4.4.3: Mean seasonal variation in cauloplane fungi at Padiyoor

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	14.4	14.40	7.2	15.20
Rainy	30.4	35.2	18.4	28
Post Monsoon	36	46.4	32.8	95.2
F(Variance ratio)	7.1	4.39	10.87	92.98
CD(P=0.05)	12.97	23.88	11.99	13.73

Table 2.4.4.4: Mean seasonal variation in cauloplane fungi at Palappilly

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	8.8	6.80	8.8	2.36
Rainy	12	7.2	10.4	5.6
Post Monsoon	16.8	8	14.4	8
F(Variance ratio)	1.75	0.18	1.07	9.29
CD(P=0.05)	9.38	4.39	8.6	2.86

Table 2.4.4.5: Mean seasonal variation in cauloplane fungi at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	8	12.80	9.6
Rainy	32	23.2	10.4
Post Monsoon	40	55.2	12
F(Variance ratio)	9.67	25.29	0.34
CD(P=0.05)	16.5	13.54	6.44

Table 2.4.4.6: Mean seasonal variation in cauloplane fungi at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	PB 260
Summer	9.6	12.80	9.20
Rainy	25.6	23.2	32.8
Post Monsoon	49.6	31.6	42.4
F(Variance ratio)	22.49	6.68	4.38
CD(P=0.05)	13.08	11.23	25.15

Table 2.4.4.7: Mean seasonal variation in cauloplane fungi at Vaikundam

Analysis of variation in relation to rubber clones:

The variation in population of cauloplane fungi in relation to rubber clones for the three seasons are presented in Tables 2.4.4.8 to 2.4.4.13.

It was observed that cauloplane fungi on mature trees of the two clones of rubber displayed significant variation during the summer at Nettana, Palappilly, Lahai and Vaikundam (*t* value -1.05, 0, -1.08 and -1.03 respectively) while no such variation was observed at Chethackal.

During the rainy season, variation in population of cauloplane fungi was significant only at Palappilly. But during the post monsoon season it was significant at Palappilly and Lahai (-0.81 and -1.77 respectively).

Immature trees belonging to the two clones had significant variation in cauloplane fungal population at Palappilly (*t* value -3.78) in the summer season. Variation was significant at Palappilly and Vaikundam during the rainy season (*t* value -3.78) in the summer season. Variation was significant at Palappilly and Vaikundam during the rainy season (*t* value -1.56 and -1.98 respectively) and during the post monsoon season (*t* value -7.88 and -2.05 respectively).

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	9.6	26.8	16	160	-1.05
Palappilly	14.4	36.8	14.4	68.8	0
Chethackal	8.8	31.7	6.8	3.2	0.76
Lahai	8	8	12.8	91.2	-1.08
Vaikundam	9.6	12.8	12.8	35.2	-1.03

Table 2.4.4.8: Clonal variation in cauloplane fungi during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	67.6	416.8	56.8	347.2	0.87
Palappilly	30.4	28.8	35.2	203.2	-0.70
Chethackal	12	64	7.2	11.2	1.24
Lahai	32	160	23.2	1.2	1.24
Vaikundam	25.6	92.8	23.2	75.2	0.41

Table 2.4..4.9: Clonal variation in cauloplane fungi during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	98.4	1692.8	96	70	0.13
Palappilly	36	200	46.4	628.8	-0.81
Chethackal	16.8	43.2	8	16	2.56
Lahai	40	262	55.2	107.2	-1.77
Vaikundam	49.6	164.8	31.6	88.8	2.53

Table 2.4.4.10: Clonal variation in cauloplane fungi during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	7.2	11.2	15.2	11.2	-3.78
Chethackal	8.8	43.2	2.36	0.148	2.19

Table 2.4.4.11: Clonal variation in cauloplane fungi during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	18.4	172.8	28	16	-1.56
Chethackal	10.4	36.8	5.6	4.8	1.66
Vaikundam	14.4	36.8	32.8	395.2	-1.98

Table 2.4.4.12: Clonal variation in cauloplane fungi during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	32.8	43.2	95.2	270.7	-7.88
Chethackal	14.4	36.8	8	8	2.14
Vaikundam	20.4	84.8	42.4	492.8	-2.05

Table 2.4.4.13: Clonal variation in cauloplane fungi during post-monsoon season on immature trees

2.4.5. Seasonal variation in rhizosphere bacteria

Table 2.4.5.1 indicates average rhizosphere bacterial counts observed during the three seasons.

Sample Name	Mature / Immature	Clone	Location	Mean Population [c f u x 10^5]		
				Summer	Rainy	Post-monsoon
NET/RH-BC/105/M	Mature	RRII 105	Nettana	187.8	61.7	199.8
NET/RH-BC/105/IM	Immature	RRII 105	Nettana	90.2	30.5	163.6
NET/RH-BC/260/M	Mature	PB 260	Nettana	516.6	209.6	179.2
PAD/RH-BC/105/M	Mature	RRII 105	Padiyoor	284.9	205.9	493.9
PAD/RH-BC/105/IM	Immature	RRII 105	Padiyoor	104.9	297.7	70.6
PAL/RH-BC/105/M	Mature	RRII 105	Palappilly	124.2	39.5	29.2
PAL/RH-BC/105/IM	Immature	RRII 105	Palappilly	407.8	118.4	163.6
PAL/RH-BC/260/M	Mature	PB 260	Palappilly	33.8	78.2	332.6
PAL/RH-BC/260/IM	Immature	PB 260	Palappilly	114.9	32.9	324.1
CHE/RH-BC/105/M	Mature	RRII 105	Chethackal	656.5	145.3	163.5
CHE/RH-BC/105/IM	Immature	RRII 105	Chethackal	516.8	140.5	245.1
CHE/RH-BC/260/M	Mature	PB 260	Chethackal	189.6	42.1	578.9
CHE/RH-BC/260/IM	Immature	PB 260	Chethackal	66.8	155.2	313.7
LAH/RH-BC/105/M	Mature	RRII 105	Lahai	298.6	137.0	915.7
LAH/RH-BC/105/IM	Immature	RRII 105	Lahai	177.8	51.1	108.7
LAH/RH-BC/260/M	Mature	PB 260	Lahai	767.7	55.1	591.2
VAI/RH-BC/105/M	Mature	RRII 105	Vaikundam	141.7	121.3	35.9
VAI/RH-BC/105/IM	Immature	RRII 105	Vaikundam	117.6	47.3	35.1
VAI/RH-BC/260/M	Mature	PB 260	Vaikundam	171.4	17.1	266.5
VAI/RH-BC/260/IM	Immature	PB 260	Vaikundam	735.7	37.5	150.7

Table 2.4.5.1: Occurrence of rhizosphere bacteria

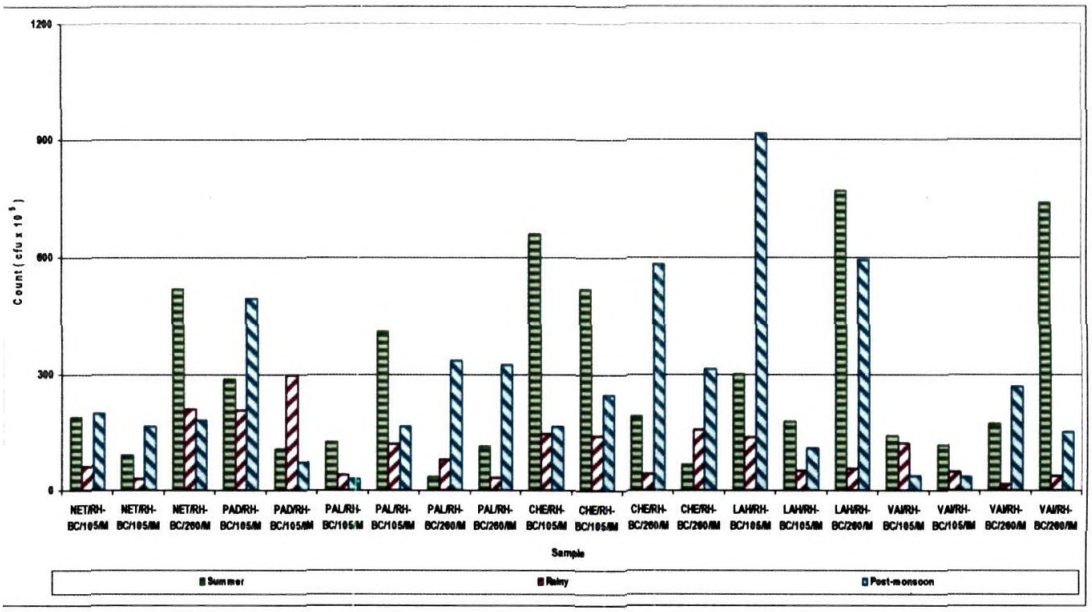

Fig. 2.4.5.1 - Seasonal variation of rhizosphere bacteria in rubber trees

Fig. 2.4.5.1 indicates average trends of rhizosphere bacteria found over the three seasons across all six locations and varying ages of rubber trees. In general, bacterial count was observed to be low during the rainy season. Highest bacterial count was found during the post monsoon season at Lahai. The population was higher in areas under mature rubber (Fig. 2.4.5.2 and Fig. 2.4.5.3).

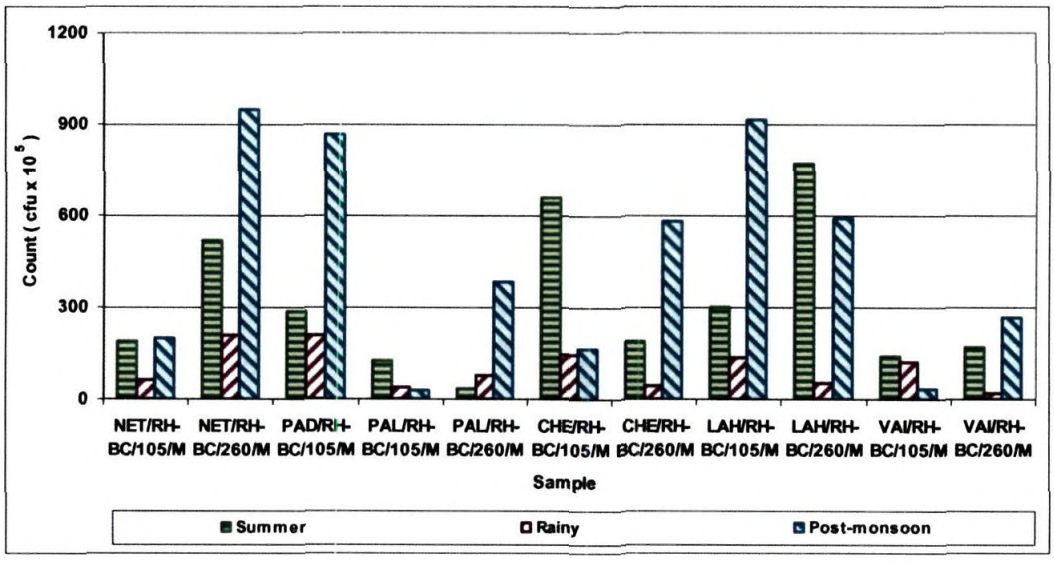

Fig. 2.4.5.2 - Seasonal variation of rhizosphere bacteria in mature rubber trees

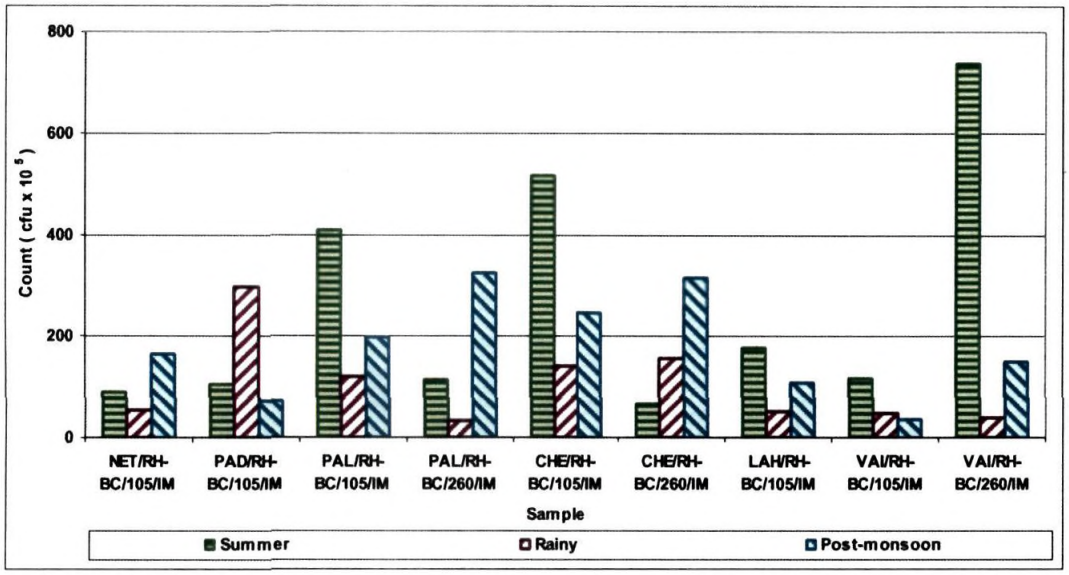

Fig. 2.4.5.3 - Seasonal variation of rhizosphere bacteria in immature rubber trees

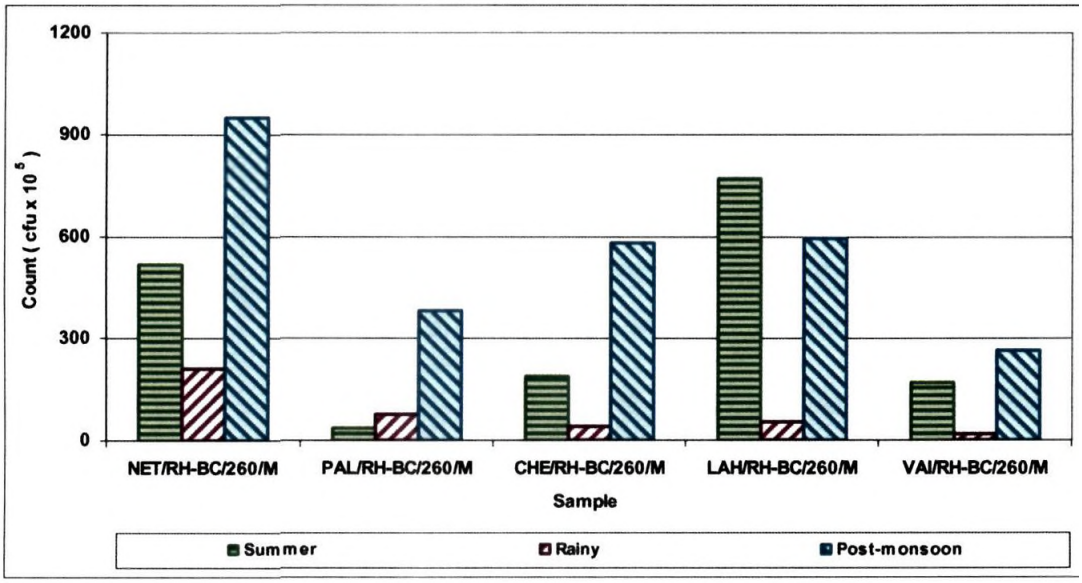

Fig. 2.4.5.4 - Seasonal variation of rhizosphere bacteria in PB 260 rubber trees

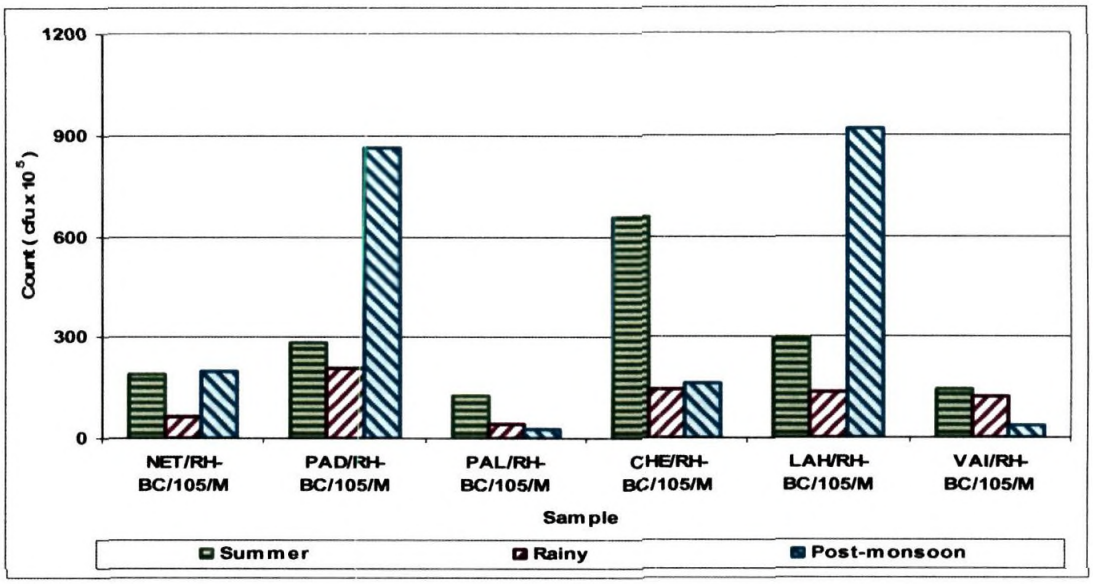

Fig. 2.4.5.5 -Seasonal variation of rhizosphere bacteria in RRII 105 rubber trees

In general, fields under the clone PB 260 had lower rhizosphere bacterial population than those under RRII 105 during rainy season. The population in the fields of mature PB 260 and RRII 105 were observed to be higher at Lahai (Fig. 2.4.5.4 and Fig. 2.4.5.5).

Analysis of seasonal variation:

Analysis of seasonal variation of rhizosphere bacteria of each clone in relation to their age is given in Tables 2.4.5.2 to 2.4.5.7

The results of statistical analysis of seasonal variation of rhizosphere bacteria were as shown in the following tables.

It was observed that population of rhizosphere bacteria on RRII 105 mature trees had significant variation at Nettana, Padiyoor, Palappilly, Chethackal, Lahai as well as Vaikundam.

Immature RRII 105 clones had significant variation in rhizosphere bacterial population at all the above regions. Rhizosphere bacteria on PB 260 mature trees had significant seasonal variation at Nettana, Chethackal, Lahai and Vaikundam. But at Palappilly the variation was not significant.

In the case of immature PB 260 trees, there was significant variation of rhizosphere bacteria at Palappilly, Chethackal and Vaikundam.

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	187.76	516.56	90.16
Rainy	61.7	209.58	30.46
Post Monsoon	199.8	179.18	163.6
F(Variance ratio)	47.7	38.77	88.5
CD(P=0.05)	33.6	90.92	21.5

Table 2.4.5.2: Mean seasonal variation in rhizosphere bacteria at Nettana

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	284.88	104.88
Rainy	205.92	297.72
Post Monsoon	493.88	70.6
F(Variance ratio)	5.39	7.06
CD(P=0.05)	197.44	142.02

Table 2.4.5.3: Mean seasonal variation in rhizosphere bacteria at Padiyoor

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	124.24	33.80	407.76	114.94
Rainy	39.5	78.16	118.4	32.9
Post Monsoon	29.16	332.58	163.64	324.14
F(Variance ratio)	35.13	4.23	24.11	61.15
CD(P=0.05)	27.12	241.59	97.69	59.18

Table 2.4.5.4: Mean seasonal variation in rhizosphere bacteria at Palappilly

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	656.48	189.64	516.78	66.80
Rainy	145.3	42.1	140.46	155.2
Post Monsoon	163.54	578.9	245.1	313.7
F(Variance ratio)	55.94	216.77	28.62	1588.34
CD(P=0.05)	119.49	58.04	111.89	9.67

Table 2.4.5.5: Mean seasonal variation in rhizosphere bacteria at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	298.56	767.74	177.76
Rainy	136.98	55.06	51.08
Post Monsoon	915.7	591.2	108.7
F(Variance ratio)	710.62	315.75	69.87
CD(P=0.05)	47.51	64.37	23.38

Table 2.4.5.6: Mean seasonal variation in rhizosphere bacteria at Lahai

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	141.7	171.42	117.58	735.66
Rainy	121.26	17.12	47.34	37.54
Post Monsoon	35.9	266.5	35.08	150.7
F(Variance ratio)	68.29	62.45	27.92	251.77
CD(P=0.05)	20.93	49.08	25.96	72.77

Table 2.4.5.7: Mean seasonal variation in rhizosphere bacteria at Vaikundam

Analysis of variation in relation to rubber clones:

The variation in rhizosphere bacterial population in relation to rubber clones for the three seasons are presented in Tables 2.4.5.8 to 2.4.5.13

There was significant variation regarding rhizosphere bacteria colonizing the roots of mature RRII 105 and PB 260 clones of rubber at Nettana (*t* value -6.53), Lahai (*t* value -10.55) as well as Vaikundam (*t* value -0.99), during the summer season. During the rainy season, the variation of rhizosphere bacterial population was significant at Nettana (*t* value -11.30) and Palappilly (*t* value -8.99) whereas it was not significant at Chethackal, Lahai and Vaikundam. During the post monsoon season, significant variation was present at Palappilly (-2.231), Chethackal (-33.75) and Vaikundam (-248.66) while it was not significant at Nettana and Lahai.

With regard to bacterial colonies in the rhizosphere of immature rubber trees belonging to the two clones under study, significant variation during the summer season was observed at Vaikundam (-14.52) and during the rainy season at Chethackal (-2.011). The three regions Palappilly, Chethackal and Vaikundam showed significant variation (*t* value -2.515, -27.44 and -69.45 respectively) during the post monsoon season.

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	187.76	1797.85	516.56	10861.88	-6.53
Palappilly	124.24	833.423	33.804	6.936	6.98
Chethackal	656.48	21894.5	189.64	5199.5	6.34
Lahai	298.56	3528	767.74	6365.8	-10.55
Vaikundam	141.7	675.55	171.42	3785.9	-0.99

Table 2.4.5.8: Clonal variation in rhizosphere bacteria during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	61.7	8.43	209.58	848.31	-11.3
Palappilly	39.5	79.75	78.16	12.63	-8.99
Chethackal	145.3	5.01	42.1	18.55	47.54
Lahai	136.98	29.63	55.06	166.63	13.07
Vaikundam	121.26	13.19	17.12	17.8	41.83

Table 2.4.5.9: Clonal variation in rhizosphere bacteria during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	199.8	33.7	179.18	1764.73	1.09
Palappilly	29.16	248.71	332.58	92180.8	-2.23
Chethackal	163.54	653.49	578.9	103.8	-33.75
Lahai	915.7	7.2	591.2	11.7	166.9
Vaikundam	35.9	3.05	266.5	1.25	-248.66

Table 2.4.5.10: Clonal variation in rhizosphere bacteria during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	407.76	124.09	114.94	101.45	43.6
Chethackal	516.78	19576.5	66.8	46.26	7.18
Vaikundam	117.58	1051.01	735.66	8007.18	-14.52

Table 2.4.5.11: Clonal variation in rhizosphere bacteria during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	118.4	0.54	32.9	18.03	44.37
Chethackal	140.46	181.2	155.2	87.33	-2.01
Vaikundam	47.34	2.63	37.54	354.08	1.16

Table 2.4.5.12: Clonal variation in rhizosphere bacteria during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	163.64	14948.5	324.14	5412.9	-2.51
Chethackal	245.1	17.05	313.7	14.2	-27.44
Vaikundam	35.08	10.91	150.7	2.95	-69.45

Table 2.4.5.13: Clonal variation in rhizosphere bacteria during post-monsoon season on immature trees

2.4.6. Seasonal variation in rhizosphere fungi

Sample Name	Mature / Immature	Clone	Location	Mean Population [c f u x 10^6]		
				Summer	Rainy	Post-monsoon
NET/RH-FG/105/M	Mature	RRII 105	Nettana	40.0	115.5	42.9
NET/RH-FG/105/IM	Immature	RRII 105	Nettana	132.7	3.6	222.9
NET/RH-FG/260/M	Mature	PB 260	Nettana	556.8	12.1	279.5
PAD/RH-FG/105/M	Mature	RRII 105	Padiyoor	38.1	19.8	165.7
PAD/RH-FG/105/IM	Immature	RRII 105	Padiyoor	81.5	24.5	13.1
PAL/RH-FG/105/M	Mature	RRII 105	Palappilly	142.7	4.1	25.7
PAL/RH-FG/105/IM	Immature	RRII 105	Palappilly	122.2	8.7	78.2
PAL/RH-FG/260/M	Mature	PB 260	Palappilly	11.4	31.0	29.1
PAL/RH-FG/260/IM	Immature	PB 260	Palappilly	16.4	9.5	71.1
CHE/RH-FG/105/M	Mature	RRII 105	Chethackal	35.9	9.5	71.1
CHE/RH-FG/105/IM	Immature	RRII 105	Chethackal	3.5	34.1	43.6
CHE/RH-FG/260/M	Mature	PB 260	Chethackal	157.9	7.1	385.4
CHE/RH-FG/260/IM	Immature	PB 260	Chethackal	2.1	16.1	195.3
LAH/RH-FG/105/M	Mature	RRII 105	Lahai	18.6	34.1	43.6
LAH/RH-FG/105/IM	Immature	RRII 105	Lahai	38.1	7.1	385.4
LAH/RH-FG/260/M	Mature	PB 260	Lahai	35.5	16.1	195.3
VAI/RH-FG/105/M	Mature	RRII 105	Vaikundam	58.1	68.4	156.8
VAI/RH-FG/105/IM	Immature	RRII 105	Vaikundam	134.9	7.5	78.2
VAI/RH-FG/260/M	Mature	PB 260	Vaikundam	159.0	2.0	39.1
VAI/RH-FG/260/IM	Immature	PB 260	Vaikundam	131.3	2.0	215.0

Table 2.4.6.1: Occurrence of rhizosphere fungi

In general, lowest rhizosphere fungal population was recorded during the rainy season. Their number was markedly high during the summer. The details are given in Table 2.4.6.1. In some locations the post monsoon season was congenial for the fungi than the summer season. At Lahai, immature and mature RRII 105 and mature PB 260 exhibited more fungal population during the post monsoon season which was 385.4 $x10^5$, 43.6 $x10^5$ and 195.3 x 10^5 respectively.

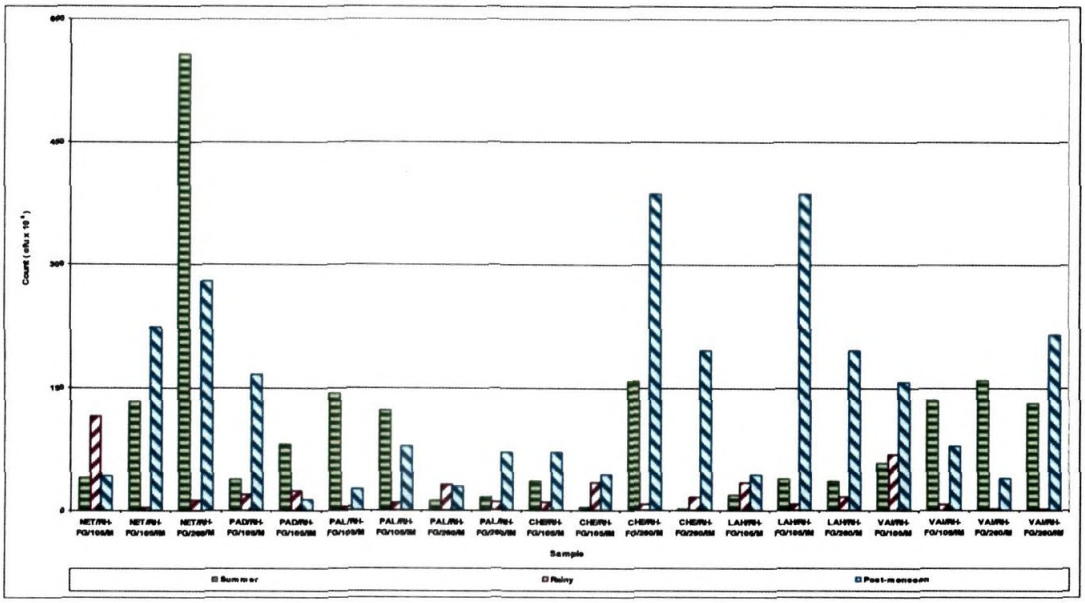

Fig. 2.4.6.1 - Seasonal variation of rhizosphere fungi in rubber trees

Fig. 2.4.6.1 depicts the variation in rhizosphere fungal population in mature and immature rubber trees of RRII 105 and PB 260 clones across the summer, rainy and post monsoon seasons.

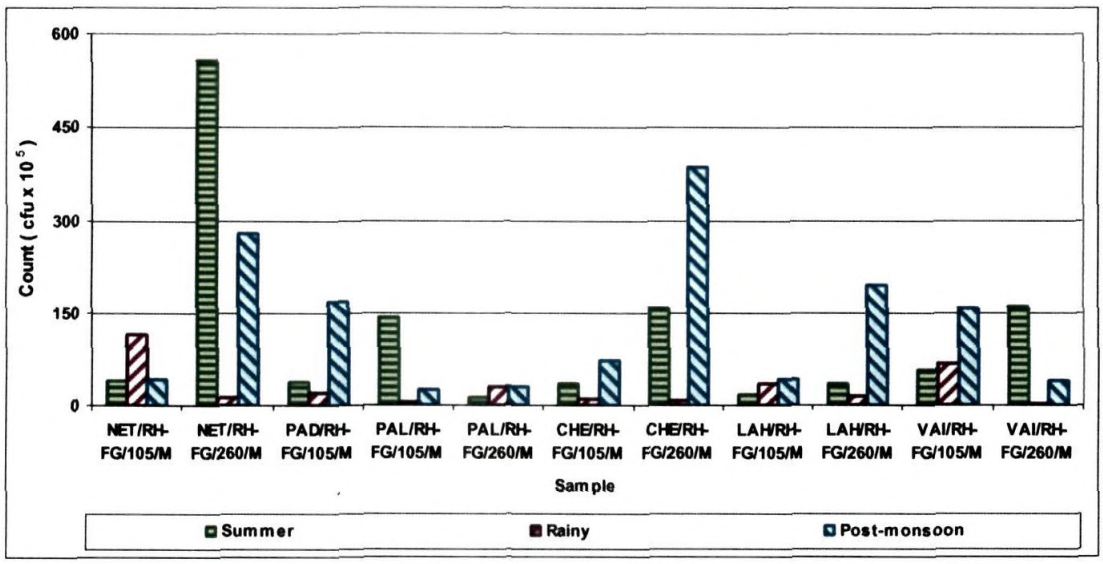

Fig. 2.4.6.2 - Seasonal variation of rhizosphere fungi in mature rubber trees

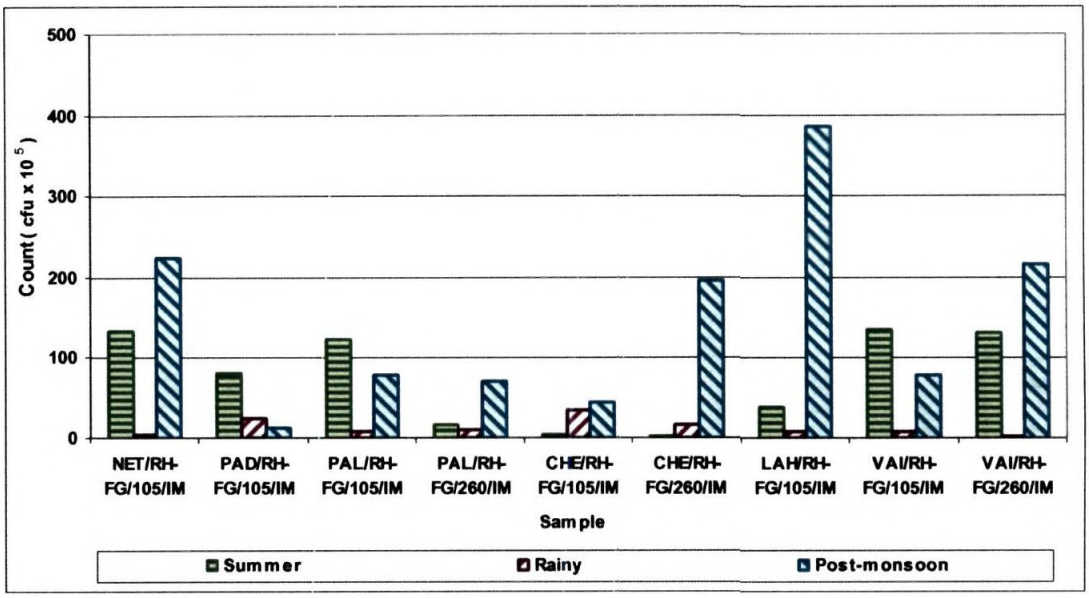

Fig. 2.4.6.3 - Seasonal variation of rhizosphere fungi in immature rubber trees

The rhizosphere fungal population of immature RRII 105 was observed to be very high during post monsoon season. The population in other places did not show much variation across the seasons.

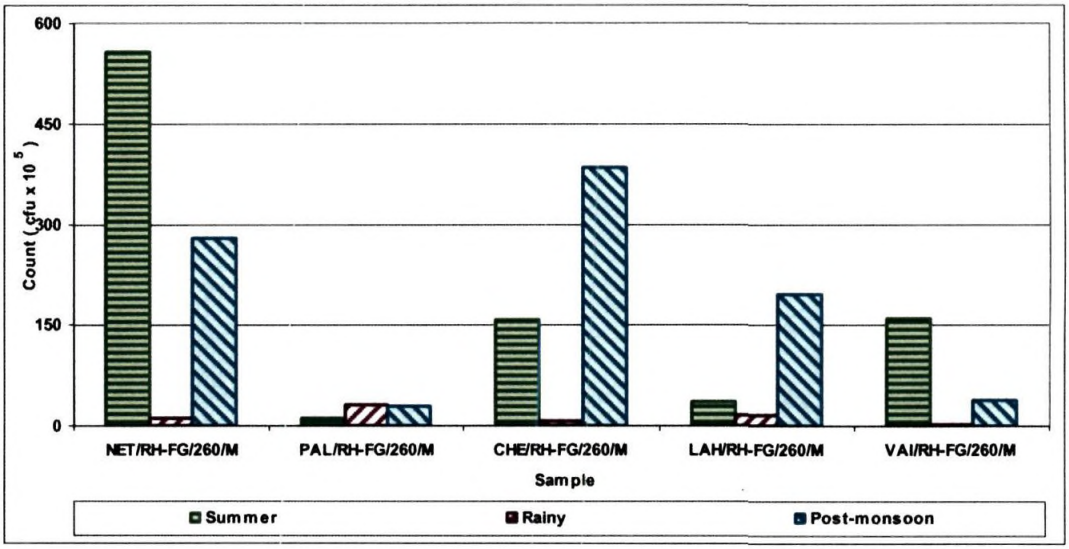

Fig. 2.4.6.4 - Seasonal variation of rhizosphere fungi in PB 260 mature rubber trees

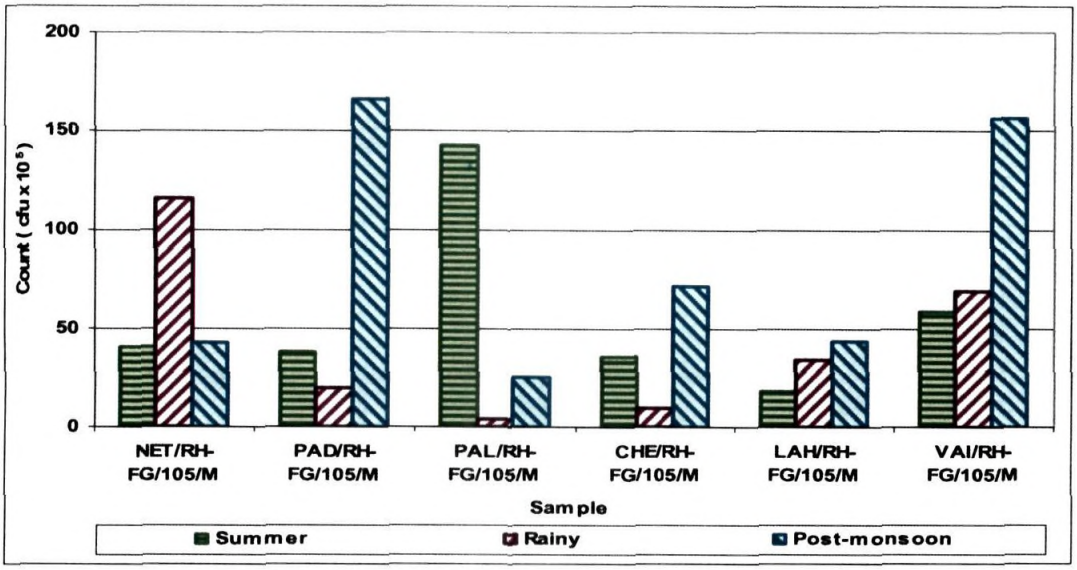

Fig. 2.4.6.5 - Seasonal variation of rhizosphere fungi in RRII 105 mature rubber trees

While the patterns of fungal population in mature PB 260 during summer and post monsoon seasons were similar, the population was very less during the rainy season (Fig. 2.4.6.4). The population showed variation at Padiyoor and Vaikundam during post monsoon and at Palappilly during the summer season (Fig. 2.4.6.5).

Analysis of seasonal variation

Locationwise analysis of seasonal variation in rhizosphere fungi was attempted for each clone in relation to their age (Tables 2.4.6.2 to 2.4.6.7).

It was observed that seasonal variation in rhizosphere fungi was significant in all the samples from Nettana, Padiyoor, Palappilly, Chethackal and Lahai as seen in the following tables. Significant variation was present at Vaikundam in mature and immature RRII 105 and immature PB 260 (*F* value 63.18, 78.01 and 8.98 respectively) while mature PB 260 had no significant variation.

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	39.98	556.80	132.68
Rainy	115.5	12.08	3.56
Post Monsoon	42.86	279.5	222.88
F(Variance ratio)	235.49	54.17	323.72
CD(P=0.05)	8.59	114.04	18.88

Table 2.4.6.2: Mean seasonal variation in rhizosphere fungi at Nettana

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	38.1	81.48
Rainy	19.8	24.48
Post Monsoon	165.72	13.1
F(Variance ratio)	226.43	20.49
CD(P=0.05)	16.28	24.94

Table 2.4.6.3: Mean seasonal variation in rhizosphere fungi at Padiyoor

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	142.72	11.4	122.18	16.44
Rainy	4.06	31.04	8.68	9.54
Post Monsoon	25.7	29.06	78.2	71.1
F(Variance ratio)	40.48	9.61	86.75	39.01
CD(P=0.05)	36.13	10.75	18.93	16.64

Table 2.4.6.4: Mean seasonal variation in rhizosphere fungi at Palappilly

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	35.9	157.90	3.54	2.14
Rainy	9.52	7.1	34.12	16.12
Post Monsoon	71.1	385.4	43.56	195.28
F(Variance ratio)	12.51	44.83	230.11	100.33
CD(P=0.05)	26.91	87.65	4.25	33.13

Table 2.4.6.5: Mean seasonal variation in rhizosphere fungi at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	18.64	35.52	38.12
Rainy	34.1	16.1	7.06
Post Monsoon	43.64	195.3	385.38
F(Variance ratio)	5.62	94.22	18.4
CD(P=0.05)	16.4	31.22	150.88

Table 2.4.6.6: Mean seasonal variation in rhizosphere fungi at Lahal

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	58.14	159.02	134.86	131.26
Rainy	68.38	1.962	7.48	2.04
Post Monsoon	156.84	39.14	78.16	215.04
F(Variance ratio)	63.18	4.04	78.01	8.98
CD(P=0.05)	21.04	125.78	22.27	110.33

Table 2.4.6.7: Mean seasonal variation in rhizosphere fungi at Valkundam

Analysis of variation in relation to rubber clones:

Analysis of variation in rhizosphere fungi in relation to rubber clones for the three seasons are as in Tables 2.4.6.8 to 2.4.6.13

During the summer season, mature trees belonging to the two clones under study had significant variation in fungal colonies in the rhizosphere at Nettana, Chethackal, Lahai and Vaikundam (*t* value -187.22, -9.262, -0.957 and -1.420 respectively). But the samples from Palappilly had no such significant variation. During the rainy season no significant difference was seen at all the regions except in the samples from Palappilly. Rhizosphere fungal colonies varied significantly during the post monsoon at Nettana, Palappilly, Chethackal and Lahai (*t* value -3.689, -0.80, -6.31 and -18.89 respectively). But at Vaikundam the variation was not significant.

Immature rubber trees of the two clones showed no significant variation with regard to the rhizosphere fungal population at three regions namely Palappilly, Chethackal and Vaikundam during the summer season. During the rainy season, variation in rhizosphere fungal population was significant only at Palappilly (*t* value -0.31). But during the post monsoon season, significant variation was seen in samples from Chethackal and Vaikundam (*t* value -8.16 and -3.96 respectively).

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	39.98	25.4	556.8	12.7	-187.22
Palappilly	142.72	199.16	11.4	11.68	6.56
Chethackal	35.9	593.58	157.9	273.96	-9.26
Lahai	18.64	52.77	35.52	1503.41	-0.957
Vaikundam	58.14	273.19	159.02	24973	-1.42

Table 2.4.6.8: Clonal variation in rhizosphere fungi during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	115.5	4.06	12.08	41.33	34.32
Palappilly	4.06	2.85	31.04	148.88	-4.9
Chethackal	9.52	4.462	7.1	6.74	1.62
Lahai	34.1	78.19	16.1	7.54	4.35
Vaikundam	68.38	10.83	1.96	2.1	41.31

Table 2.4.6.9: Clonal variation in rhizosphere fungi during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	42.86	87.18	279.5	20489.7	-3.69
Palappilly	25.7	66.28	29.06	21.91	-0.80
Chethackal	71.1	546.23	385.4	11855	-6.31
Lahai	43.64	293.65	195.3	28.48	-18.89
Vaikundam	156.84	415.2	39.14	16.88	12.66

Table 2.4.6.10: Clonal variation in rhizosphere fungi during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	122.18	136.15	16.44	37.19	17.96
Chethackal	3.54	0.99	2.14	0.92	2.26
Vaikundam	134.86	299.73	131.26	13727	0.07

Table 2.4.6.11: Clonal variation in rhizosphere fungi during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	8.68	23.63	9.54	15.12	-0.31
Chethackal	34.12	15.58	16.12	16.97	7.05
Vaikundam	7.48	4.40	2.04	0.81	5.33

Table 2.4.6.12: Clonal variation in rhizosphere fungi during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	8.68	23.63	9.54	15.12	-0.31
Chethackal	34.12	15.58	16.12	16.97	7.05
Vaikundam	7.48	4.40	2.04	0.81	5.33

Table 2.4.6.13: Clonal variation in rhizosphere fungi during post-monsoon season on immature trees

2.4.7. Seasonal variation in rhizosphere actinomycete

Rhizosphere actinomycetes showed variation in the samples collected during the three seasons (Table 2.6.7.1). Many of the summer season samples were devoid of actinomycetes while all the samples in the post monsoon and all the samples except one in the rainy season possessed actinomycetes.

Sample Name	Mature / Immature	Clone	Location	Mean Population [c f u x 10^5]		
				Summer	Rainy	Post-monsoon
NET/RH-AC/105/M	Mature	RRII 105	Nettana	12.3	0.0	42.9
NET/RH-AC/105/IM	Immature	RRII 105	Nettana	7.8	3.6	222.9
NET/RH-AC/260/M	Mature	PB 260	Nettana	15.8	12.1	279.5
PAD/RH-AC/105/M	Mature	RRII 105	Padiyoor	0.0	19.8	165.7
PAD/RH-AC/105/IM	Immature	RRII 105	Padiyoor	19.9	24.5	13.1
PAL/RH-AC/105/M	Mature	RRII 105	Palappilly	0.0	4.1	25.7
PAL/RH-AC/105/IM	Immature	RRII 105	Palappilly	0.0	8.7	78.2
PAL/RH-AC/260/M	Mature	PB 260	Palappilly	0.0	31.0	29.1
PAL/RH-AC/260/IM	Immature	PB 260	Palappilly	0.0	9.5	71.1
CHE/RH-AC/105/M	Mature	RRII 105	Chethackal	0.0	9.5	71.1
CHE/RH-AC/105/IM	Immature	RRII 105	Chethackal	2.7	34.1	43.6
CHE/RH-AC/260/M	Mature	PB 260	Chethackal	52.7	7.1	385.4
CHE/RH-AC/260/IM	Immature	PB 260	Chethackal	5.0	16.1	195.3
LAH/RH-AC/105/M	Mature	RRII 105	Lahai	12.2	34.1	43.6
LAH/RH-AC/105/IM	Immature	RRII 105	Lahai	1.7	7.1	385.4
LAH/RH-AC/260/M	Mature	PB 260	Lahai	0.0	16.1	195.3
VAI/RH-AC/105/M	Mature	RRII 105	Vaikundam	0.0	68.4	656.8
VAI/RH-AC/105/IM	Immature	RRII 105	Vaikundam	0.0	7.5	78.2
VAI/RH-AC/260/M	Mature	PB 260	Vaikundam	0.0	2.0	39.1
VAI/RH-AC/260/IM	Immature	PB 260	Vaikundam	0.0	2.0	215.0

Table 2.4.7.1: Occurrence of rhizosphere actinomycete

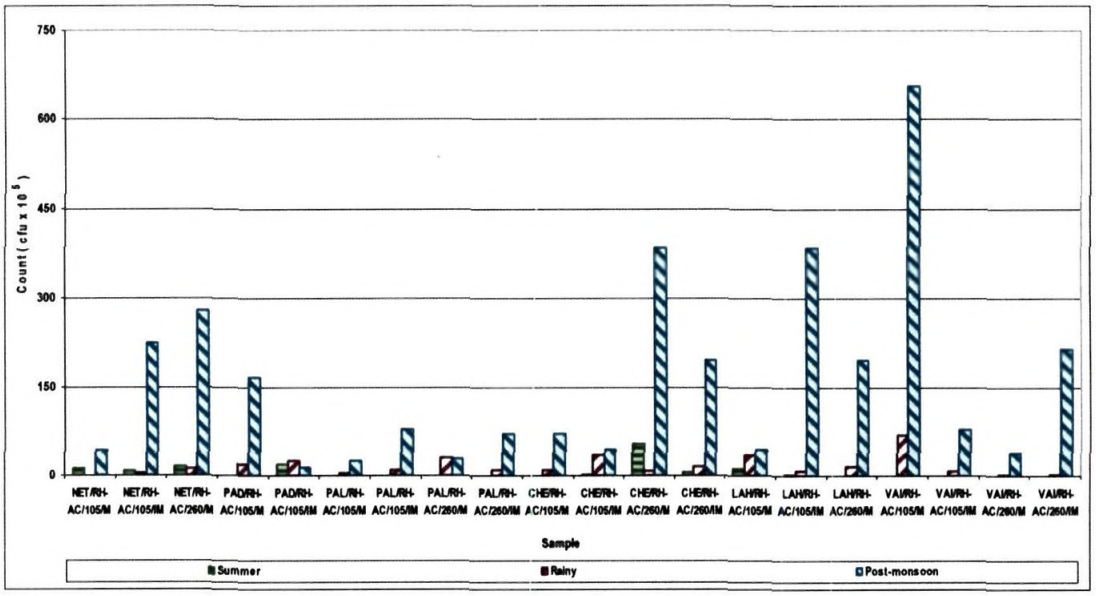

Fig. 2.4.7.1: Seasonal variation of rhizosphere actinomycetes in rubber trees

Figure 2.4.7.1 clearly shows the absence of actinomycetes in many locations. Highest actinomycetes population was recorded in RRII 105 mature samples from Vaikundam (656.8 $x10^5$/g soil).

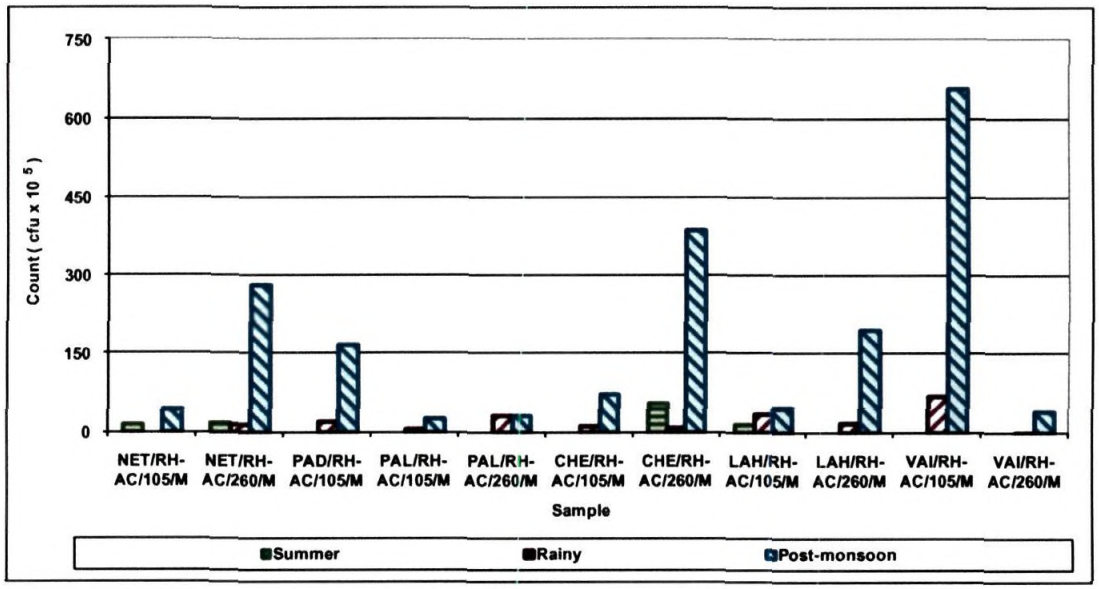

Fig. 2.4.7.2 -Seasonal variation of rhizosphere actinomycetes in mature rubber trees

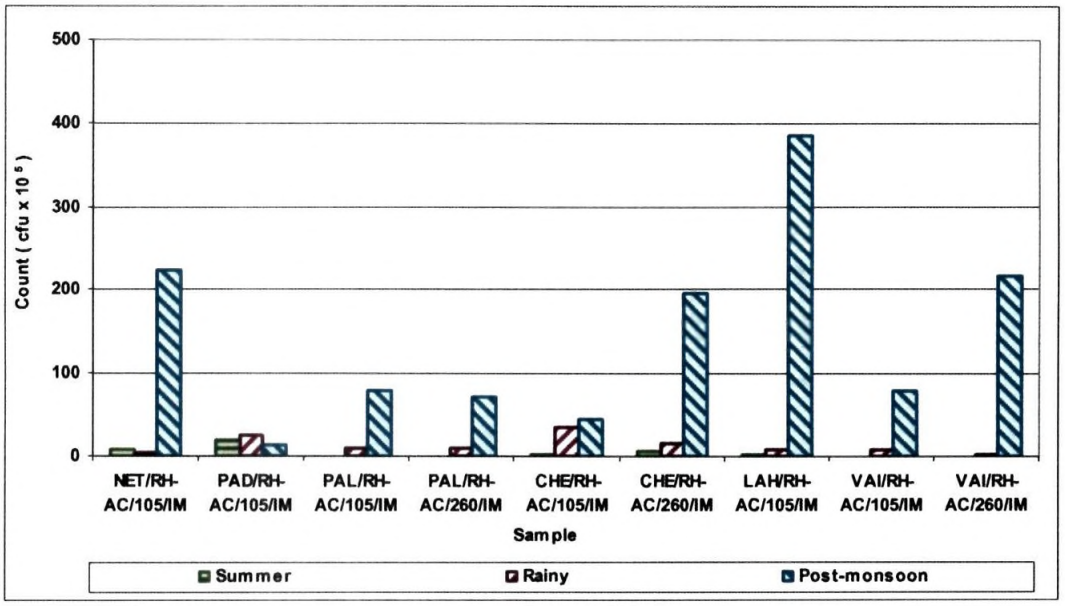

Fig. 2.4.7.3 - Seasonal variation of rhizosphere actinomycetes in immature rubber trees

The comparison of mature and immature rubber trees has been shown in Figure 2.4.7.2 and Figure 2.4.7.3.

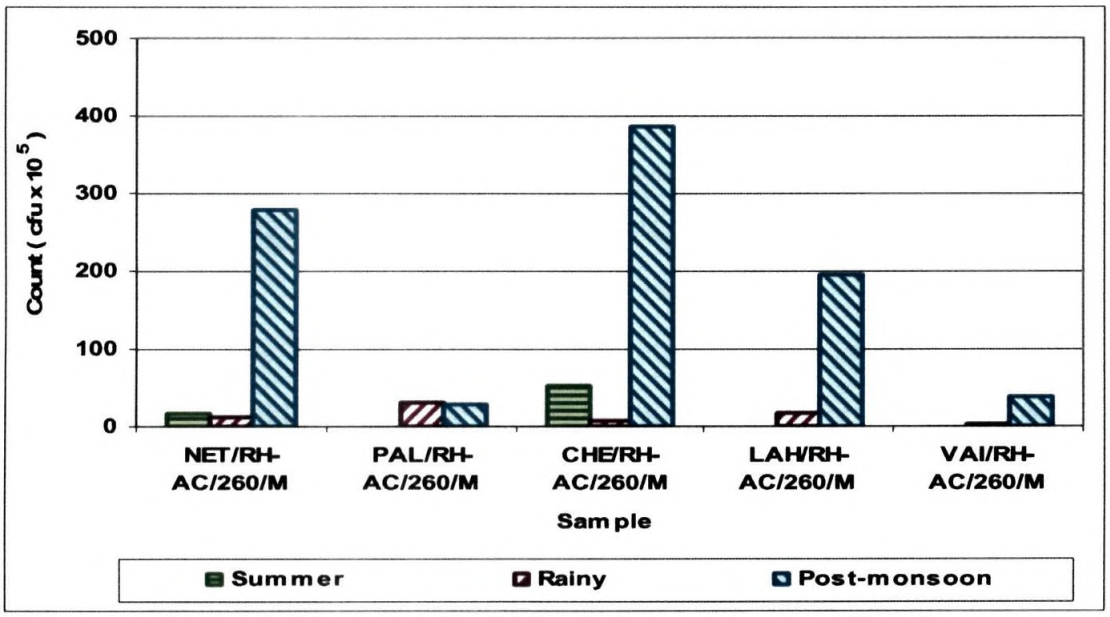

Fig. 2.4.7.4 - Seasonal variation of rhizosphere actinomycetes in PB 260 mature rubber trees

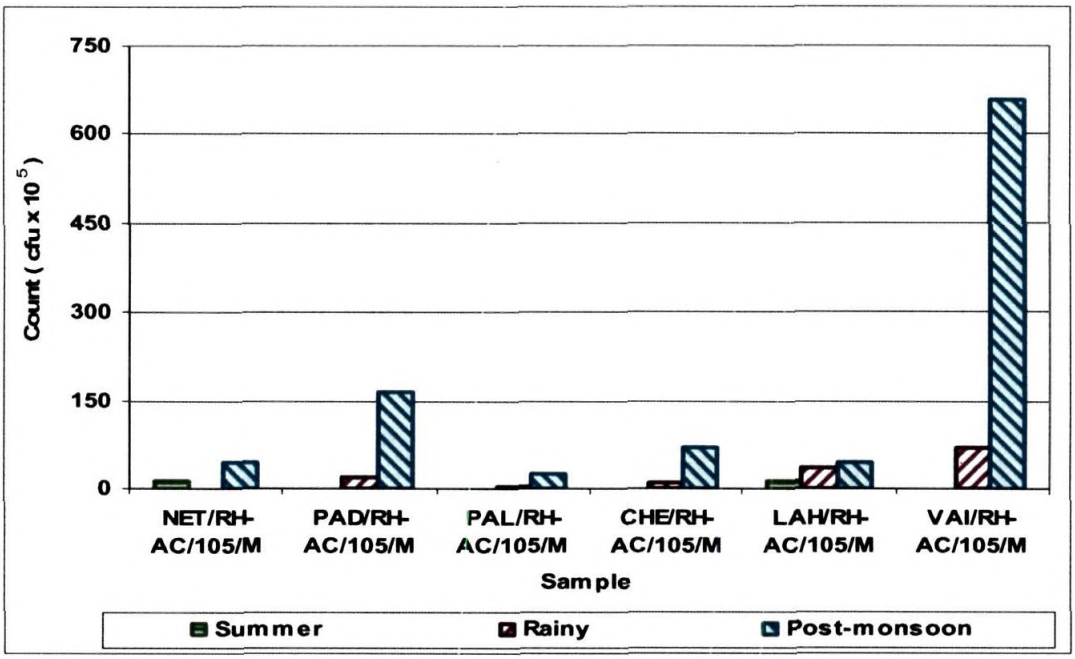

Fig. 2.4.7.5 - Seasonal variation of rhizosphere actinomycetes in RRII 105 mature trees

In general, actinomycete population was less in summer and high during the post monsoon season.

Analysis of seasonal variation:

The analysis of seasonal variation of rhizosphere actinomycetes residing on each clone in relation to their age are as in Tables 2.4.7.2 to 2.4.7.5

There was significant variation of rhizosphere actinomycetes in mature RRII 105 trees at Lahai and immature trees of the same clone at Nettana, Padiyoor, Chethackal and Lahai. In PB 260 trees, seasonal variation in rhizosphere actinomycetes was significant in mature trees at Nettana, and both mature and immature trees at Chethackal.

Season	Growth Stage of Plantations	
	Mature	Immature
	PB 260	RRII 105
Summer	15.78	7.84
Rainy	12.14	3.6
Post Monsoon	279.5	222.9
F(Variance ratio)	9284.02	96959.79
CD(P=0.05)	4.9	1.24

Table 2.4.7.2: Mean seasonal variation in rhizosphere actinomycetes at Nettana

Season	Growth Stage of Plantations
	Immature
	RRII 105
Summer	19.88
Rainy	24.48
Post Monsoon	13.14
F(Variance ratio)	94.9
CD(P=0.05)	1.8

Table 2.4.7.3: Mean seasonal variation in rhizosphere actinomycetes at Padiyoor

Season	Growth Stage of Plantations		
	Mature	Immature	
	PB 260	RRII 105	PB 260
Summer	52.74	2.74	5.00
Rainy	7.12	34.08	16.14
Post Monsoon	385.36	43.62	195.28
F(Variance ratio)	164681.14	818.53	20988.26
CD(P=0.05)	1.57	2.3	2.27

Table 2.4.7.4: Mean seasonal variation in rhizosphere actinomycetes at Chethackal

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	12.24	1.66
Rainy	34.06	7.08
Post Monsoon	43.62	385.36
F(Variance ratio)	623.08	250475.1767
CD(P=0.05)	1.99	1.35

Table 2.4.7.5: Mean seasonal variation in rhizosphere actinomycetes at Lahal

Analysis of variation in relation to rubber clones:

Variation in actinomycete population in relation to rubber clones for the three seasons are as in Tables 2.4.7.8 to 2.4.7.13

Mature rubber trees belonging to RRII 105 and PB 260 showed significant clonal variation in the number of actinomycetes harbouring the rhizosphere, at Nettana during the summer (*t*-value -1.22) during the summer and at Palappiliy during the rainy season (*t*-value -12) while those at Chethackal, Lahai and Vaikundam had no significant variation as shown in the following tables.

During the post monsoon season at Nettana, Palappilly, Chethackal and Lahai, significant variation (*t* value -248.12, -5.467, -218.76 and -156.53 respectively) was observed while there was no significant variation at Vaikundam.

Immature trees of the two clones at Chethackal during the summer season showed significant variation (*t*-value -1.98). Those at Palappilly showed significant variation (*t* value -3.727) in actinomycete population, during the rainy season, while there was no significant variation in trees at Chethackal and Vaikundam.

During the post monsoon season, immature trees at Chethackal and Vaikundam showed significant variation (*t* values -136.76 and -184.09 respectively). But no significant clonal variation was shown by the two clones at Palappilly.

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	12.26	7.03	15.78	34.78	-1.22

Table 2.4.7.6: Clonal variation in rhizosphere actinomycetes during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	4.08	1.87	30.96	23.2	-12.0
Chethackal	9.5	0.21	7.12	0.58	6.00
Lahai	34.06	0.72	16.14	1.95	24.54
Vaikundam	68.36	1.77	2	0.10	108.57

Table 2.4.7.7: Clonal variation in rhizosphere actinomycetes during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	42.86	3.30	279.50	1.25	-248.12
Palappilly	25.68	0.067	29.08	1.867	-5.467
Chethackal	71.1	8.55	385.36	1.77	-218.76
Lahai	43.62	3.92	195.28	0.772	-156.53
Vaikundam	656.78	5.24	39.08	1.91	516.58

Table 2.4.7.8: Clonal variation in rhizosphere actinomycetes during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Chethackal	2.74	1.053	5	5.48	-1.98

Table 2.4.7.9: Clonal variation in rhizosphere actinomycetes during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	8.68	0.067	9.52	0.187	-3.73
Chethackal	34.08	1.91	16.14	1.95	20.43
Vaikundam	7.48	0.147	2.02	0.092	24.97

Table 2.4.7.10: Clonal variation in rhizosphere actinomycetes during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	78.18	0.292	71.1	44.8	2.35
Chethackal	43.62	5.42	195.28	0.73	-136.76
Vaikundam	78.18	0.262	215	2.5	-184.09

Table 2.4.7.11: Clonal variation in rhizosphere actinomycetes during post-monsoon season on immature trees

2.4.8. Seasonal variation in rhizosphere phosphobacteria

Rhizosphere phosphobacteria were not present in the samples from Palappilly RRII 105 immature PB 260 mature trees and Vaikundam PB 260 mature soil during the summer season (Table 2.4.8.1).

Sample Name	Mature / Immature	Clone	Location	Mean Population [c f u x 10^5]		
				Summer	Rainy	Post-monsoon
NET/RH-PB/105/M	Mature	RRII 105	Nettana	132.0	99.2	169.2
NET/RH-PB/105/IM	Immature	RRII 105	Nettana	9.6	66.7	586.2
NET/RH-PB/260/M	Mature	PB 260	Nettana	31.3	228.1	507.8
PAD/RH-PB/105/M	Mature	RRII 105	Padiyoor	10.4	183.9	376.2
PAD/RH-PB/105/IM	Immature	RRII 105	Padiyoor	195.7	266.9	574.2
PAL/RH-PB/105/M	Mature	RRII 105	Palappilly	90.6	124.6	181.2
PAL/RH-PB/105/IM	Immature	RRII 105	Palappilly	NIL	77.8	93.5
PAL/RH-PB/260/M	Mature	PB 260	Palappilly	NIL	194.1	376.2
PAL/RH-PB/260/IM	Immature	PB 260	Palappilly	0.1	20.1	205.7
CHE/RH-PB/105/M	Mature	RRII 105	Chethackal	79.3	226.1	348.5
CHE/RH-PB/105/IM	Immature	RRII 105	Chethackal	17.6	135.2	273.1
CHE/RH-PB/260/M	Mature	PB 260	Chethackal	158.1	443.7	748.5
CHE/RH-PB/260/IM	Immature	PB 260	Chethackal	3.4	101.4	192.7
LAH/RH-PB/105/M	Mature	RRII 105	Lahai	158.5	76.3	610.6
LAH/RH-PB/105/IM	Immature	RRII 105	Lahai	52.7	55.3	146.4
LAH/RH-PB/260/M	Mature	PB 260	Lahai	86.8	82.7	102.4
VAI/RH-PB/105/M	Mature	RRII 105	Vaikundam	36.6	38.3	76.3
VAI/RH-PB/105/IM	Immature	RRII 105	Vaikundam	90.7	91.1	163.5
VAI/RH-PB/260/M	Mature	PB 260	Vaikundam	NIL	52.8	90.8
VAI/RH-PB/260/IM	Immature	PB 260	Vaikundam	38.1	81.0	101.7

Table 2.4.8.1: Occurrence of rhizosphere phosphobacteria

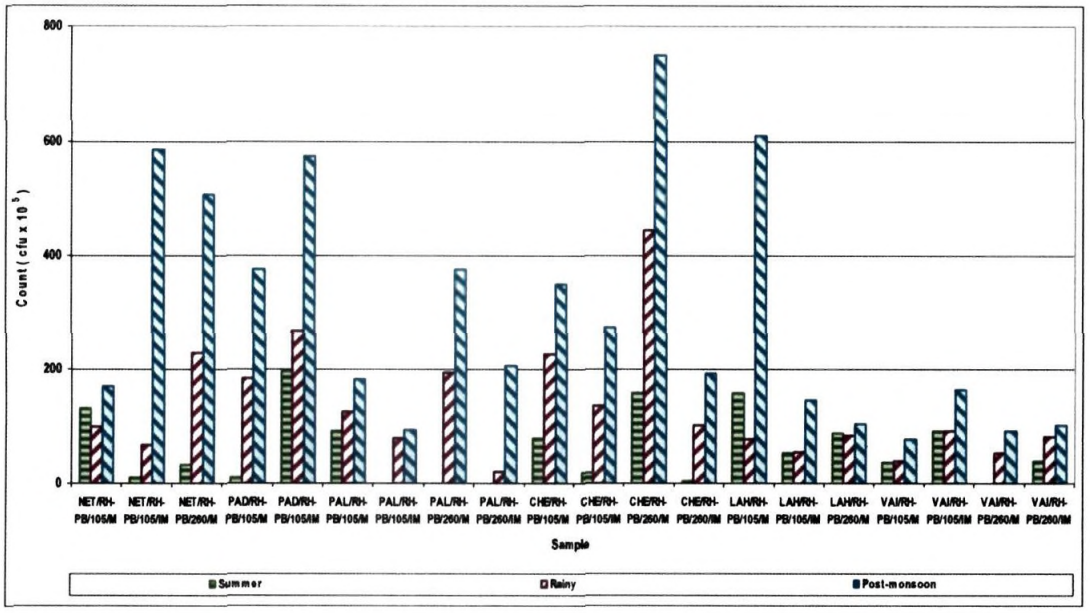

Fig. 2.4.8.1 - Seasonal variation of rhizosphere phosphobacteria in rubber trees

There was no significant variation between the phosphobacteria samples of summer and rainy seasons in PB 260 mature trees of Lahai. Maximum population of phosphobacteria was found at Chethackal during the post monsoon season in PB 260 mature trees as shown in Figure 2.4.8.1.

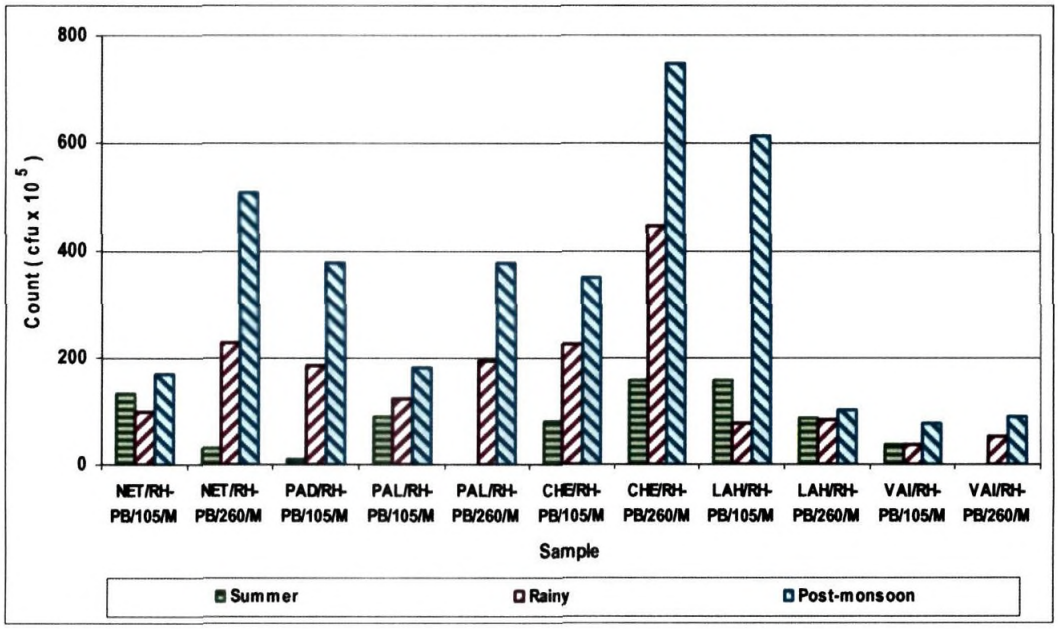

Fig. 2.4.8.2 -Seasonal variation of rhizosphere phosphobacteria in mature rubber trees

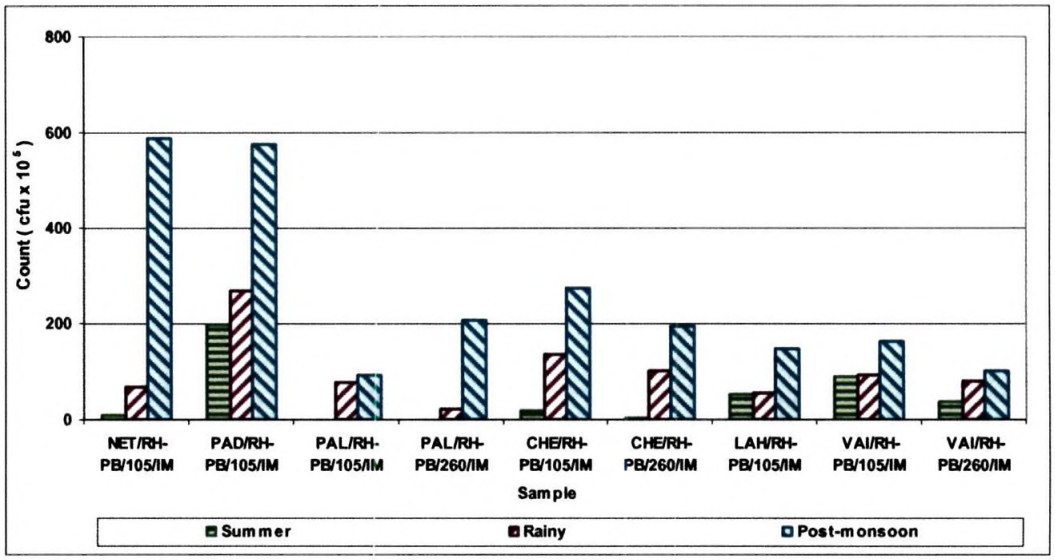

Fig. 2.4.8.3 - Seasonal variation of rhizosphere phosphobacteria in immature rubber trees

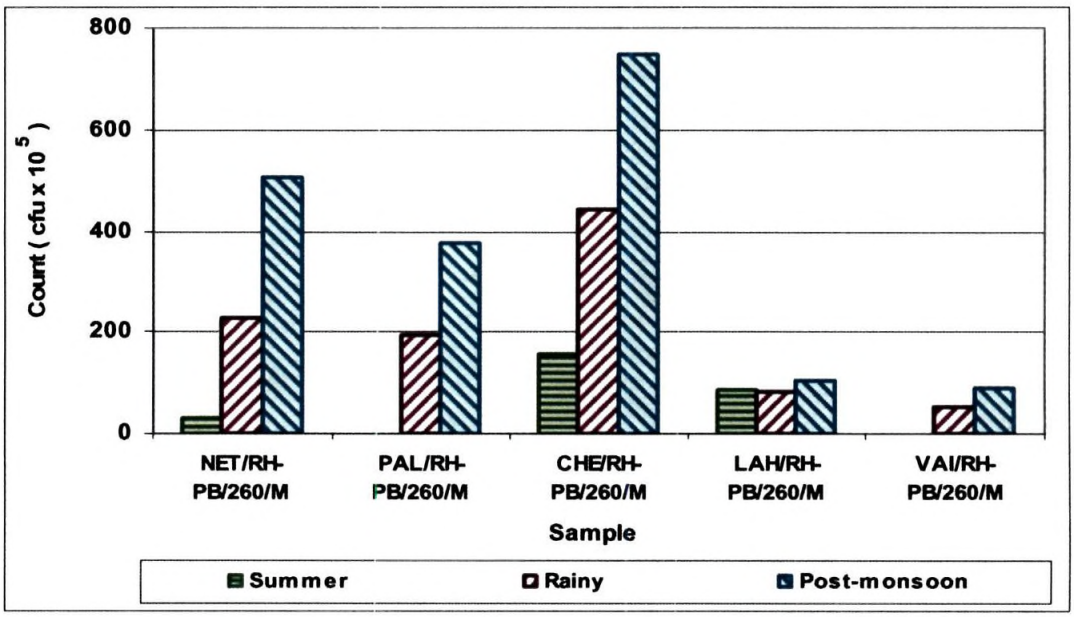

Fig. 2.4.8.4 - Seasonal variation of rhizosphere phosphobacteria in PB 260 mature rubber trees

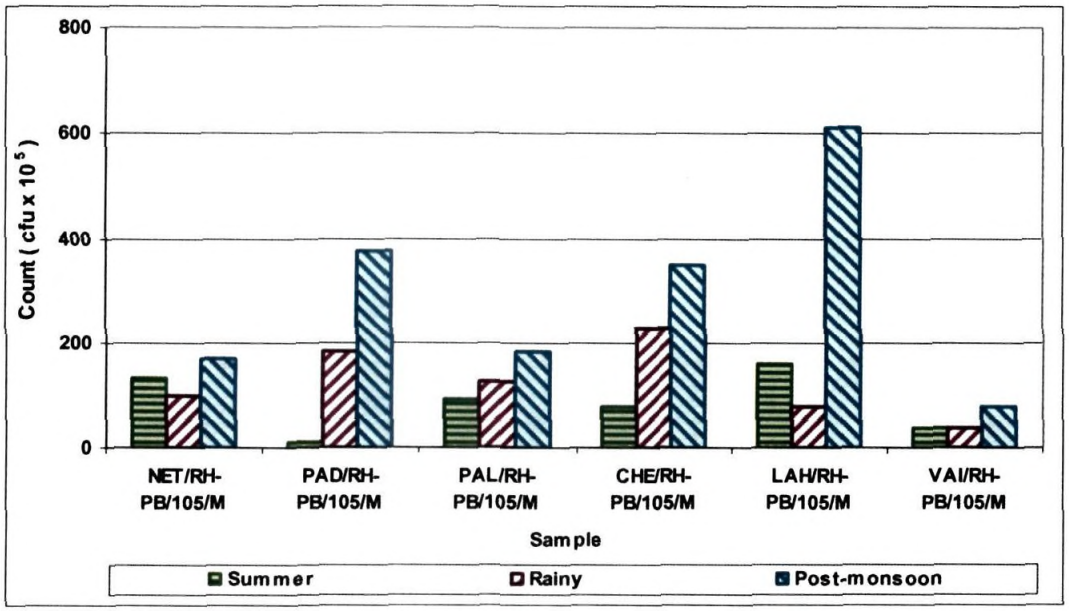

Fig. 2.4.8.5 - Seasonal variation of rhizosphere phosphobacteria in RRII 105 mature rubber trees

In general, PB 260 at Chethackal had more rhizosphere phosphobacteria followed by RRII 105 at Lahai during the post monsoon season (Fig. 2.4.8.4 and Fig. 2.4.8.5).

Analysis of seasonal variation:

Analysis of seasonal variation in phosphobacterial population for each clone in relation to their age are presented in Tables 2.4.8.2 to 2.4.8.7

The samples from mature trees of Nettana, Padiyoor, Palappally, Chethackal, Lahai and Vaikundam had significant variation as seen in the following tables. RRII 105 immature rubber trees of Nettana, Padiyoor, Chethackal, Lahai and Vaikundam also showed highly significant variation. In case of PB 260 mature samples, highly significant variation was found in rhizosphere samples from Nettana and Chethackal. In the case of immature PB 260 trees, significant variation of rhizosphere phosphobacteria was observed at Palappally, Chethackal and Vaikundam while at Lahai there was no significant variation in PB 260 mature trees.

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	132	31.34	9.56
Rainy	99.16	228.1	66.66
Post Monsoon	169.2	507.84	586.24
F(Variance ratio)	573.24	48410.06	741.2
CD(P=0.05)	4.51	3.35	35.97

Table 2.4.8.2: Mean seasonal variation in rhizosphere phosphobacteria at Nettana

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	10.38	195.7
Rainy	183.9	266.9
Post Monsoon	376.2	574.24
F(Variance ratio)	18414.12	56166.69
CD(P=0.05)	4.16	2.62

Table 2.4.8.3: Mean seasonal variation in rhizosphere phosphobacteria at Padiyoor

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	PB 260
Summer	90.6	0.14
Rainy	124.6	20.1
Post Monsoon	181.24	205.72
F(Variance ratio)	167.03	79.09
CD(P=0.05)	10.92	39.28

Table 2.4.8.4: Mean seasonal variation in rhizosphere phosphobacteria at Palappilly

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	79.26	158.08	17.6	3.36
Rainy	226.1	443.7	135.2	101.4
Post Monsoon	348.52	748.5	273.12	192.74
F(Variance ratio)	109.96	476.26	1481.82	11958.85
CD(P=0.05)	39.62	41.69	10.24	2.67

Table 2.4.8.5: Mean seasonal variation in rhizosphere phosphobacteria at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	158.5	86.78	52.7
Rainy	76.3	82.7	55.3
Post Monsoon	610.56	102.42	146.42
F(Variance ratio)	10576.3	2.55	9107.55
CD(P=0.05)	8.62	20.1	1.72

Table 2.4.8.6: Mean seasonal variation in rhizosphere phosphobacteria at Lahal

Season	Growth Stage of Plantations		
	Mature	Immature	
	RRII 105	RRII 105	PB 260
Summer	36.6	90.74	38.10
Rainy	38.28	91.12	80.98
Post Monsoon	76.26	163.52	101.7
F(Variance ratio)	321.02	349.58	4481.1
CD(P=0.05)	3.86	6.91	1.49

Table 2.4.8.7: Mean seasonal variation in rhizosphere phosphobacteria at Valkundam

Analysis of variation in relation to rubber clones:

The variation in phosphobacterial population in relation to rubber clones for the three seasons are presented in Tables 2.4.8.8 to 2.4.8.13

Phosphobacterial colonies on mature RRII 105 and PB 260 clones of rubber trees showed significant variation during the rainy season in samples from Nettana, Palappilly, Chethackal, Lahai and Vaikundam (*t* value -61.45, -51.52, -164.49, - 3.51 and -10.88 respectively). But during the post monsoon season highest significant variation of phosphobacterial population was shown in samples from Chethackal (*t* value -639.33) and Nettana (*t* value -429.52). *t value* was -45.03 at Palappilly and -6.29 at Vaikundam. Variation was not significant at Lahai.

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	132	10	31.34	14.78	45.22
Chethackal	79.26	2476.4	158.08	2738.05	-2.44
Lahai	158.5	104	86.78	628.62	5.92

Table 2.4.8.8: Clonal variation in rhizosphere phosphobacteria during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	99.16	19.71	228.1	2.3	-61.45
Palappilly	124.6	6.8	194.1	2.3	-51.52
Chethackal	226.1	2.05	443.7	6.7	-164.49
Lahai	76.3	9.7	82.7	6.95	-3.51
Vaikundam	38.28	0.727	52.76	8.12	-10.88

Table 2.4.8.9: Clonal variation in rhizosphere phosphobacteria during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	169.2	2.42	507.84	0.688	-429.52
Palappilly	181.24	90.69	376.24	3.06	-45.03
Chethackal	348.52	0.937	748.5	1.02	-639.33
Lahai	610.56	3.673	102.42	2.682	450.72
Vaikundam	76.26	21.47	90.76	5.093	-6.29

Table 2.4.8.10: Clonal variation in rhizosphere phosphobacteria during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Chethackal	17.6	143.11	3.36	1.61	2.65
Vaikundam	90.74	70.14	38.1	0.55	14

Table 2.4.8.11: Clonal variation in rhizosphere phosphobacteria during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	77.8	3.7	20.1	2.05	53.81
Chethackal	135.2	3.7	101.4	4.8	25.92
Vaikundam	91.12	0.297	80.98	2.752	12.99

Table 2.4.8.12: Clonal variation in rhizosphere phosphobacteria during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	93.5	10.25	205.72	2435.49	-5.074
Chethackal	273.12	18.77	192.74	4.838	36.99
Vaikundam	163.52	4.93	101.7	0.22	60.9

Table 2.4.8.13: Clonal variation in rhizosphere phosphobacteria during post-monsoon season on immature trees

2.4.9. Seasonal variation in rhizosphere azotobacter

Rhizosphere azotobacter variation during the summer, rainy and post-monsoon seasons are presented in Table 2.4.9.1

Sample Name	Mature / Immature	Clone	Location	Mean Population [c f u x 10^5]		
				Summer	Rainy	Post-monsoon
NET/RH-AZ/105/M	Mature	RRII 105	Nettana	700.0	41.4	120.4
NET/RH-AZ/105/IM	Immature	RRII 105	Nettana	190.9	16.5	88.1
NET/RH-AZ/260/M	Mature	PB 260	Nettana	365.0	71.9	397.0
PAD/RH-AZ/105/M	Mature	RRII 105	Padiyoor	233.2	129.6	188.8
PAD/RH-AZ/105/IM	Immature	RRII 105	Padiyoor	144.9	155.4	46.6
PAL/RH-AZ/105/M	Mature	RRII 105	Palappilly	76.8	28.8	37.8
PAL/RH-AZ/105/IM	Immature	RRII 105	Palappilly	435.8	142.4	280.6
PAL/RH-AZ/260/M	Mature	PB 260	Palappilly	553.6	158.5	180.9
PAL/RH-AZ/260/IM	Immature	PB 260	Palappilly	300.2	119.2	290.2
CHE/RH-AZ/105/M	Mature	RRII 105	Chethackal	37.1	108.5	29.7
CHE/RH-AZ/105/IM	Immature	RRII 105	Chethackal	1.0	169.4	470.8
CHE/RH-AZ/260/M	Mature	PB 260	Chethackal	63.9	87.0	392.3
CHE/RH-AZ/260/IM	Immature	PB 260	Chethackal	0.0	215.7	128.7
LAH/RH-AZ/105/M	Mature	RRII 105	Lahai	53.9	36.7	155.0
LAH/RH-AZ/105/IM	Immature	RRII 105	Lahai	135.6	197.4	100.7
LAH/RH-AZ/260/M	Mature	PB 260	Lahai	41.5	35.3	230.4
VAI/RH-AZ/105/M	Mature	RRII 105	Vaikundam	0.9	34.0	215.7
VAI/RH-AZ/105/IM	Immature	RRII 105	Vaikundam	1.9	34.5	63.5
VAI/RH-AZ/260/M	Mature	PB 260	Vaikundam	45.7	41.3	11.9
VAI/RH-AZ/260/IM	Immature	PB 260	Vaikundam	30.0	20.5	81.5

Table 2.4.9.1: Occurrence of rhizosphere azotobacter

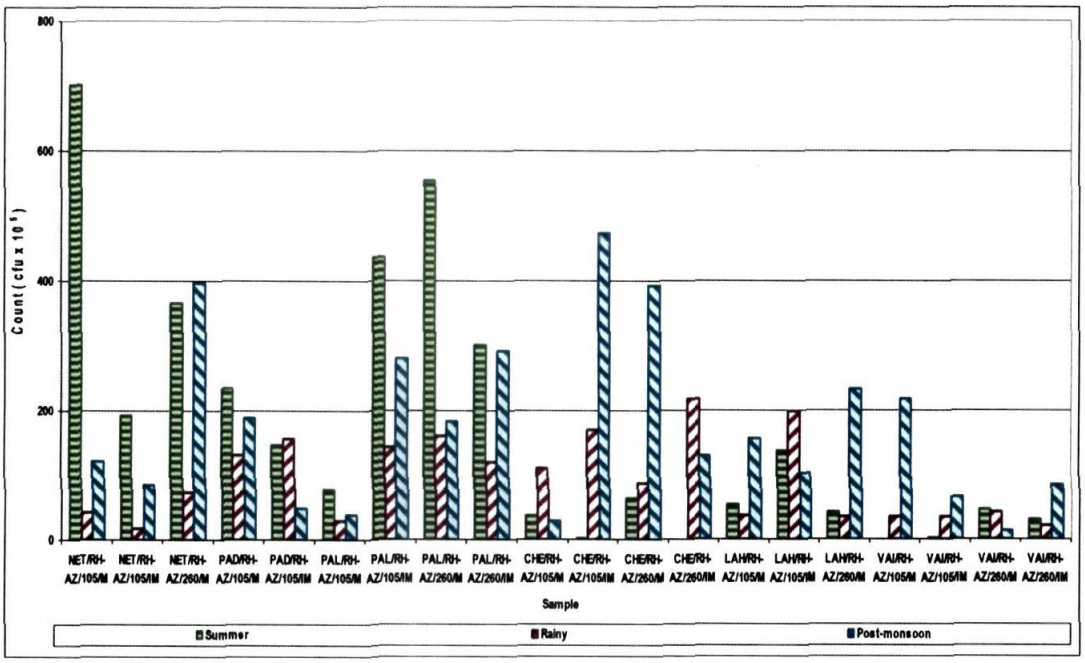

Fig. 2.4.9.1 - Seasonal variation of rhizosphere azotobacter in rubber trees

In general, rhizosphere azotobacter count was observed to be low during the rainy season. Highest azotobacter count was found during the summer season in Nettana and Palappilly, whereas higher azotobacter count was observed during the post-monsoon season in Chethackal and Lahai, as is evident from Fig.2.4.9.1.

A comparison of mature and immature rubber trees has been shown in Figure 2.4.9.2 and Figure 2.4.9.3. Azotobacter was not present in samples from immature PB 260 rubber trees from Chethackal while the count was very low in Chethackal RRII 105 immature tree samples.

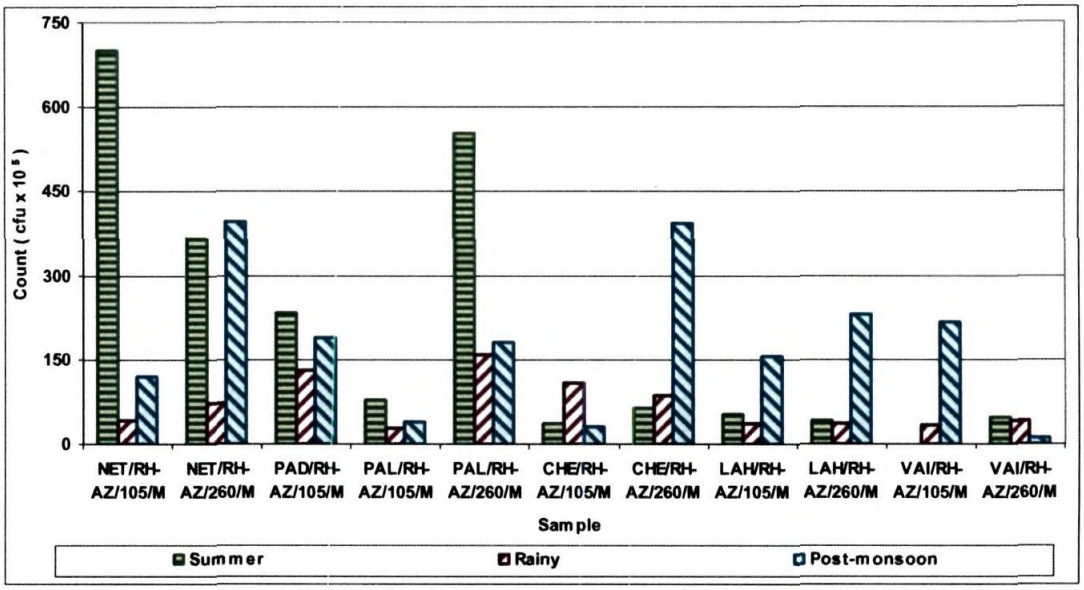

Fig. 2.4.9.2 - Seasonal variation of rhizosphere azotobacter in mature rubber trees

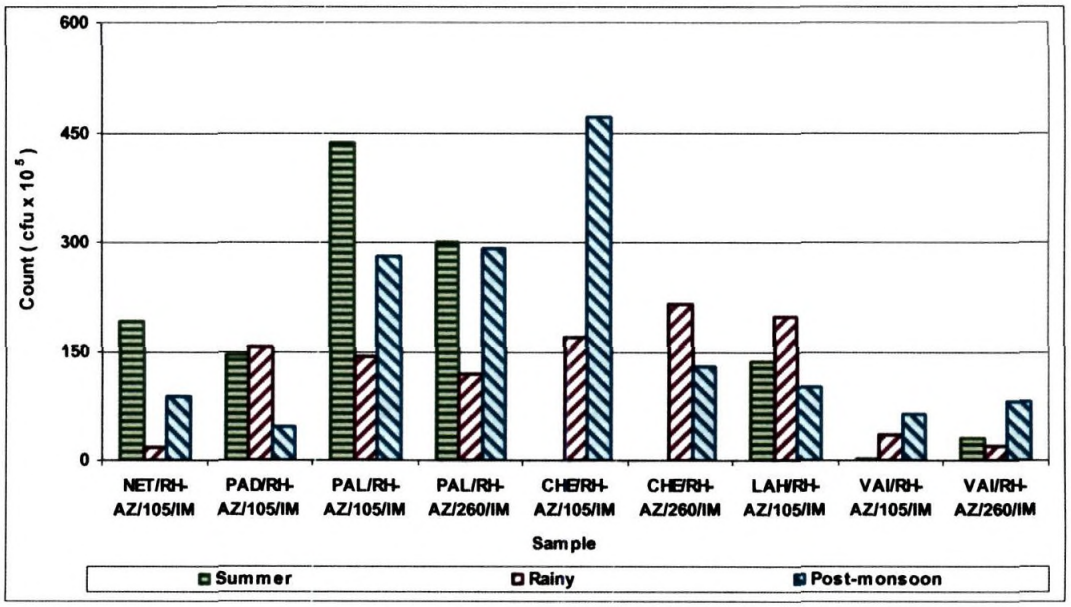

Fig. 2.4.9.3 - Seasonal variation of rhizosphere azotobacter in immature rubber trees

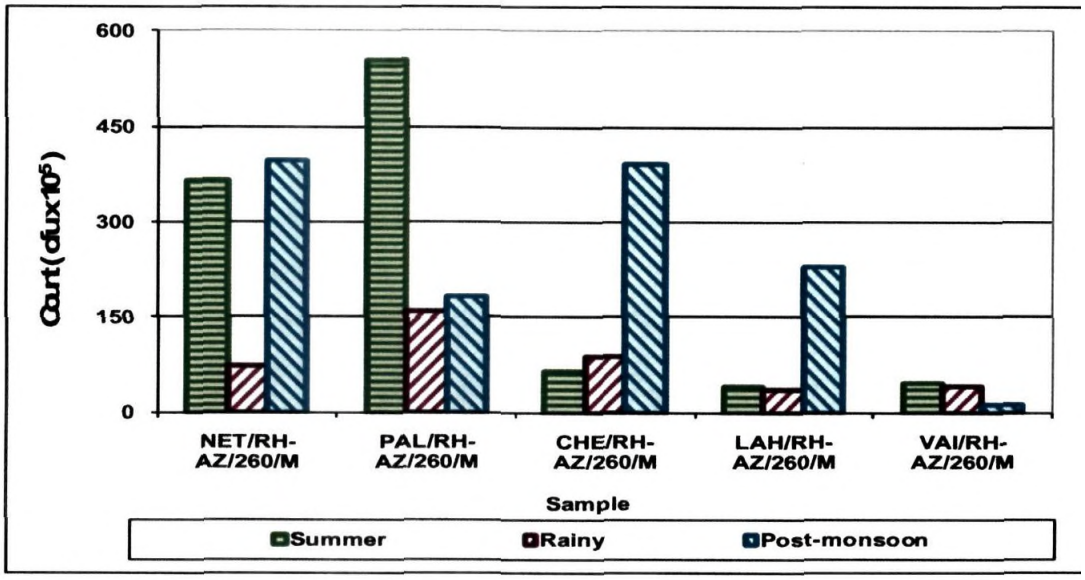

Fig. 2.4.9.4 - Seasonal variation of rhizosphere azotobacter in PB 260 mature rubber trees

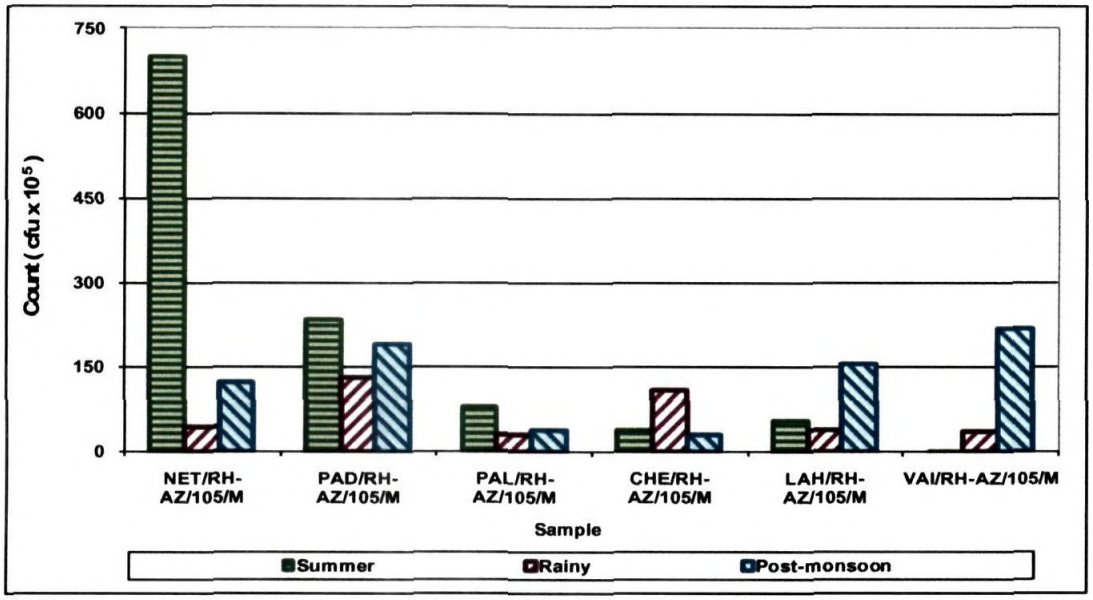

Fig. 2.4.9.5 - Seasonal variation of rhizosphere azotobacter in RRII 105 mature rubber trees

From Figure 2.4.9.4 it can be observed that among the rhizosphere samples of mature PB 260 rubber clones, lowest azotobacter population was recorded at Vaikundam

Rhizosphere azotobacter present on RRII 105 mature rubber trees at Vaikundam showed least population (0.9 X 10^5/g soil) as observed in Figure 2.4.9.5.

Analysis of seasonal variation:

Analysis of seasonal variation of azotobacter in each clone in relation to their age are presented in Tables 2.4.9.2 to 2.4.9.7

Mature RRII 105 trees at Nettana, Palappilly, Chethackal, Lahai and Vaikundam showed highly significant variation while only those at Padiyoor had no significant variation, as observed in the following tables. Similarly rhizosphere azotobacter on immature 105 trees at Nettana, Padiyoor, Chethackal and Vaikundam showed highly significant seasonal variation while those at Lahai showed slightly significant variation and those at Palappilly had no significant variation.

In all the rhizosphere samples of PB 260 from Nettana, Palappilly, Chethackal, Lahai and Vaikundam there was highly significant seasonal variation of rhizosphere azotobacter. PB 260 immature tree samples from Palappilly and Vaikundam also showed highly significant seasonal variation.

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	700	364.98	190.88
Rainy	41.36	71.9	16.54
Post Monsoon	120.4	397	88.1
F(Variance ratio)	41081.15	1376.38	2378.88
CD(P=0.05)	5.47	14.88	5.54

Table 2.4.9.2: Mean seasonal variation in rhizosphere azotobacter at Nettana

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	233.2	144.9
Rainy	129.56	155.4
Post Monsoon	188.8	46.6
F(Variance ratio)	**2.5**	4356.97
CD(P=0.05)	101.39	2.8

Table 2.4.9.3: Mean seasonal variation In rhizosphere azotobacter at Padiyoor

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	76.8	553.60	435.78	300.20
Rainy	28.78	158.48	142.4	119.2
Post Monsoon	37.84	180.9	280.56	290.24
F(Variance ratio)	69.48	995.07	3.78	17822.47
CD(P=0.05)	9.43	21.68	232.68	2.35

Table 2.4.9.4: Mean seasonal variation In rhizosphere azotobacter at Palappilly

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	37.06	63.90	1
Rainy	108.46	87.02	169.36
Post Monsoon	29.72	392.3	470.8
F(Variance ratio)	92.12	307.83	5786.44
CD(P=0.05)	13.97	32.19	9.22

Table 2.4.9.5: Mean seasonal variation in rhizosphere azotobacter at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	53.88	41.48	135.62
Rainy	36.72	35.28	197.4
Post Monsoon	154.96	230.4	100.7
F(Variance ratio)	104.7	565.62	6.36
CD(P=0.05)	19.24	14.37	59.85

Table 2.4.9.6: Mean seasonal variation in rhizosphere azotobacter at Lahal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	0.92	45.70	1.92
Rainy	33.96	41.3	34.48
Post Monsoon	215.7	11.9	63.5
F(Variance ratio)	10333.7	1009.04	1007.22
CD(P=0.05)	3.51	1.78	2.99

Table 2.4.9.7: Mean seasonal variation in rhizosphere azotobacter at Vaikundam

Analysis on clonal variation:

The variation in azotobacter popuiation in relation to rubber clones for the three seasons are presented in Tabie 2.4.9.8 to 2.4.9.13

Mature trees of RRII 105 and PB 260 showed significant variation in Azotobacter count in the rhizosphere during summer season at Palappilly, Chethackal and Vaikundam. But at Nettana and Lahai there was no significant variation.

Clonal variation was significant during the rainy season in Azotobacter population at Nettana, Palappiliy and Vaikundam (*t* value -17.8, -10.68 and -6.86 respectively) while it was not significant at Lahai and Chethackal.

Variation of Azotobacter population was significant at Nettana, Palappiliy, Chethackal and Lahai (*t* values -246.41, -27.10, -24.25 and -7.14 respectively) during the post monsoon season.

Regarding immature trees, significant variation between the two clones under study was observed (*t* value -6.83) during the rainy season, only at Chethackal. At Palappilly and Vaikundam the variation of rhizosphere azotobacter was not significant. But during the post monsoon season, there was significant variation at Palappiliy (*t* value -9.02) and Vaikundam (*t* value -14.7).

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	700	36.5	364.98	339.55	38.63
Palappilly	76.8	3.7	553.6	2.3	-435.26
Chethackal	37.06	282.47	63.9	166.69	-2.83
Lahai	53.88	2.5	41.48	313.53	1.56
Vaikundam	0.92	0.007	45.7	1.45	-82.95

Table 2.4.9.8: Clonal variation in rhizosphere azotobacter during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	41.36	9.42	71.9	5.30	-17.8
Palappilly	28.78	1.81	158.48	735.88	-10.68
Chethackal	108.46	2.41	87.02	375.68	2.47
Lahai	36.72	25.78	35.28	10.37	0.54
Vaikundam	33.96	4.21	41.3	1.52	-6.86

Table 2.4.9.9: Clonal variation in rhizosphere azotobacter during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	120.4	1.3	397	5	-246.4
Palappilly	37.84	135.03	180.9	4.3	-27.1
Chethackal	29.72	23.19	392.3	1094.7	-24.25
Lahai	154.96	556.51	230.4	2.3	-7.14
Vaikundam	215.7	15.2	11.9	2.05	109.72

Table 2.4.9.10: Clonal variation in rhizosphere azotobacter during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	435.78	85503	300.2	2.82	1.04
Vaikundam	1.92	0.007	30.02	20.847	-13.76

Table 2.4.9.11: Clonal variation in rhizosphere azotobacter during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	142.4	15.3	119.2	3.7	11.9
Chethackal	169.36	119.32	215.7	110.7	-6.83
Vaikundam	34.48	9.13	20.54	0.423	10.09

Table 2.4.9.12: Clonal variation in rhizosphere azotobacter during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	280.56	3.568	290.24	2.19	-9.02
Chethackal	470.8	3.7	128.68	64.54	92.61
Vaikundam	63.5	5	81.5	2.5	-14.7

Table 2.4.9.13: Clonal variation in rhizosphere azotobacter during post-monsoon season on immature trees

3. STUDIES ON VAM

3.1. Introduction

Mycorrhizae are symbiotic fungi and have been recognized as a beneficial component of crop husbandry. They help in mining non-mobile nutrients and water for the plant. Endomycorrhizae help the plant to combat diseases by competing with the root pathogens for the portals of entry into the root system and thus augment the health of the plant. Some ectomycorrhizhae are known to physically cover the growing region of roots preventing pathogens. Mycorrhizae may also elaborate growth promoting substances and also interact favorably with other soil microflora to enhance crop growth through synergy.

3.2. Review of literature

Vesicular arbuscular mycorrhizal (VAM) fungi are associated with more than 80% of terrestrial plant families (Taber and Trappe, 1982; Pendleton and Smith, 1983; Trappe, 1987; Mohankumar *et al.,* 1988; Regupathy and Mahadevan, 1993). The beneficial effects of microflora on higher plants have been studied by Chiarriello *et al.* (1988) and Bagyaraj (1995).

Giovannetti (1985) studied VAM association in the roots and rizhosphere soil of three plants, *Helichrysum stoechas, Ammophila arenaria* and *Eryngium maritimum* over a year, in a stable sand dune located in Tuscany, Italy. VA mycorrhizal infection decreased in summer, remained relatively constant in autumn and increased slowly from January until June during flowering. Spore numbers followed the same trend, being low in summer when root growth was poor.

Seasonal pattern in the formation of arbuscular mycorrhizae have been found to vary considerably (Lekshman *et al.,* 2006). Spore population has been found to be lowest during the monsoon season (Singh and Varma, 1981). Kumar(2002) studied the spore population of AM fungi in soils from forest area at Alagar hills, Madurai, Tamil Nadu for a year and

found that the highest AM fungal spore population occurred during winter season(December to January) whereas lowest was observed during the rainy season(August to September). Khade and Rodrigues (2004) studied the seasonal variation of AM fungi in the rhizosphere of banana in Goa and found that the species richness and spore density was maximum during pre monsoon and minimum during post monsoon season. Distribution of various species of AM fungi varies considerably with the rainfall pattern.

Guadarrama and Alvarez (1999) on studying the abundance of arbuscular mycorrhizal fungi spores in different environments in a tropical rain forest in Mexico reported that the highest number of species and spores were observed during the dry season, with a marked decrease during the rainy season.

Harinikumar and Bagyaraj (1988) studied the effect of annual season on mycorrhizal colonization and sporulation in two perennial tree hosts, mango and leucaena. Maximum colonization and sporulation occurred during the winter (November to January) months while summer months (April to June) were unfavourable for the proliferation of VA mycorrhizal fungi. There was a positive corrrelation between relative humidity and mycorrhizal activity, while the correlation between temperature and mycorrhizal activity was negative.

The mycorrhizal infection was found higher towards the base of the rubber trees and away from the site of fertilizer placement (Deka *et al.*, 1998)

3.3. Materials and methods

The percentage of vesicular and arbuscular mycorrhizal (VAM) spores in soils and their colonization in the roots of rubber plants were studied. Roots of rubber were collected from five trees in a plot from the six locations after removing the top soil. These fine roots were collected in polythene bags, labelled and brought to the lab in order to assay the

infection by vesicular arbuscular mycorrhizae (VAM). Soil samples were collected separately and labelled so as to find out the spore count of VAM.

3.3.1. Root infection assay of vesicular arbuscular mycorrhizae (VAM)

About 5 g of feeder roots were collected and cleaned thoroughly using tap water. The roots were cut into 1 cm bits and placed in a beaker which was properly labelled. 10% KOH solution was added to the roots and boiled in a water bath for about 45 min. It was washed again thoroughly using tap water and the excess water was drained off. Poured 2% HCl to neturalize the alkali and attain clarity for the roots. After 10 min, washed again thoroughly, added cotton blue stain and heated on a water bath for about 3 min. The excess stain was removed by adding plain lactophenol. The stained roots were labelled properly and retained for infection assay later. The roots were mounted on glass slides and examined under a light microscope to observe the fungal mycelia, vesicles and arbuscules. Ten bits were examined per sample and the average infection was expressed as percentage.

Cotton blue stain in Lactophenol

Lactic acid	-	20 ml
Phenol	-	20 g
Glycerol	-	40 ml
Distilled water	-	20 ml
Cotton blue	-	0.05 g

Added while stirring glycerol, lactic acid, phenol and distilled water and heated gently to dissolve.Then cotton blue dye was added.

3.3.2. VAM spore count

The soil collected from different locations for VAM spore count were air dried properly, labelled and stored for determination of spore count later.

Spores of vesicular arbuscular mycorrhizae were collected using the wet sieving and decanting method described by Gerdemann and Nicolson (1963). Soil samples (10g) was taken in a 500 ml beaker into which water was added, stirred well and allowed to settle for about 30 seconds. The supernatant along with the floating particles was decanted through a series of meshes (710, 250, 105 ad 45 µm) with the largest sieve on the top. This process was repeated 4 times and the debris collected in the top mesh was discarded. The residues on the three lower meshes were collected. The spores were collected into a beaker and the solution was made up to 100 ml. Aliquotes of 10 ml each were pipetted on to a Whatman No.1 filter paper placed in a funnel kept over a conical flask. The spores collected on the filter paper were examined under a microscope. The average count from five such filter papers was recorded.

The percentage of spores in 1 g of soil was calculated using the following formula:

$$\text{Percentage VAM spores} = \frac{n \times 10\ (ml) \times 100}{10\ (g)}$$

Where, n = number of VAM spores in 10 ml

3.4. Observations and results

Observations of mycorrhizal incidence on the roots collected from the different regions are presented in Table 3.4.1

3.4.1. VAM incidence

Sample	Clone	Mature / Immature	Place	Percentage Incidence of VAM in roots		
				Summer	Rainy	Post Monsoon
NET/VAM-R/105/M	RRII 105	Mature	Nettana	40	60	100
NET/VAM-R/105/IM	RRII 105	Immature	Nettana	40	50	80
NET/VAM-R/260/M	PB 260	Mature	Nettana	70	100	90
PAD/VAM-R/105/M	RRII 105	Mature	Padiyoor	60	90	90
PAD/VAM-R/105/IM	RRII 105	Immature	Padiyoor	70	100	80
PAL/VAM-R/105/M	RRII 105	Mature	Palappilly	50	90	80
PAL/VAM-R/105/IM	RRII 105	Immature	Palappilly	60	80	70
PAL/VAM-R/260/M	PB 260	Mature	Palappilly	50	60	60
PAL/VAM-R/260/IM	PB 260	Immature	Palappilly	80	70	50
CHE/VAM-R/105/M	RRII 105	Mature	Chethackal	50	100	20
CHE/VAM-R/105/IM	RRII 105	Immature	Chethackal	40	80	60
CHE/VAM-R/260/M	PB 260	Mature	Chethackal	60	50	90
CHE/VAM-R/260/IM	PB 260	Immature	Chethackal	50	20	80
LAH/VAM-R/105/M	RRII 105	Mature	Lahai	40	50	50
LAH/VAM-R/105/IM	RRII 105	Immature	Lahai	30	20	100
LAH/VAM-R/260/M	260M	Mature	Lahai	30	40	80
VAI/VAM-R/105/M	RRII 105	Mature	Vaikundam	60	80	100
VAI/VAM-R/105/IM	RRII 105	Immature	Vaikundam	60	70	100
VAI/VAM-R/260/M	260M	Mature	Vaikundam	70	90	90
VAI/VAM-R/260/IM	PB 260	Immature	Vaikundam	60	80	100

Table 3.4.1.1: Occurrence of VAM incidence

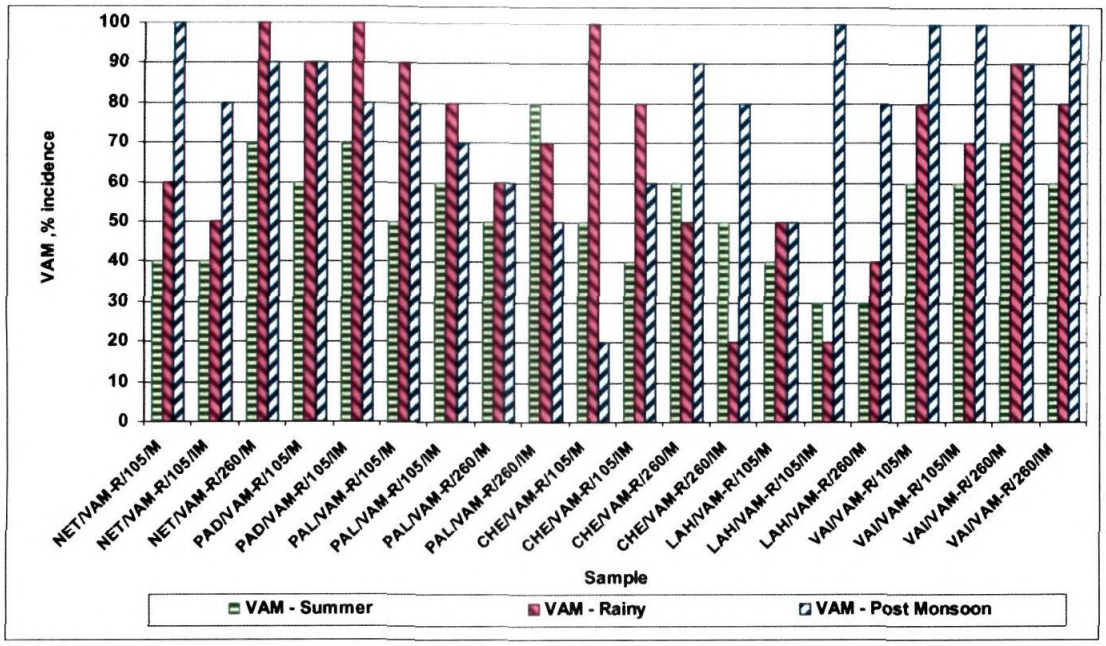

Fig. 3.4.1.1: Seasonal variation of VAM incidence

The seasonal variation of VAM incidence on the roots of rubber trees was not consistent across the regions (Fig. 3.4.1.1). In many samples there was hundred percent incidence of VAM.

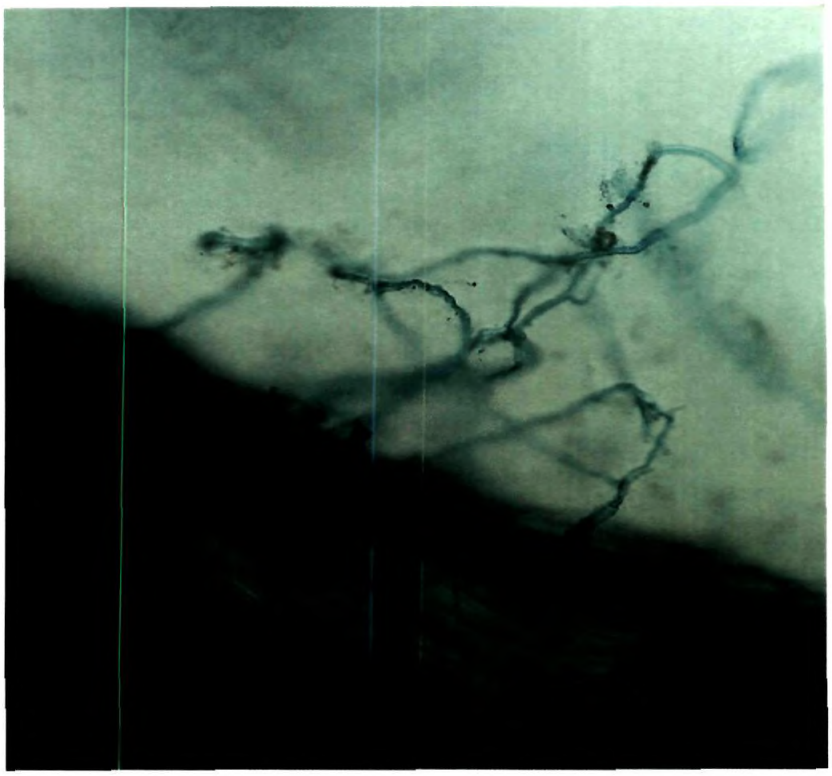

Fig.3.4.1.2: Appresorium along with hyphae extending out of the roots of rubber

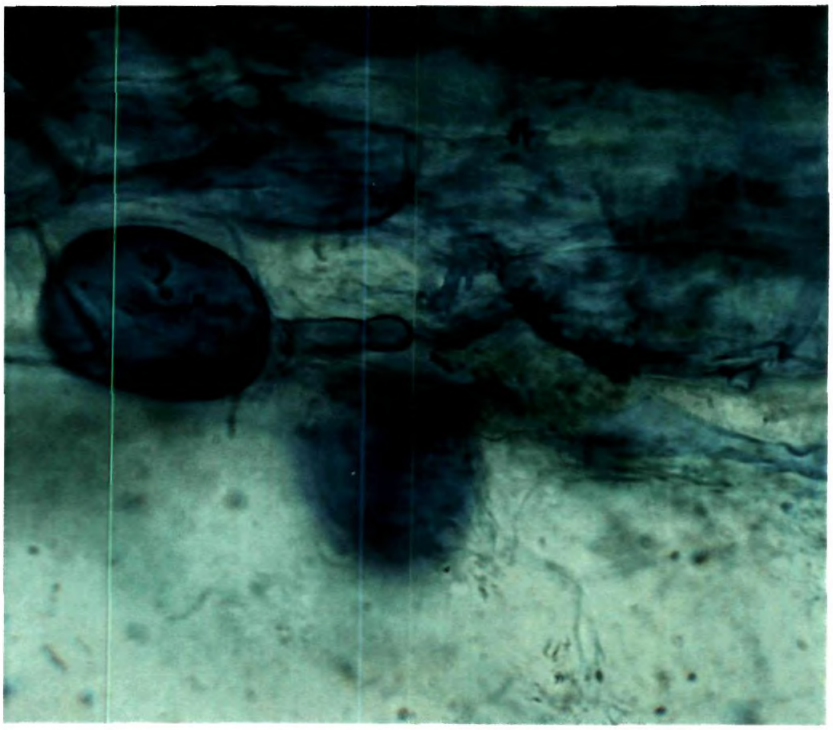

Fig.3.4.1.3: Vesicle and hyphae of VAM

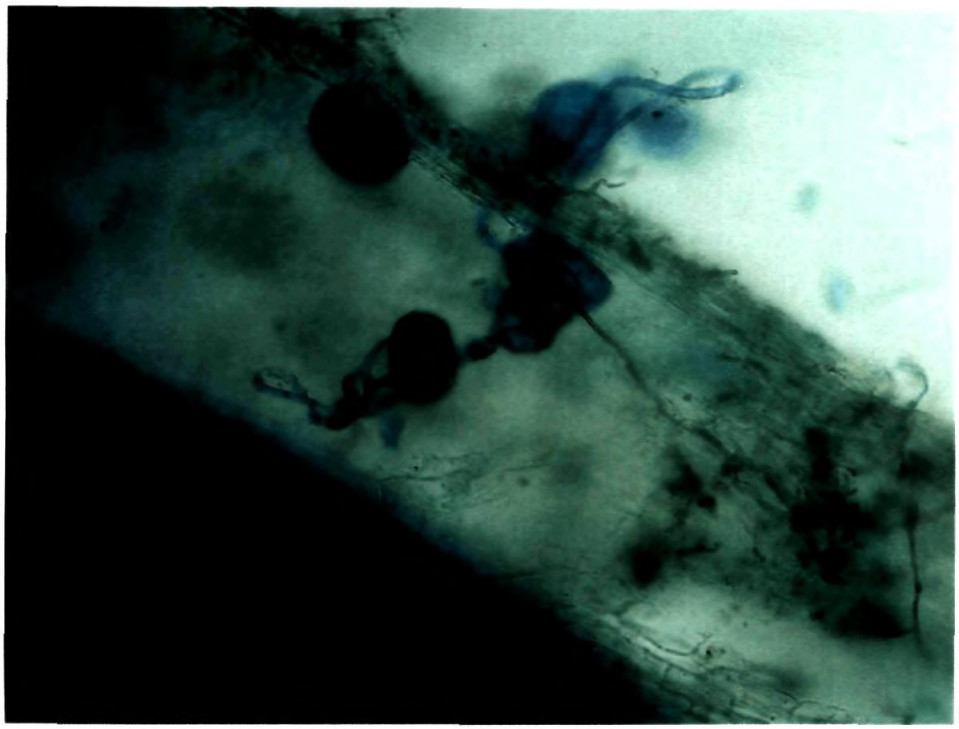

Fig.3.4.1.4: VAM in roots of *Hevea brasiliensis*

Fig.3.4.1.5: Vesicle of VAM in roots of *Hevea brasiliensis*

Seasonal variation in VAM incidence:

Analysis of seasonal variation of VAM incidence have shown that mature RRII 105 and PB260 rubber trees at Nettana, Padiyoor, Palappilly, Chethackai, Lahai and Vaikundam showed highly significant variation (Tables 3.4.1.2 to 3.4.1.7)

Immature rubber trees at Nettana, Palappilly, Padiyoor, Chethackal, Lahai and Vaikundam also showed highly significant seasonal variation in VAM incidence.

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	40	70	40
Rainy	60	100	50
Post Monsoon	100	90	80
F(Variance ratio)	1866.67	466.67	866.67
CD(P=0.05)	2.18	2.18	2.18

Table 3.4.1.2: Mean seasonal variation in VAM incidence at Nettana

Season	Growth Stage of Plantations	
	Mature	Immature
	RRII 105	RRII 105
Summer	60	70
Rainy	90	100
Post Monsoon	90	80
F(Variance ratio)	600	466.67
CD(P=0.05)	2.18	2.18

Table 3.4.1.3: Mean seasonal variation in VAM incidence at Padiyoor

Season	Growth Stage of Plantations		
	Mature	Immature	
	PB 260	RRII 105	PB 260
Summer	50	60	80.00
Rainy	60	80	70
Post Monsoon	60	70	50
F(Variance ratio)	66.67	200	466.67
CD(P=0.05)	2.18	2.18	2.18

Table 3.4.1.4: Mean seasonal variation in VAM incidence at Palappilly

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	50	60	40	50.00
Rainy	100	50	80	20
Post Monsoon	20	90	60	80
F(Variance ratio)	3266.67	866.67	800	1800
CD(P=0.05)	2.18	2.18	2.18	2.18

Table 3.4.1.5: Mean seasonal variation in VAM incidence at Chethackal

Season	Growth Stage of Plantations		
	Mature		Immature
	RRII 105	PB 260	RRII 105
Summer	40	30	30
Rainy	50	40	20
Post Monsoon	50	80	100
F(Variance ratio)	66.67	1235.29	3800
CD(P=0.05)	2.18	2.32	2.18

Table 3.4.1.6: Mean seasonal variation in VAM incidence at Lahai

Season	Growth Stage of Plantations			
	Mature		Immature	
	RRII 105	PB 260	RRII 105	PB 260
Summer	60	70.00	60	60.00
Rainy	80	90	70	80
Post Monsoon	100	90	100	100
F(Variance ratio)	800	266.67	650	80
CD(P=0.05)	2.18	2.18	2.52	2.18

Table 3.4.1.7: Mean seasonal variation in VAM incidence at Vaikundam

Variation in mycorrhizal association on the two rubber clones:

Studies on variation of percentage incidence of vesicular arbuscular mycorrhizae in relation to rubber clones for the three seasons are presented in Tables 3.4.1.8 to 3.4.1.13

Analysis of percentage incidence of vesicular arbuscular mycorrhizae on the roots of mature rubber trees of RRII105 and PB 260 during the summer season have shown that at Nettana, Chethackal and Vaikundam the variation was significant (*t* value -30, -10 and -10 respectively). But there was no significant variation at Palappilly and Lahai.

During the rainy season, variation in the percentage incidence of VAM between the two clones of mature rubber trees was significant at Nettana and Vaikundam.There was no significant variation at Palappilly, Chethackal and Lahai.

Percentage incidence of VAM during the post monsoon season in mature rubber trees of the two clones showed significant variation at Chethackal and Lahai (*t* values -70 and -27.39) while there was no significant variation at Nettana, Palappilly and Vaikundam.

Immature trees of the two clones at Palappiily and Chethackal showed significant variation (*t* value -20 and -10 respectively) with regard to percentage incidence of VAM during summer. But during the rainy season there was no significant variation.

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	40	2.5	70	2.5	-30
Palappilly	50	2.5	50	2.5	0
Chethackal	50	2.5	60	2.5	-10
Lahai	40	2.5	30	2.5	10
Vaikundam	60	2.5	70	2.5	-10

Table 3.4.1.8: Clonal variation in VAM incidence during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	60	2.5	100	2.5	-40
Palappilly	90	2.5	60	2.5	30
Chethackal	100	2.5	50	2.5	50
Lahai	50	2.5	40	2.5	10
Vaikundam	80	2.5	90	2.5	-10

Table 3.4.1.9: Clonal variation in VAM incidence during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	100	2.5	90	2.5	10
Palappilly	80	2.5	60	2.5	20
Chethackal	20	2.5	90	2.5	-70
Lahai	50	2.5	80	3.5	-27.39
Vaikundam	100	2.5	90	2.5	10

Table 3.4.1.10: Clonal variation in VAM incidence during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	60	2.5	80	2.5	-20
Chethackal	40	2.5	50	2.5	-10
Vaikundam	60	2.5	60	2.5	0

Table 3.4.1.11: Clonal variation in VAM incidence during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	80	2.5	70	2.5	10
Chethackal	80	2.5	20	2.5	60
Vaikundam	70	2.5	80	2.5	-10

Table 3.4.1.12: Clonal variation in VAM incidence during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	70	2.5	50	2.5	20
Chethackal	60	2.5	80	2.5	-20
Vaikundam	100	5	100	2.5	0

Table 3.4.1.13: Clonal variation in VAM incidence during post-monsoon season on immature trees

3.4.2. Seasonal variation of VAM spores

VAM spore count during the three seasons are presented in Table 3.4.2.1

Sample	Clone	Mature / Immature	Place	Percentage Incidence of VAM spores		
				Summer	Rainy	Post Monsoon
NET/VAM-S/105/M	RRII 105	Mature	Nettana	80	30	40
NET/VAM-S/105/IM	RRII 105	Immature	Nettana	70	20	40
NET/VAM-S/260/M	PB 260	Mature	Nettana	90	15	30
NET/VAM-S/Vrgn	Virgin	N.A	Nettana	100	25	35
PAD/VAM-S/105/M	105M	Mature	Padiyoor	80	20	30
PAD/VAM-S/105/IM	RRII 105	Immature	Padiyoor	50	10	20
PAD/VAM-S/Vrgn	Virgin	N.A	Padiyoor	90	20	40
PAL/VAM-S/105/M	105M	Mature	Palappilly	75	10	25
PAL/VAM-S/105/IM	RRII 105	Immature	Palappilly	60	5	20
PAL/VAM-S/260/M	PB 260	Mature	Palappilly	50	10	30
PAL/VAM-S/260/IM	PB 260	Immature	Palappilly	70	20	35
PAL/VAM-S/Vrgn	Virgin	N.A	Palappilly	90	30	50
CHE/VAM-S/105/M	RRII 105	Mature	Chethackal	30	10	20
CHE/VAM-S/105/IM	RRII 105	Immature	Chethackal	25	5	10
CHE/VAM-S/260/M	PB 260	Mature	Chethackal	20	10	15
CHE/VAM-S/260/IM	PB 260	Immature	Chethackal	15	5	10
CHE/VAM-S/Vrgn	Virgin	N.A	Chethackal	50	10	20
LAH/VAM-S/105/M	RRII 105	Mature	Lahai	50	5	15
LAH/VAM-S/105/IM	RRII 105	Immature	Lahai	80	15	30
LAH/VAM-S/260/M	260M	Mature	Lahai	70	5	20
LAH/VAM-S/Vrgn	Virgin	N.A	Lahai	85	10	25
VAI/VAM-S/105/M	RRII 105	Mature	Vaikundam	85	30	50
VAI/VAM-S/105/IM	RRII 105	Immature	Vaikundam	80	20	40
VAI/VAM-S/260/M	260M	Mature	Vaikundam	90	35	55
VAI/VAM-S/260/IM	PB 260	Immature	Vaikundam	85	35	40
VAI/VAM-S/Vrgn	Virgin	N.A	Vaikundam	100	30	45

Table 3.4.2.1 Seasonal variation of VAM spore count

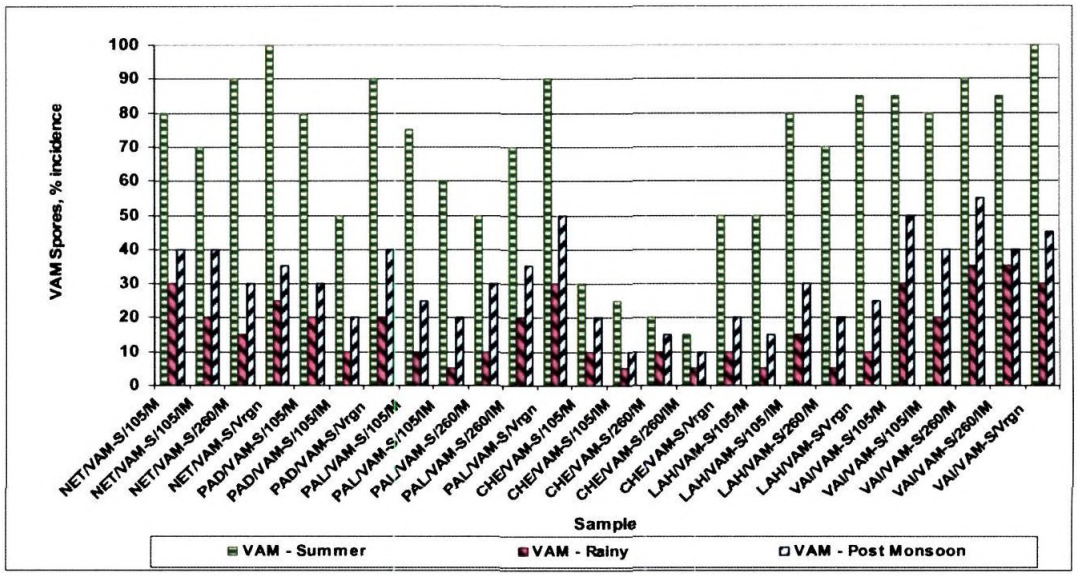

Fig. 3.4.2.1 – Seasonal variation of VAM spores

The VAM spores could be collected easily on the filter paper (Fig. 3.4.2.2) and observed. Occasionally broken spores also were observed (Fig. 3.4.2.3). The spore count was consistently high across all the regions during summer season and consistently low during the rainy season.

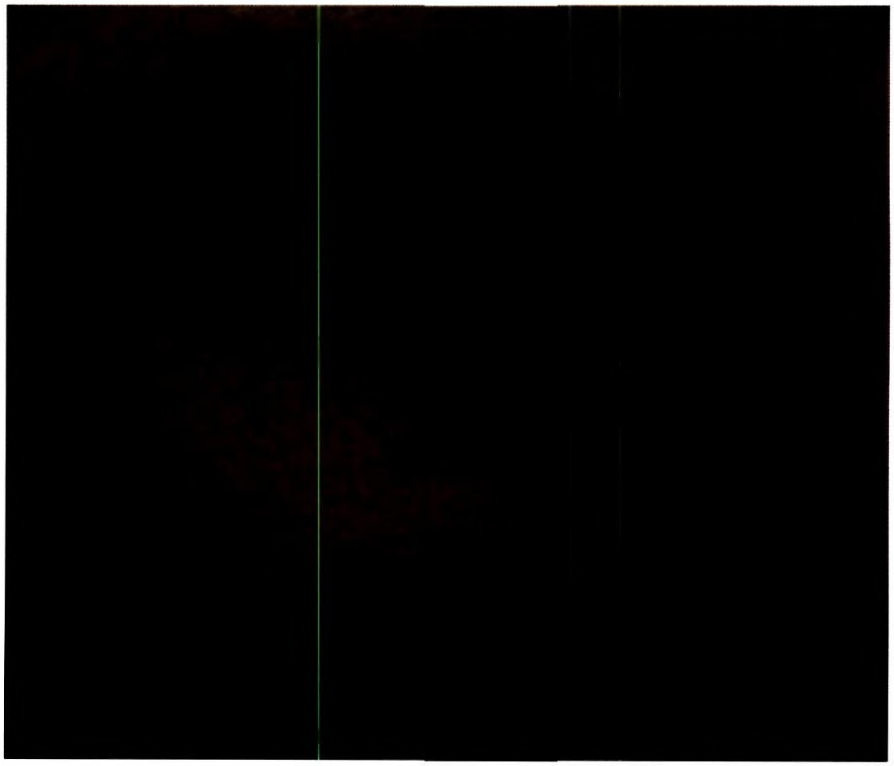

Fig.3.4.2.2: VAM spores isolated from soil

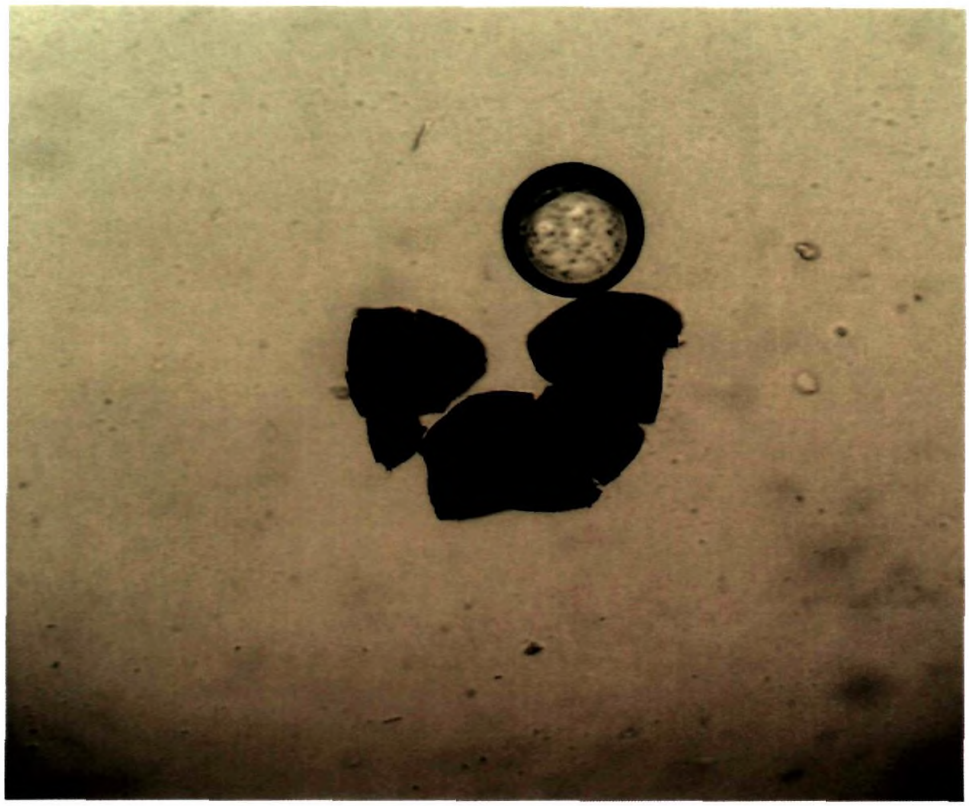

Fig.3.4.2.3: Broken VAM spore

Seasonal variation in occurrence of VAM spores:

Analysis of seasonal variation of VAM spores in the rhizosphere of mature RRII 105 and PB 260 rubber trees at Nettana, Padiyoor, Palappilly, Chethackal, Lahai and Vaikundam showed highly significant variation(Tables 3.4.2.2.to 3.4.2.7).

Similarly in the rhizosphere of immature rubber trees at Nettana, Palappilly, Padiyoor, Chethackal, Lahai and Vaikundam there was highly significant seasonal variation in VAM spores.

Season	Growth Stage of Plantations			
	Mature		Immature	Virgin
	RRII 105	PB 260	RRII 105	
Summer	80	90	70	100
Rainy	30	15	20	25
Post Monsoon	40	30	40	35
F(Variance ratio)	1400	3150	1266.67	3316.67
CD(P=0.05)	2.18	2.8	2.18	2.18

Table 3.4.2.2: Mean seasonal variation in VAM spore count at Nettana

Season	Growth Stage of Plantations		
	Mature	Immature	Virgin
	RRII 105	RRII 105	
Summer	80	50	90
Rainy	20	10	20
Post Monsoon	30	20	40
F(Variance ratio)	2066.67	866.67	2600
CD(P=0.05)	2.18	2.18	2.18

Table 3.4.2.3: Mean seasonal variation in VAM spore count at Padiyoor

Season	Growth Stage of Plantations				
	Mature		Immature		Virgin
	RRII 105	PB 260	RRII 105	PB 260	
Summer	80	90	70	70	90
Rainy	30	15	20	20	30
Post Monsoon	40	30	40	35	50
F(Variance ratio)	1400	3150	3166.67	1316.67	1866.67
CD(P=0.05)	2.18	2.8	2.18	2.18	2.18

Table 3.4.2.4: Mean seasonal variation in VAM spore count at Palappilly

Season	Growth Stage of Plantations				
	Mature		Immature		Virgin
	RRII 105	PB 260	RRII 105	PB 260	
Summer	30	20	25	15	50
Rainy	10	20	5	5	10
Post Monsoon	20	15	10	10	20
F(Variance ratio)	200	16.67	295.45	44.12	866.67
CD(P=0.05)	2.18	2.18	1.87	2.32	2.18

Table 3.4.2.5: Mean seasonal variation in VAM spore count at Chethackal

Season	Growth Stage of Plantations			
	Mature		Immature	Virgin
	RRII 105	PB 260	RRII 105	
Summer	50	70.00	80	85
Rainy	5	5	15	10
Post Monsoon	15	20	30	25
F(Variance ratio)	1116.67	2316.67	2316.67	3150
CD(P=0.05)	2.18	2.18	2.18	2.18

Table 3.4.2.6: Mean seasonal variation in VAM spore count at Lahai

Season	Growth Stage of Plantations				
	Mature		Immature		Virgin
	RRII 105	PB 260	RRII 105	PB 260	
Summer	85	90.00	80	85.00	100
Rainy	30	35	20	35	30
Post Monsoon	50	55	40	40	45
F(Variance ratio)	1550	1550	1866.67	1516.67	2397.06
CD(P=0.05)	2.18	2.18	2.18	2.18	2.32

Table 3.4.2.7: Mean seasonal variation in VAM spore count at Vaikundam

Variation in VAM spore count in the rhizosphere of the two rubber clones:

The variation in VAM spore count in relation to rubber clones for the three seasons are as in Tables 3.4.2.8 to 3.4.2.13

VAM spore count showed significant variation between the rhizosphere of two clones of mature rubber trees at Nettana, Lahai and Vaikundam (*t*- value -10, -20 and -5 resp.) during the summer season. During the rainy season, such significant variation was observed only in samples from Chethakkal and Vaikundam (*t*- value -10 and -5 resp.)

Post-monsoon samples from Palappilly, Lahai and Vaikundam showed significant variation (*t*-value -5) while Nettana and Chethakkal samples had no significant variation.

Rhizosphere soil samples of immature rubber trees from Palappilly and Vaikundam showed significant variation in VAM spore count during the summer (*t*-value -10 and -5)and during the rainy (*t*-value -15) seasons.In the post-monsoon season also, the samples from Palappilly showed significant variation (*t*-value -15).

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	80	2.5	90	2.5	-10
Palappilly	75	2.5	50	2.5	25
Chethackal	30	2.5	20	2.5	10
Lahai	50	2.5	70	2.5	-10
Vaikundam	85	2.5	90	2.5	-5

Table 3.4.2.8: Clonal variation in VAM spores during summer on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	30	2.5	15	2.5	15
Palappilly	10	2.5	10	2.5	0
Chethackal	10	2.5	20	2.5	-10
Lahai	5	2.5	5	2.5	0
Vaikundam	30	2.5	35	2.5	-5

Table 3.4.2.9: Clonal variation in VAM spores during rainy season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Nettana	40	2.5	30	2.5	10
Palappilly	25	2.5	30	2.5	-5
Chethackal	20	2.5	15	2.5	5
Lahai	15	2.5	20	2.5	-5
Vaikundam	50	2.5	55	2.5	-5

Table 3.4.2.10: Clonal variation in VAM spores during post-monsoon season on mature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	60	2.5	70	2.5	-10
Chethackal	25	2.5	15	2.5	10
Vaikundam	80	2.5	85	2.5	-5

Table 3.4.2.11: Clonal variation in VAM incidence during summer on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	5	2.5	20	2.5	-15
Chethackal	50	2.5	5	3.5	41.08
Vaikundam	20	2.5	35	2.5	-15

Table 3.4.2.12: Clonal variation in VAM spores during rainy season on immature trees

Location	RRII 105		PB 260		t value
	Mean population	Variance	Mean population	Variance	
Palappilly	20	2.5	35	2.5	-15
Chethackal	10	2.5	10	2.5	0
Vaikundam	40	2.5	40	2.5	0

Table 3.4.2.13: Clonal variation in VAM spores during post-monsoon season on immature trees

4. INFLUENCE OF EDAPHIC FACTORS ON SOIL MICROFLORA

4.1. Introduction

Atmospheric and climatic factors exert both direct and indirect effects on plants and plant pathogens (Coakley and Scherm, 1996). Microbes need a suitable pH and moisture to thrive in their habitat.

4.2. Review of literature

Seasonal variations in microbial population and relationship of microbes with some soil parameters were studied in tea ecosystem of Assam by Gogol *et al.* (2003). The population was observed to be influenced by both the season and the soil factors like moisture and pH.

Low pH (3 to 4) inhibited the mycelial growth of mycorrhizal fungus, *Cenococcum geophilum* (Fanxiang and Min, 1996)

4.3. Materials and methods

In the present studies, the moisture content of the soil and pH were determined to observe their influence on the soil microflora.

4.3.1. Soil moisture content

Transferred about 5g of soil immediately from each of the samples from which the microbial population was assayed, into a pre-welghed weighing bottle and its accurate weight was determined using an electronic balance. The samples were dried in an electric oven at 105 ^{0}C, cooled and weighed again. Repeated the process till two consecutive weights agreed. From the loss in weight the percentage of moisture of the samples was calculated. The correlation between the soil moisture content and population of soil microorganisms was studied.

4.3.2. Soil pH

The pH of soil was determined using a pH meter. Added 10g of each of the soil samples into a 50 ml beaker, added 25 ml of distilled water and stirred at regular intervals for 20 to 30 minutes. The pH of the samples were determined using a pre-calibrated pH meter.

Correlation of soil pH with the soil microbial population was studied.

4.4. Observation and results

4.4.1. Seasonal variation in moisture content

The moisture content of soil from each plot is given in Table 4.4.1

				MOISTURE CONTENT		
SAMPLE	Soil Condition	Clone	Location	SUMMER	RAINY	POST MONSOON
NET/105/M	Mature	RRII 105	Nettana	15.22	23.80	21.98
NET/105/IM	Immature	RRII 105	Nettana	16.91	24.30	22.30
NET/260/M	Mature	PB 260	Nettana	15.58	23.70	19.20
NET/VIR	Virgin	Virgin	Nettana	16.13	25.07	21.39
PAD/105/M	Mature	RRII 105	Padiyoor	16.92	21.30	18.51
PAD/105/IM	Immature	RRII 105	Padiyoor	13.22	20.29	16.30
PAD/VIR	Virgin	Virgin	Padiyoor	10.56	19.99	15.16
PAL/105/M	Mature	RRII 105	Palappilly	13.81	18.86	16.77
PAL/105/IM	Immature	RRII 105	Palappilly	17.55	23.71	19.90
PAL/260/M	Mature	PB 260	Palappilly	16.09	22.34	21.39
PAL/260/IM	Immature	PB 260	Palappilly	12.55	21.98	19.67
PAL/VIR	Virgin	Virgin	Palappilly	14.41	18.04	17.59
CHE/105/M	Mature	RRII 105	Chethackal	16.77	23.80	18.84
CHE/105/IM	Immature	RRII 105	Chethackal	16.59	22.68	18.68
CHE/260/M	Mature	PB 260	Chethackal	17.70	24.08	19.13
CHE/260/IM	Immature	PB 260	Chethackal	17.59	23.68	18.57
CHE/VIR	Virgin	Virgin	Chethackal	15.44	22.30	19.99
LAH/105/M	Mature	RRII 105	Lahai	11.81	22.13	18.51
LAH/105/IM	Immature	RRII 105	Lahai	19.60	23.80	20.30
LAH/260/M	Mature	PB 260	Lahai	13.04	19.27	16.27
LAH/VIR	Virgin	Virgin	Lahai	18.06	24.32	21.40
VAI/105/M	Mature	RRII 105	Vaikundam	9.33	15.50	12.55
VAI/105/IM	Immature	RRII 105	Vaikundam	12.68	16.90	13.40
VAI/260/M	Mature	PB 260	Vaikundam	7.96	13.45	10.70
VAI/260/IM	Immature	PB 260	Vaikundam	10.76	15.10	11.80
VAI/VIR	Virgin	Virgin	Vaikundam	9.42	17.87	14.40

Table 4.4.1: Moisture content of soil samples

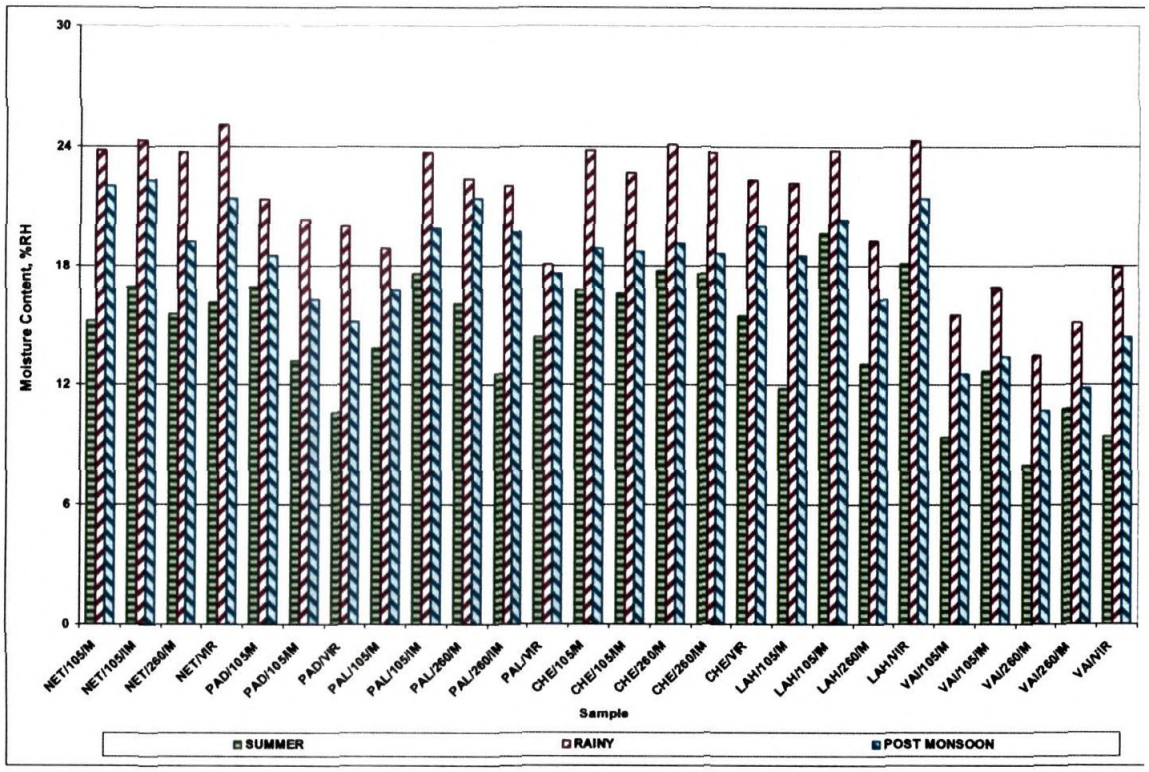

Fig. 4.4.1 - Seasonal variation of moisture content

Moisture contents in all regions were dependant on the seasons, as expected with highest value recorded during rainy season, followed by post-monsoon and summer seasons respectively, as is evident from Fig.4.4.1.

4.4.2. Seasonal variation in pH values

The pH values recorded from soil samples are given in Table 4.4.2

				pH		
SAMPLE	Soil Condition	Clone	Location	SUMMER	RAINY	POST MONSOON
NET/105/M	Mature	RRII 105	Nettana	4.21	4.96	5.43
NET/105/IM	Immature	RRII 105	Nettana	4.18	5.10	5.26
NET/260/M	Mature	PB 260	Nettana	4.62	5.60	6.02
NET/VIR	Virgin	Virgin	Nettana	5.12	4.90	6.21
PAD/105/M	Mature	RRII 105	Padiyoor	4.59	5.07	6.29
PAD/105/IM	Immature	RRII 105	Padiyoor	5.09	5.61	5.68
PAD/VIR	Virgin	Virgin	Padiyoor	5.50	5.44	5.61
PAL/105/M	Mature	RRII 105	Palappilly	3.63	5.05	4.96
PAL/105/IM	Immature	RRII 105	Palappilly	5.00	5.30	5.21
PAL/260/M	Mature	PB 260	Palappilly	4.75	4.67	4.62
PAL/260/IM	Immature	PB 260	Palappilly	4.00	4.27	4.23
PAL/VIR	Virgin	Virgin	Palappilly	5.12	5.98	5.52
CHE/105/M	Mature	RRII 105	Chethackal	4.50	4.69	4.59
CHE/105/IM	Immature	RRII 105	Chethackal	4.53	5.14	4.76
CHE/260/M	Mature	PB 260	Chethackal	4.86	5.49	5.19
CHE/260/IM	Immature	PB 260	Chethackal	4.53	5.14	4.76
CHE/VIR	Virgin	Virgin	Chethackal	5.13	5.01	5.32
LAH/105/M	Mature	RRII 105	Lahai	4.14	4.79	4.38
LAH/105/IM	Immature	RRII 105	Lahai	4.04	3.68	3.96
LAH/260/M	Mature	PB 260	Lahai	4.61	3.61	4.31
LAH/VIR	Virgin	Virgin	Lahai	5.87	4.64	4.44
VAI/105/M	Mature	RRII 105	Vaikundam	5.50	4.87	4.98
VAI/105/IM	Immature	RRII 105	Vaikundam	4.47	4.58	4.92
VAI/260/M	Mature	PB 260	Vaikundam	4.65	4.67	5.16
VAI/260/IM	Immature	PB 260	Vaikundam	4.61	4.95	4.87
VAI/VIR	Virgin	Virgin	Vaikundam	5.62	4.80	5.51

Table 4.4.2: pH of soil samples

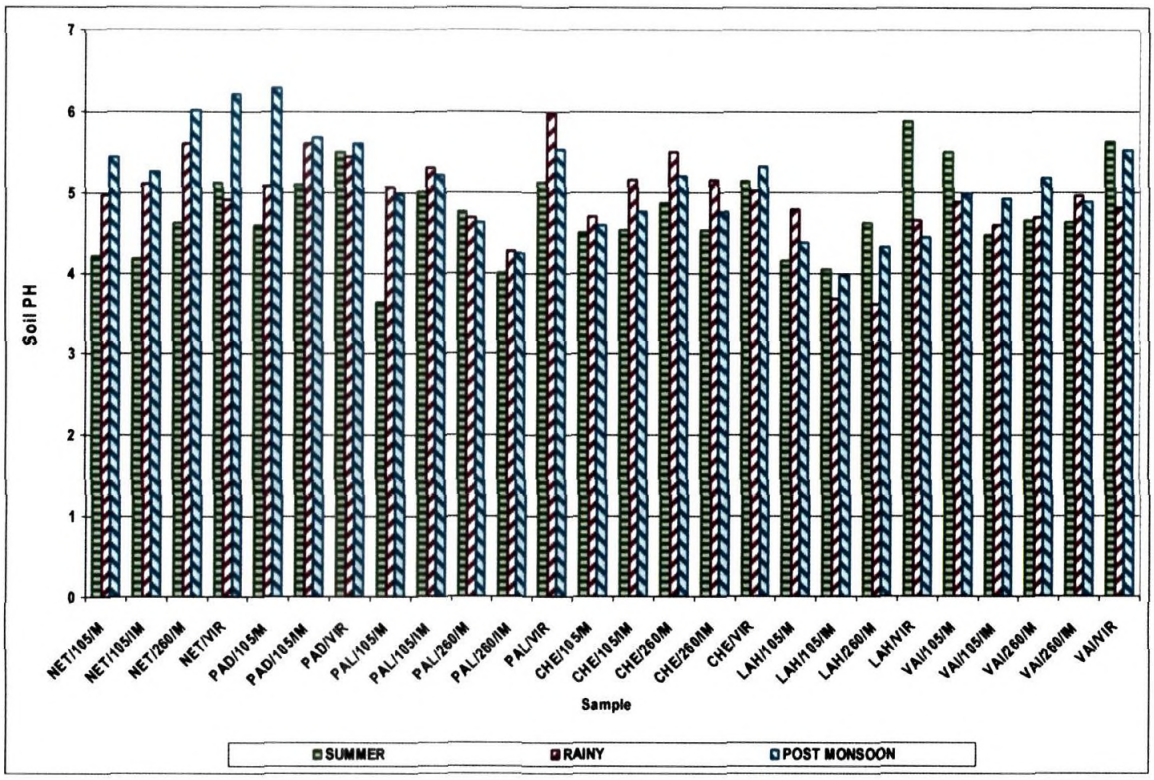

Fig.4.4.2 - Seasonal variation of soil pH

Soil pH readings were higher during the post-monsoon season in Nettana, Palappilly and Padiyoor, when compared to readings during summer and rainy seasons. However, higher readings of soil pH were recorded during the summer season in Lahai and Vaikundam regions, as is evident from Fig. 4.4.2

4.4.3. Correlation studies

Statistical analysis was performed to determine the correlation between the microbes and soil moisture content, as well as, between the microbes and soil pH value.

The results are tabulated below:

Micro-organisms	Correlation coefficient		
	Summer	Rainy	Post-monsoon
Bacteria	-0.035	0.259	0.237
Fungi	-0.048	0.087	0.129
Actinomycetes	0.236	-0.158	-0.158
Phosphobacteria	-0.157	0.325	0.418
Azotobacter	0.345	**0.477 ***	0.311

Table 4.4.3.1: Correlation of soil moisture content and microbial populatiion

(*) - Positive correlation between population and pH

From Table 4.4.3.1, it can be observed that no correlation was present between microbial population and soil moisture content (SMC), except in the case of azotobacter population during the rainy season.

Micro-organisms	Correlation coefficient		
	Summer	Rainy	Post-monsoon
Bacteria	0.1038	**0.5078 ***	-0.1569
Fungi	0.0777	0.0739	-0.0122
Actinomycetes	0.1823	0.0555	-0.0083
Phosphobacteria	0.1198	**0.5275 ***	0.3146
Azotobacter	-0.0653	0.0278	0.0909

Table 4.4.3.2: Correlation of soil pH and microbial populatiion

(*) - Positive correlation between population and pH

From Table 4.4.3.2, it can be observed that positive correlation existed between bacterial, as well as, phosphobacterial population and soil pH during the rainy season. In all other cases, no correlation was observed between the microbial population and soil pH.

5. EFFECT OF BIO-CHEMICAL COMPOUNDS ON MICROFLORA

5.1. Introduction

The leaves from different locations collected during the post monsoon season were subjected to bio-chemical tests to find out whether there was any correlation between the phenol content and sugar content of the leaves and the presence of microflora on the phylloplane.

5.2. Review of literature

Reduction of phenol content has been previously reported (Parupus, 1967, Stavely and Chaplin, 1972) to cause an undesirable aroma. Phenol content of tobacco leaves decreased due to infection (Oke, 1988).

5.2.1. Phenol

Plants being linked to the ground by means of their root system, cannot escape from their biotic and abiotic stressors. Therefore they must protect themselves. The production of chemicals that deter or kill pests and pathogens represents one way of self-protection. Phenolic compounds form one of the main classes of secondary metabolites. Plants need these compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. In contrast with basic metabolism that refers to the anabolic and catabolic processes required for cell maintenance and proliferation, secondary metabolism refers to compounds present in specialized cells that are not directly essential for basic photosynthetic or respiratory metabolism but are thought to be required for plants' survival in the environment. The pattern of secondary metabolites in a given plant is complex; it changes in a tissue- and organ specific way; regularly, differences can be seen between different developmental stages (e.g., organs important for survival and reproduction have the highest and most potent secondary metabolites), between individuals and populations. Secondary metabolites apparently act as defence (against herbivores, microbes, viruses or competing plants) and signal compounds (to attract pollinating or seed dispersing

animals), as well as protecting the plant from ultraviolet radiation and oxidants. (Lattanzio *et al.*, 2006)

Phenol is an intermediate in the metabolism of plants, but it also is a high-volume production compound frequently found in the environment. The increase in phenol concentrations might arise from the release of phenols from their glucosides by β-glucosidase of either host or pathogen. Accumulation of phenols in diseased plant is a known phenomenon in many host-pathogen interactions (Pridham, 1965).

Pre-formed antibiotic compounds such as phenolic and polyphenolic compounds are ubiquitous in plants and play an important role in non-host resistance to filamentous fungi. (Lattanzio *et al.*, 2006)

Effect of some phenolic compounds on the growth of wood inhabiting fungi associated with "esca disease" of grape wine has been studied by Mugnai *et al.* (1997).

In vitro studies reveal that phenolic compounds extracted from olive plants (*Olea europaea* L.), tyrosol, catechin, and oleuropein, showed antifungal activity, thus affecting plant resistance against *Phytophthora* sp.(Del Rio *et al.* 2003).

Flavones and rutinosides of flavonols, which are soluble phenolic compounds of young rubber leaves, are abundant in clones that show resistance to *Colletotrichum gloeosporioides* and *Microcyclus ulei.* (Berger, 2007). Hydroxycinnamic derivatives appear to be correlated with sensitivity. During leaf growth, resistance to the two fungi is reinforced, and soluble flavonols practically disappear and flavones and glucosides of p-coumaric acid accumulate. Flavones may participate in the resistance of old leaves. Phenols could participate in constitutive fungi toxicity in relation to *M. ulei.* Microscopic observations show that soluble phenols become insoluble by fixation on walls and/or through oxidation.

5.2.2. Sugar

Increase in reducing sugar content in smutted plants may be attributed to the increased invertase enzyme activity (Sankpal and Nimbalkar, 1979) In general; infection increased the reducing sugar content of plants (Sankpal and Nimbalkar, 1979; Dhumal and Nimbalkar, 1982).

Biochemical changes in powdery mildew (*Erysiphe polygon* D. C.) resistant and susceptible cultivators of pea (*Pisum sativum*) were studied by Guleria *et al.* (1997). There was a post-infection decrease in chlorophyll (a, b and total) and reducing sugar content in the leaves of both resistant and susceptible cultivators, whereas the total and non-reducing sugar content increased in all cultivators except Bonnevilla in which there was a decrease in total sugar content. The higher percentage increase in the non-reducing sugar content in resistant than susceptible cultivators indicates that they may be involved in disease resistance.

Biochemical changes and their effect on phyllosphere microflora of *Hevea brasiliensis* after application of nitrogenous fertilizers and inoculation of *Corynespora cassiicola* was investigated by Joseph (1998). The leaf samples were analyzed for reducing sugars alongwith other compounds such as starch and phenol. Application of increased levels of nitrogen fertilizer to rubber seedlings created a favorable condition for *C. cassiicola* by augmenting sugars, amino acids, enzymes and phyllosphere microorganisms.

5.3. Materials and methods

5.3.1. Estimation of phenol content of leaves

Weighed exactly 0.5 g of leaf samples and ground it in 10 time volume of 80% ethanol. Centrifuged the homogenate at 10000 rpm for 20 minutes. Saved the supernatant. Re-extracted the residue with 5 times the volume of 80% ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness. Dissolved the residue in 5ml distilled water. Pipetted out different aliquots (0.2 – 2ml) into test tubes. Made up the volume in each tube to 3ml with water. Added 0.5 ml of Folin-

Ciocalteau reagent. After 3 minutes added 2ml of 20% Na_2CO_3 solution to each tube. Mixed thoroughly and placed the tubes in boiling water for exactly 1 minute, cooled and measured the absorbance at 650nm against a reagent blank. Prepared a standard curve using different concentrations of catechol ($C_6H_6O_2$) against absorbance.

From the standard curve found out the concentration of phenols in the leaf samples and expressed as mg phenols /100g material.

5.3.2. Estimation of sugar content of leaves

The carbohydrate (sugar) content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides. Carbohydrates are first hydrolyzed into simple sugars using dilute HCl. In hot acid medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product, with an absorption maximum at 630 nm.

Weighed 100 mg of the leaf sample into a boiling tube. Hydrolyzed it by keeping it in a boiling water bath for 3 hrs with 5 ml of 2.5 N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceased. Made up the volume to 100 ml and centrifuged. Collected the supernatant and took 0.5 and 1 ml aliquots for analysis. Prepared the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' serves as blank. Made up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water. Cooled the contents of all the tubes on ice before adding ice cold anthrone reagent. Then added 4 ml of anthrone reagent. Heated for 8 min in a boiling water bath. Cooled rapidly and read green to dark green colour at 630 nm. Drew a standard graph by plotting concentration of the standard on the X-axis vs absorbance on the Y-axis. From the graph, the carbohydrate (sugar) present in the sample tube, was calculated using the following formula.

$$\textbf{Carbohydrate present in 100 mg of the sample} = \frac{\text{mg of glucose X 100}}{\textbf{volume of test sample}}$$

5.4. Observation and results

The phenol content of the leaf samples collected during the post-monsoon samples were as tabulated in Table-5.4.1

5.4.1. Phenol content in rubber tree leaf samples

Mature / Immature	Clone	Location	Sample Name	Fungal count	Phenol content (µg/l)
Immature	RRII 105	Nettana	NET/PHY-F/105/IM	500	312.5
Mature	PB 260	Nettana	NET/PHY-F/260/M	1100	786.5
Mature	RRII 105	Nettana	NET/PHY-F/105/M	1300	1124.5
Immature	RRII 105	Padiyoor	PAD/PHY-F/105/IM	800	187.5
Mature	RRII 105	Padiyoor	PAD/PHY-F/105/M	800	337.5
Immature	RRII 105	Palappilly	PAL/PHY-F/105/IM	500	912.5
Mature	PB 260	Palappilly	PAL/PHY-F/260/M	700	1162.5
Mature	RRII 105	Palappilly	PAL/PHY-F/105/M	900	1212.5
Immature	PB 260	Palappilly	PAL/PHY-F/260/IM	600	1537.5
Mature	RRII 105	Chethakal	CHE/PHY-F/105/M	200	112.0
Immature	RRII 105	Chethakal	CHE/PHY-F/105/IM	200	116.0
Immature	PB 260	Chethakal	CHE/PHY-F/260/IM	400	378.5
Mature	PB 260	Chethakal	CHE/PHY-F/260/M	1300	986.5
Immature	RRII 105	Lahai	LAH/PHY-F/105/IM	1500	200.0
Mature	PB 260	Lahai	LAH/PHY-F/260/M	500	412.5
Mature	RRII 105	Lahai	LAH/PHY-F/105/M	900	600.0
Mature	RRII 105	Vaikundam	VAI/PHY-F/105/M	700	187.5
Mature	PB 260	Vaikundam	VAI/PHY-F/260/M	700	198.0
Immature	RRII 105	Vaikundam	VAI/PHY-F/105/IM	900	387.5
Immature	PB 260	Vaikundam	VAI/PHY-F/260/IM	1400	787.0

Table 5.4.1: Phylloplane fungi recorded during post-monsoon period and the phenol content of the corresponding leaf samples

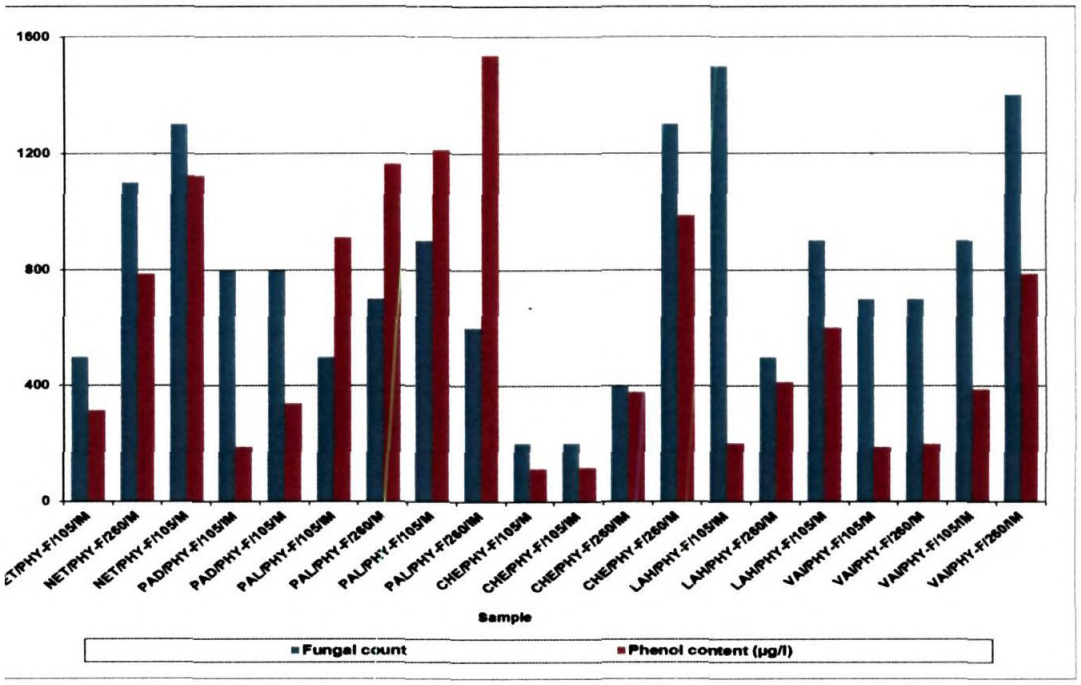

Fig. 5.4.1 Variation of phylloplane fungal count and phenol content

A proportional increase in phylloplane fungi was observed with ıcreasing phenol content in some regions, as observed in Figure 5.4.1.

5.4.2. Sugar content test on rubber tree leaf samples

The sugar content in the post-monsoon leaf samples of the two clones under study is shown along with the phylloplane count obtained, in Table 5.4.2

Mature / Immature	Clone	Location	Sample Name	Fungal Count ($x10^2$)	Sugar Content (mg/100ml)
Mature	RRII 105	Nettana	NET/PHY-F/105/M	13.0	0.59
Immature	RRII 105	Nettana	NET/PHY-F/105/IM	5.0	0.30
Mature	PB 260	Nettana	NET/PHY-F/260/M	11.0	0.49
Mature	RRII 105	Padiyoor	PAD/PHY-F/105/M	8.0	0.45
Immature	RRII 105	Padiyoor	PAD/PHY-F/105/IM	8.0	0.40
Mature	RRII 105	Palappilly	PAL/PHY-F/105/M	9.0	0.45
Immature	RRII 105	Palappilly	PAL/PHY-F/105/IM	5.0	1.04
Mature	PB 260	Palappilly	PAL/PHY-F/260/M	7.0	0.60
Immature	PB 260	Palappilly	PAL/PHY-F/260/IM	6.0	0.87
Mature	RRII 105	Chethackal	CHE/PHY-F/105/M	2.0	0.26
Immature	RRII 105	Chethackal	CHE/PHY-F/105/IM	2.0	0.33
Mature	PB 260	Chethackal	CHE/PHY-F/260/M	13.0	0.42
Immature	PB 260	Chethackal	CHE/PHY-F/260/IM	4.0	0.40
Mature	RRII 105	Lahai	LAH/PHY-F/105/M	9.0	0.92
Immature	RRII 105	Lahai	LAH/PHY-F/105/IM	15.0	0.35
Mature	PB 260	Lahai	LAH/PHY-F/260/M	5.0	0.30
Mature	RRII 105	Vaikundam	VAI/PHY-F/105/M	7.0	0.57
Immature	RRII 105	Vaikundam	VAI/PHY-F/105/IM	9.0	0.68
Mature	PB 260	Vaikundam	VAI/PHY-F/260/M	7.0	0.38
Immature	PB 260	Vaikundam	VAI/PHY-F/260/IM	14.0	0.43

Table 5.4.2: Phylloplane fungi recorded during post-monsoon period and the sugar content of the corresponding leaf samples

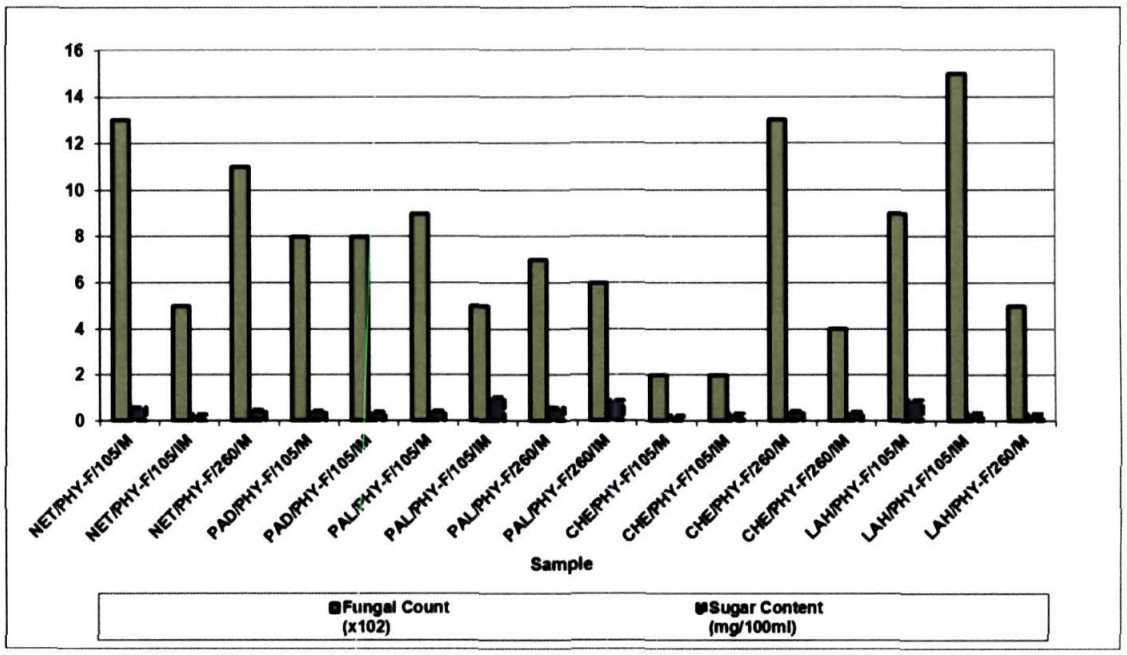

Fig. 5.4.2 Variation of phylloplane fungi and sugar content in rubber trees

Positive correlation could not be established between reducing sugar content in the leaf samples and the fungal count in the samples.

6. EFFECT OF CULTURAL PRACTICES ON MICROFLORA

6.1. Introduction

Cultural practices and chemical treatments can greatly affect the severity of several soil-borne diseases by directly acting on the pathogen and, in a more complex way, by interfering with microbiological and environmental factors. Rhizosphere microflora has a profound influence on plant growth as it has an important role in making soil nutrients available to plants. The rate and extent of root colonisation of soil, type of root system, presence of root exudates, etc. influence the soil microflora. Cultural practices like type and method of fertilizer application, soil disturbances also influence microbial population in the rhizosphere of cultivated plants.

6.2. Review of literature

Venkataram (1960) has reported that susceptibility of plants to fungi causing root rot has been conditioned by the rhizosphere microflora. Changes in the microflora can result from derangement in plant metabolism caused by foliar treatment with chemicals, fungal infection of the roots or even systemic infection by plant viruses. There is possibility to stimulate artificially the multiplication of specific microorganisms in the rhizosphere by selective plant treatment such as foliar application of nutrients designated to act directly on the plant without affecting the soil. Mycorrhizal association with plant roots favour nutrient uptake. Mycorrhizal association in rubber has been cited by Wastie (1965), Jayaratnae (1982) and Ikram and Mahmud(1984). Cultivation of cover crops like *Pueraria phaseoloides* and *Mucuna bracteata* are reported to improve the microbial population in the rubber plantation (Kothandaramen *et al.,* 1989). Intercropping has an overall beneficial effect on growth of *Hevea* due to the combined effect of added fertilizers and lime (Zainol *et al.*, 1993) However, in the case of an intercrop of *Hevea brasiliensis* with *Pueraria phaseoloides,* there was not a significant transfer of nutrients between rubber and the cover crop plants (Ikram *et al.*, 1994).

Small scale disturbances, such as the application of large amounts of ammonia (Suzuki *et al.,* 2002), and trenching of the forest floor (Laiho, 1970) cause changes in the fungal community, without changes in the whole plant community.

In studies on soil microbial dynamics and biogeochemistry in tropical forests and pastures of Southwestern Costa Rica, it was found that land conversion led to fundamental changes in the size and activity of microbial community (Cleveland *et al.* 2003). Microbial biomass was consistently higher in forests than in pastures. Forest sites had a microbial community that was more active and showed strong seasonal variation.

The microbial population is influenced by insecticide application.

Bacterial, *Azotobacter,* actinomycetes, and fungal populations were determined in groundnut (*Arachis hypogaea* L.) fields after application of Diazinon and Lindane as both seed and soil treatments (Singh and Singh, 2005). The bacterial population was not affected by this treatment. The Diazinon soil treatment had indicated some significant adverse effects on fungi and actinomycetes population, which recovered after 30 days. The population of bacteria and azotobacter increased significantly in this treatment. Lindane had no effect on bacterial and fungal population. However, its adverse effects were observed in actinomycetes and azotobacter populations between 30 to 60 days.

Performance of cinnamon as an inter-crop under rubber planted with different inter-row spacings ranging from 7.2 m to 18.0 m was investigated for eight years by Pathiratna *et al.* (2007). Having cinnamon as the intercrop, showed an increased growth and yield of rubber. Use of wider spacings for intercropping cinnamon helped to obatain high economic gain. Growth of rubber in banana/rubber intercrops was superior to that of sole crop rubber (Senevirathna *et al.*, 2010).

6.3. Materials and methods

In order to study the effect of different cultural practices on the population of microflora associated with rubber trees, plots with different cropping systems planted in the Central Experimental Station of Rubber Research Institute of India at Chethackal were selected. Five rubber trees each from plots with two inter-cropping systems (banana planted alongwith rubber trees and coffee planted with rubber trees) and control plots where there was no inter-crop (rubber trees alone) were selected and samples collected during the summer and rainy seasons. Also, samples were collected from virgin soil of nearby forest areas for comparison purpose.

6.3.1. Collection and preparation of samples

Phylloplane, cauloplane and rhizosphere samples were collected and prepared as mentioned earlier in Sections 2.3.1 to 2.3.4

6.3.2. Isolation, culturing and enumeration of natural microflora

The methods adopted for isolating, culturing and enumerating the different microbes were as described earlier in Sections 2.3.5 and 2.3.6

6.3.3. Purification of colonies

The isolated colonies were labelled and purified using the steps described in Sections 2.3.7 and 2.3.8. These colonies were allowed to grow for 4 to 5 days.

6.4. Observations and results

The colony forming units of bacteria and fungi residing on the phylloplane, cauloplane, rhizosphere and soil in the different cropping systems are tabulated in Table 6.4. Bacteria, fungi, actinomycetes, phosphobacteria and azotobacter population from the soil of uncultivated plots (virgin) are also shown.

Microflora	Coffee with rubber		Banana with rubber		Non intercrop rubber		Virgin Soil	
	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy
Bacteria in phylloplane	6.0	45.0	9.0	70.0	3.0	32.0		
Bacteria in cauloplane	23.0	42.0	77.0	148.0	10.0	33.0		
Fungi in phylloplane	26.0	33.0	4.0	22.0	2.0	15.0		
Fungi in cauloplane	9.0	76.0	7.0	57.0	4.0	25.0		
Bacteria in rhizosphere	13.3	40.8	19.5	92.3	8.15	24.1		
Bacteria in soil	10.1	31.2	12.2	75.3	5.3	21.1	2.8	19.5
Fungi in rhizosphere	121.5	213.7	60.9	82.2	29.7	32.1		
Fungi in soil	33.8	87.5	30.5	60.8	21.3	23.7	20	22.3
Actinomycetes in rhizosphere	3.4	6.8	3.1	9.2	5.4	10.9		
Actinomycetes in soil	3.1	6.9	4.7	6.1	2.8	5.7	8.57	16.75
Phosphobacteria in rhizosphere	33	71	39	89	19	37		
Phosphobacteria in soil	16.7	26.5	18.3	28.9	17.3	31.5	31.4	39.1
Azotobacter in rhizosphere	126.6	231	91.2	198.7	29.7	60.2		
Azotobacter in soil	23.7	34.3	15.2	26	10.6	21.1	25.7	30.7

Table 6.4: Microbial population in different cropping systems

6.4.1. Phylloplane and cauloplane bacteria

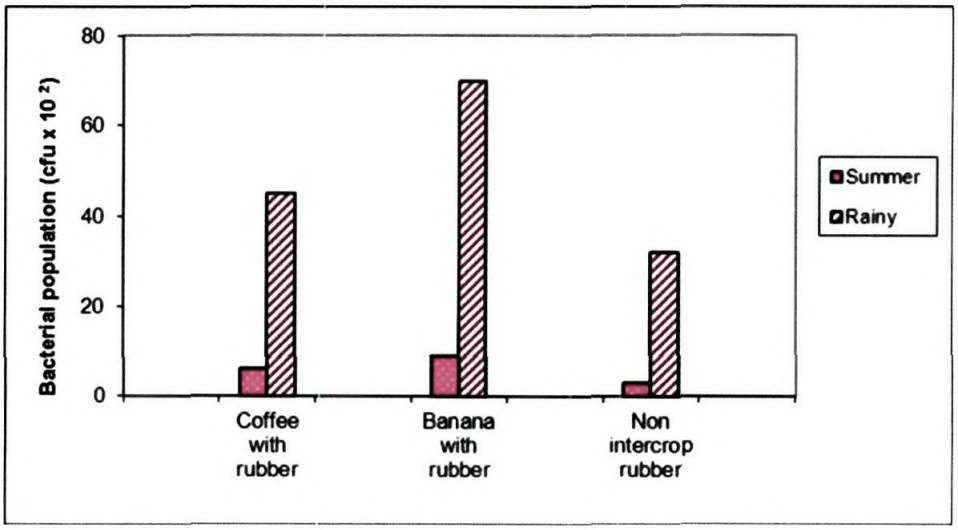

Fig. 6.4.1.a: Bacterial population in phylloplane

Bacterial population was high during the rainy season than during the summer season. It was found to be highest in plots where banana were grown along with rubber. Lowest number was recorded in plots where rubber alone were planted (Fig.6.4.1.a).

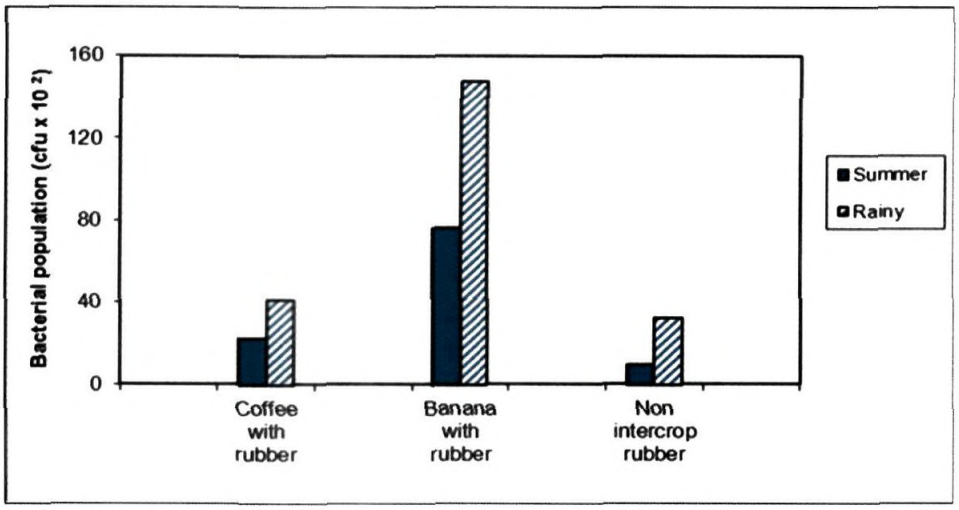

Fig.6.4.1.b: Bacterial population in cauloplane

There was an increase in bacterial population during the rainy season in cauloplane of rubber trees as indicated in fig. 6.4.1.b. Cauloplane bacteria was highest in plots with banana and rubber followed by those with coffee and rubber. Least population was in the non - intercrop plot.

6.4.2. Phylloplane and cauloplane fungi

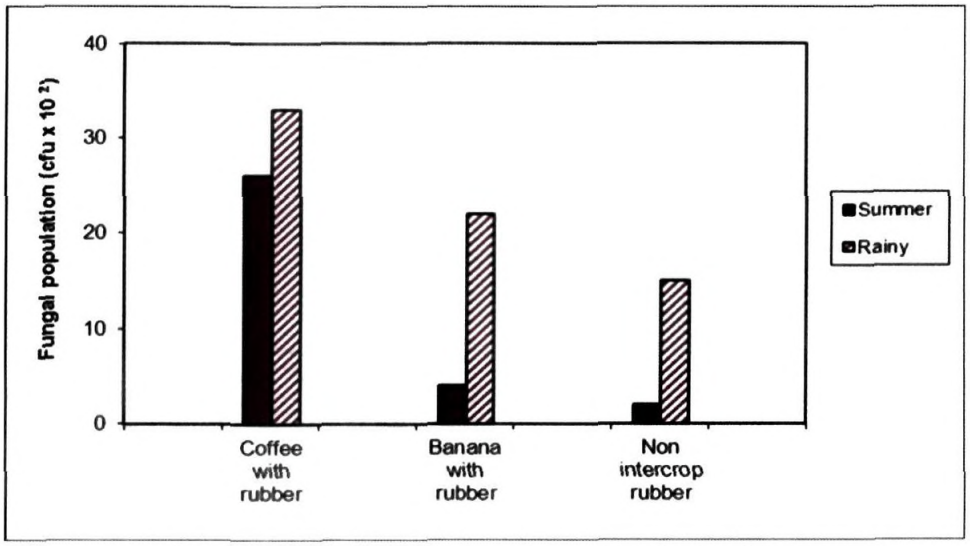

Fig. 6.4.2.a: Fungal population in phylloplane

More fungal population was recorded during the rainy season than in the summer. From Fig. 6.4.2.a, it can be observed that phylloplane fungi was highest in plots where coffee was grown with rubber, followed by banana with rubber and the least in non - intercrop areas.

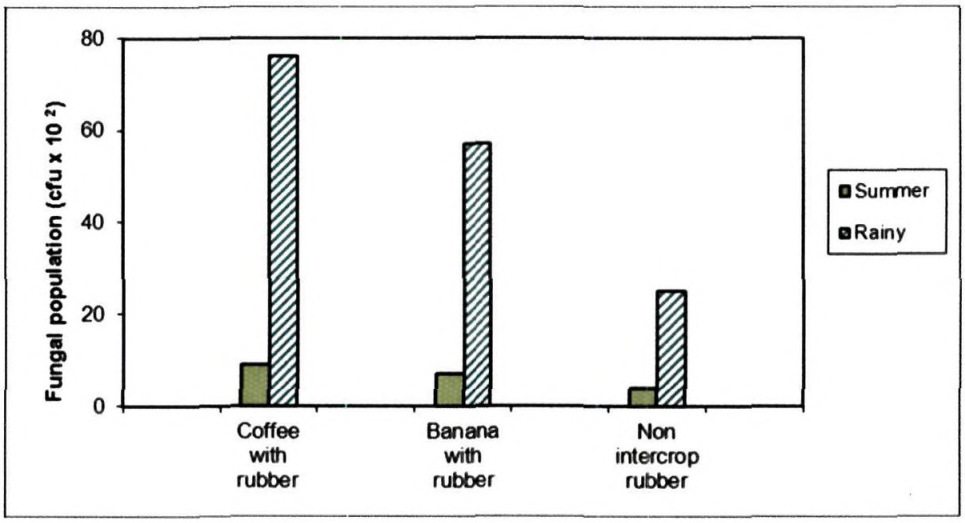

Fig. 6.4.2.b: Fungal population in cauloplane

Fungi residing in the cauloplane were much more during the rainy season than in the summer season (Fig. 6.4.2.b). Cauloplane fungi were found to be highest in plots with coffee and rubber, lesser in plots with banana and rubber and least in plots without intercrop.

6.4.3. Rhizosphere bacteria

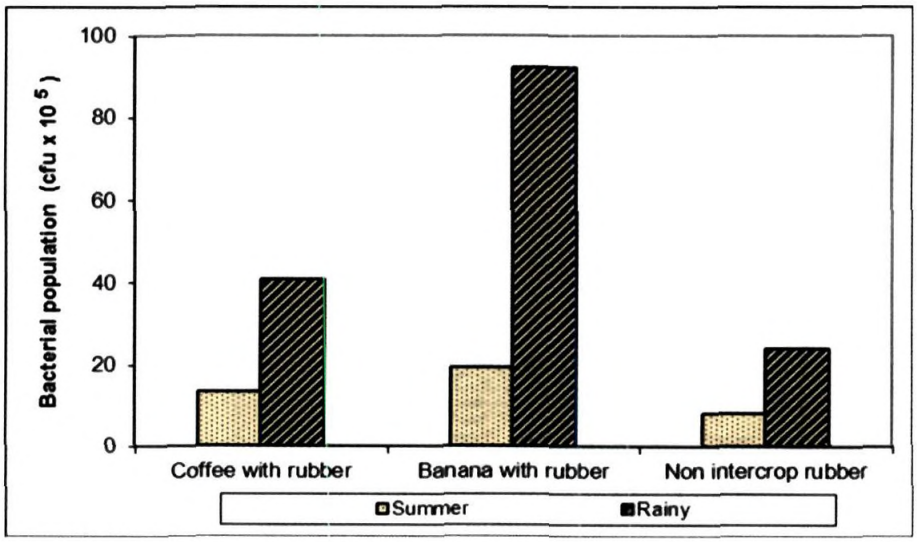

Fig. 6.4.3.a: Bacterial population in rhizosphere

Bacterial population in the rhizosphere was less in summer compared to that in the rainy season. Fig. 6.4.3.a depicts that highest population of rhizosphere bacteria occurred in plots with banana and rubber, lesser in coffee with rubber and least in non-intercrop plots.

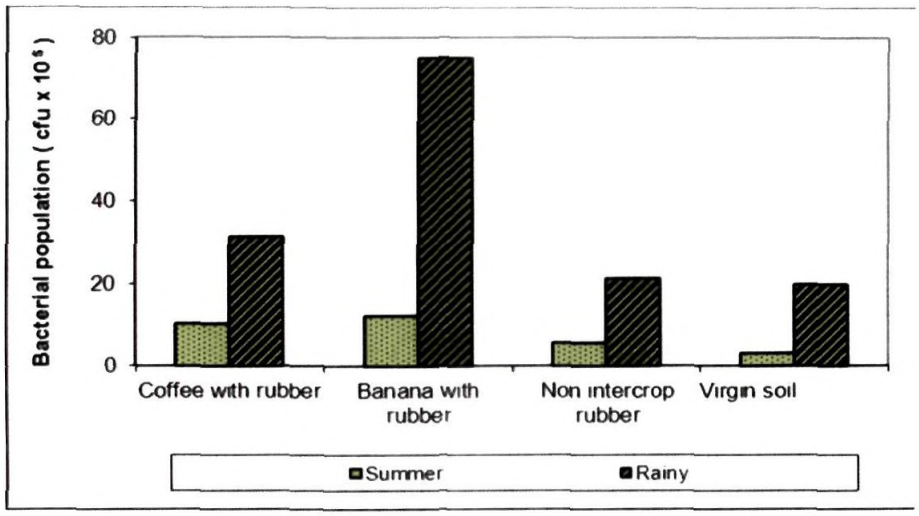

Fig. 6.4.3.b: Bacterial population in soil

Presence of bacteria was consistently high during the rainy season in all the plots than in the summer. Bacterial population in soil was significantly high in areas where intercrop of banana with rubber were planted as indicated in fig. 6.4.3.b. Next highest was recorded in coffee with rubber, followed by rubber with no intercrop and least in virgin soil.

6.4.4. Rhizosphere fungi

Colonies of different types of fungi developed in the medium is shown in Fig. 6.4 4.a.

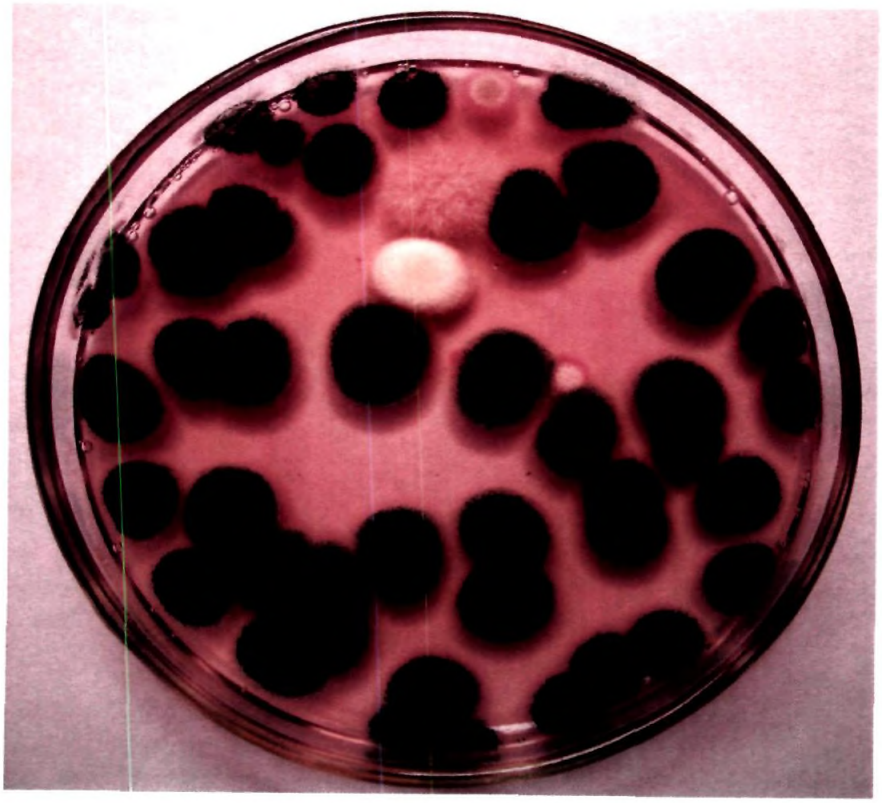

Fig. 6.4.4.a: Rhizosphere fungi in Rose Bengal Agar medium

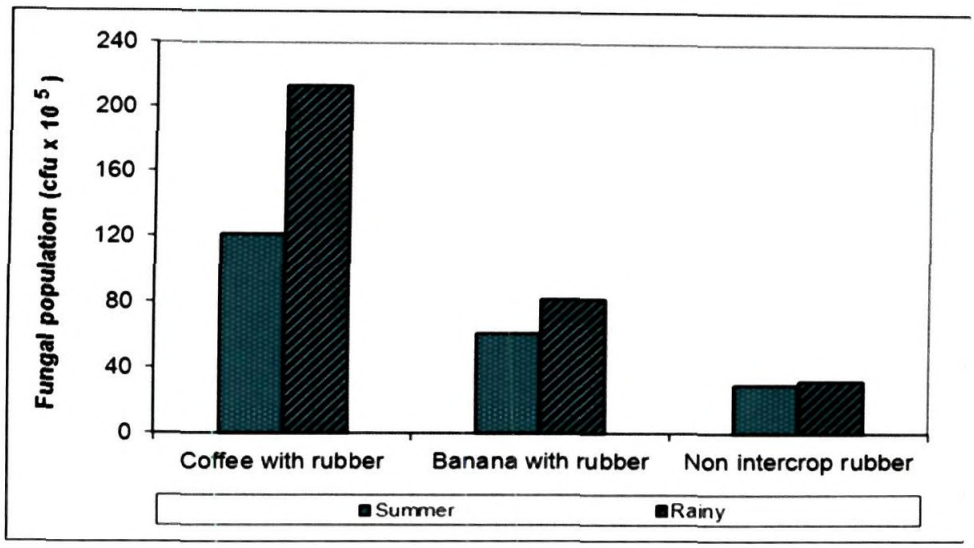

Fig. 6.4.4.b: Fungal population in rhizosphere

Rhizosphere fungal population was observed to be more during the rainy season than in summer in plots where intercrops were grown. But there was not much difference in the plots without intercrops (Fig. 6.4.4.b).

The highest population was recorded in plots with coffee and rubber followed by those with banana and rubber. Least count was observed in non - intercrop plots.

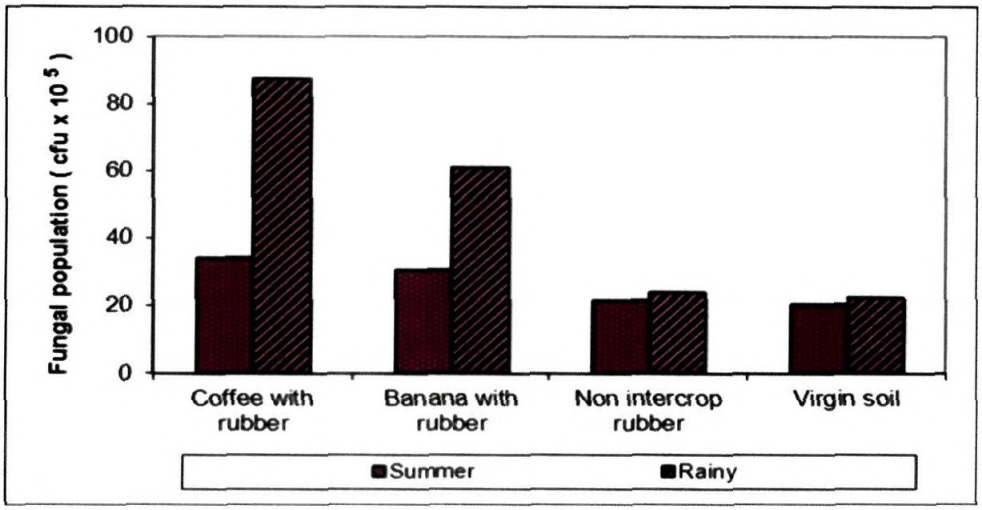

Fig. 6.4.4.c: Fungal population in soil

The fungal population in soil was significantly high in areas where intercrop of coffee with rubber were planted as indicated in Fig. 6.4.4.c. Lesser population was observed in banana with rubber followed by non-intercrop and virgin soil, in both the seasons.

6.4.5. Rhizosphere actinomycetes

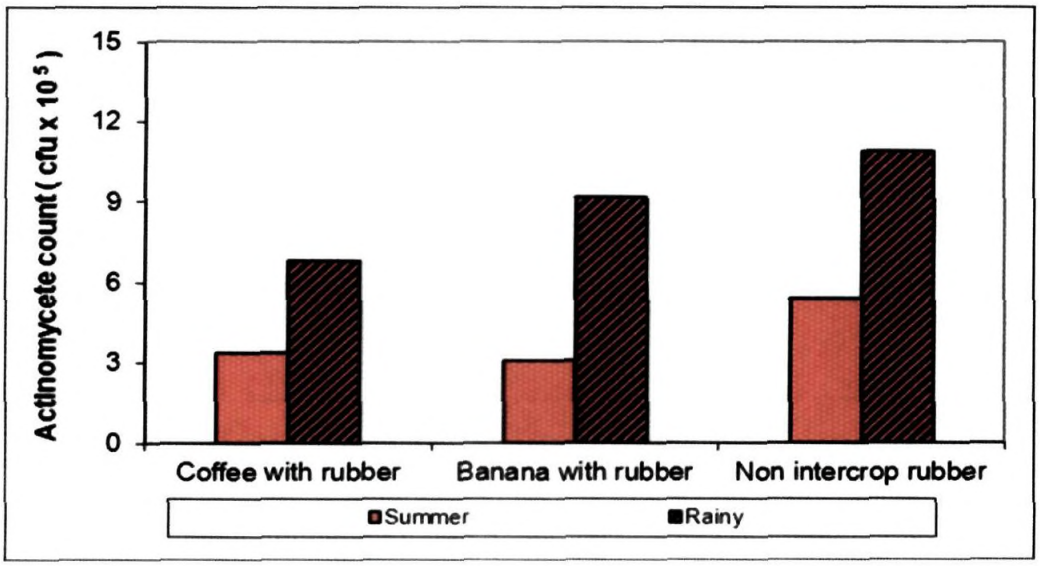

Fig. 6.4.5.a: Actinomycetes count in rhizosphere

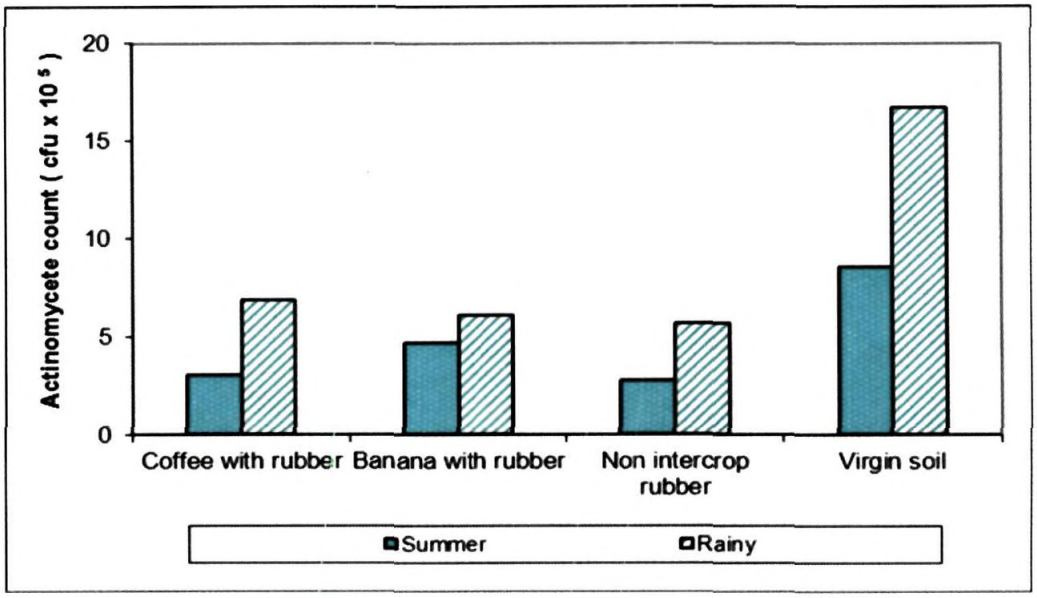

Fig. 6.4.5.b: Actinomycetes count in soil

Figure 6.4.5.a indicated that there was overall increase of actinomycetes in rhizosphere. However as per Figure 6.4.5.b increase of actinomycetes was highest in virgin soil, while there was marginal increase in areas with plantation crops.

6.4.6. Rhizosphere phosphobacteria

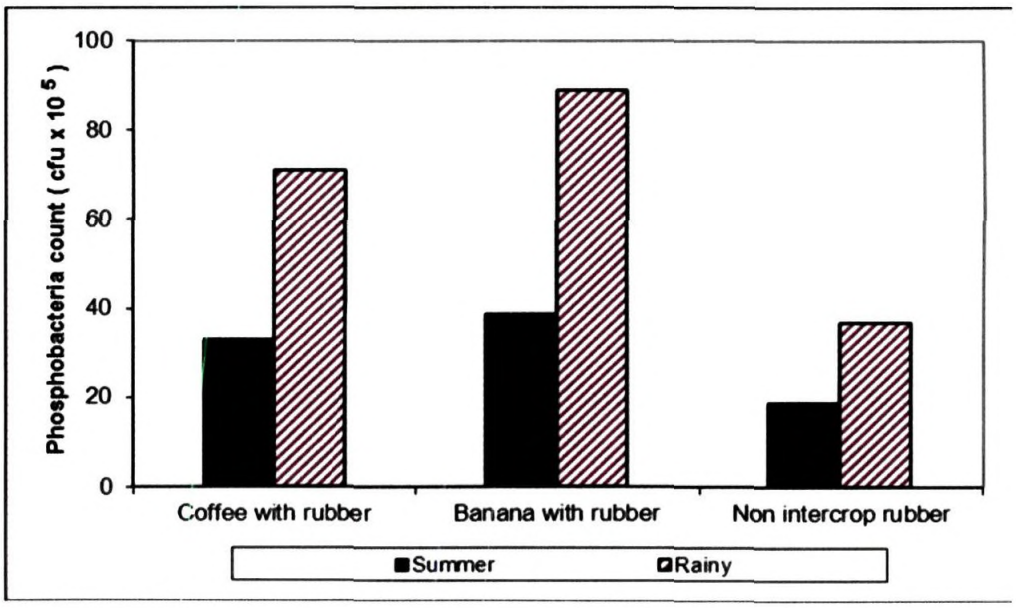

Fig. 6.4.6.a: Phosphobacterial count in rhizosphere

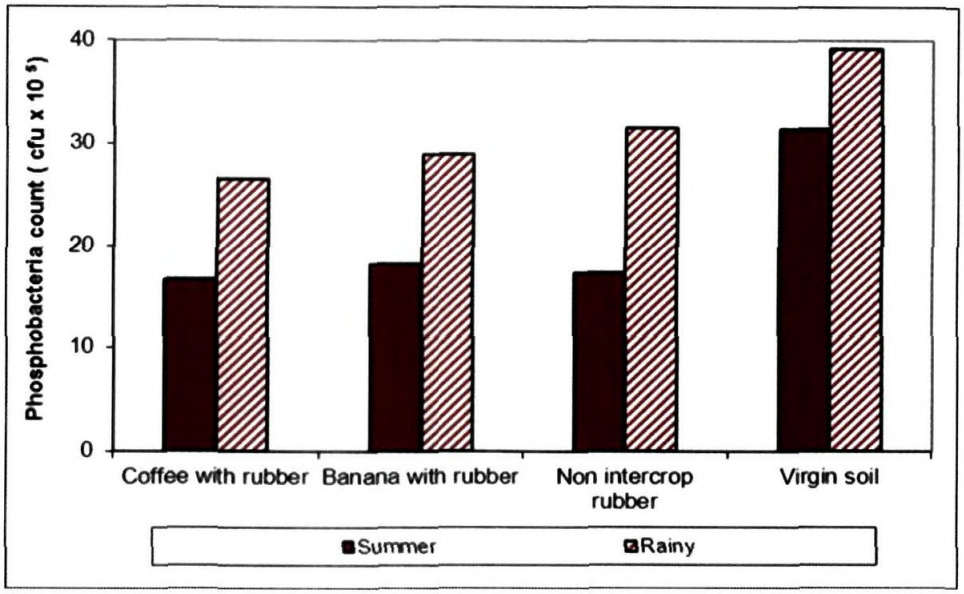

Fig. 6.4.6.b: Phosphobacterial count in soil

Fig 6.4.6.a indicated that phosphobacterial count in non intercrop areas was higher than areas with inter crop during the rainy season. Fig. 6.4.6.b indicated an overall increase in phosphobacterial count in soil during the rainy season with virgin soil recording the highest count.

6.4.7. Rhizosphere azotobacter

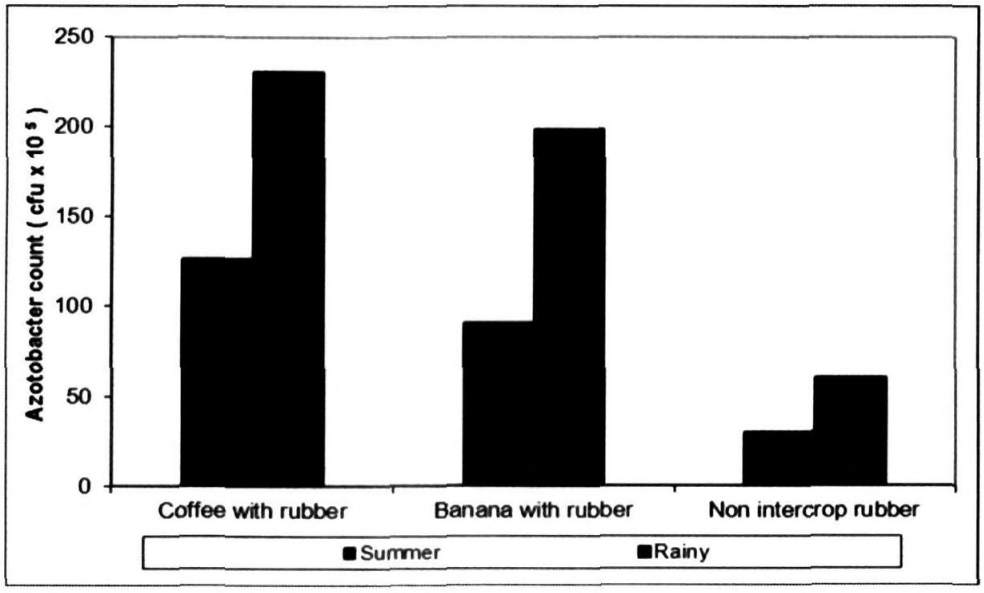

Fig. 6.4.7.a: Azotobacter count in rhizosphere

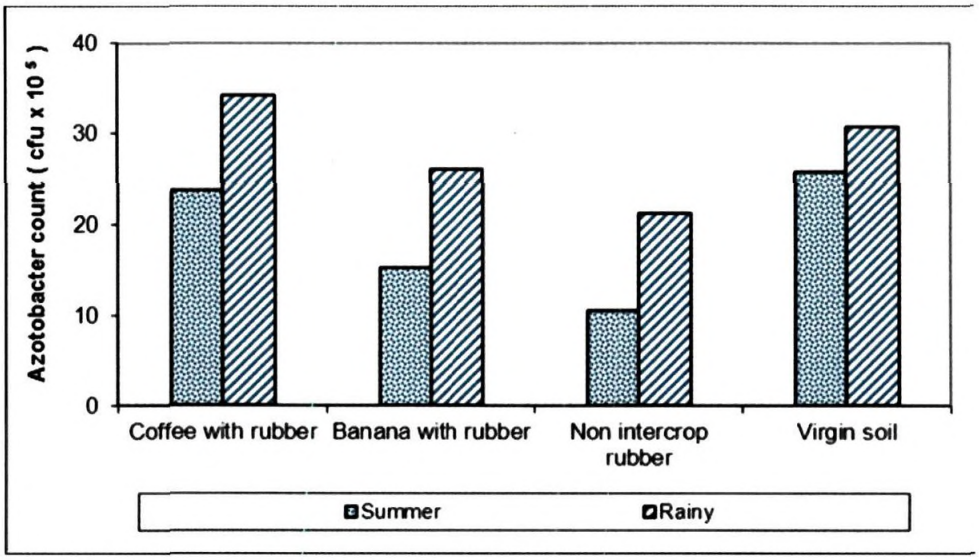

Fig. 6.4.7.b: Azotobacter count in soil

Fig 6.4.7.a indicated that Azotobacter count in intercrop areas was higher than non inter crop areas during the rainy season. Fig. 6.4.7.b indicated an overall increase in Azotobacter count in soil during the rainy seasons, with inter-crop areas containing coffee with rubber trees recording the highest count.

7. STUDIES ON BENEFICIAL EFFECTS OF NATURAL MICROFLORA

7.1. Introduction

A wide range of microorganisms including bacteria, fungi, mycorrhizae etc., are present in the environment of rubber plants. These may be pathogenic or non-pathogenic to the plants. Some of the microbes produce certain chemicals or toxins which may affect the growth of pathogenic microbes and thus may become beneficial to the plants. In the present study, such microbes were isolated from the leaf, bark and root surfaces, and their antagonistic properties evaluated, to determine whether they could be used as bio-control agents against major diseases of rubber.

Detailed studies were conducted on the beneficial aspects of microorganisms to the rubber trees. These were (1) antagonism against five major pathogens of rubber namely *Corynespora cassiicola, Phytophthora meadii, Phellinus noxius, Corticium salmonicolor* and *Colletotrichum acutatum,* (2) HCN production (3) siderophore production (4) production of volatile compounds and (5) ability for phosphate solubilization.

7.2. Review of literature

Biological control is a potent means of reducing the damage caused by plant pathogens. The following biocontrol agents have already been registered for commercial use; *Peniophora gigantea* against *Fommes annosus* (Hodges,1964); *Trichoderma harzianum/Polysporum* against wood decay (Dennis and Webster,1971); *Bacillus subtilis* for growth enhancement(Krebs *et al.*,1998); *Gliodadium virens* for seedling diseases (Zhihe *et al.*, 1998); *Pseudomonas fluorescens* for seedling diseases(Howell and Stipanovic, 1980); *Pythium oligandrum* against *Pythium* spp.(Foley and Deacon,1986); *Pseudomonas fluorescens* against bacterial blotch(Haas and Keei, 2003); *Agrobacterium radiobactor*

against crown gall (Loyter *et al.*, 2005); *Fusarium* spp. against *Fusarium oxysporum*(Kaur *et al.*, 2007)); *Trichoderma* spp. for root diseases (Ubalua and Oti, 2007).

Antagonism of phylloplane fungi against Colietotrichum leaf disease of rubber has been observed(Evueh and Ogbebor, 2008). *Aspergillus* spp. lysed the cytoplasm of *Colletotrichum gloeosporioides. Trichocladium* spp. and *Trichophyton* spp. aiso showed antagonism against this pathogen. *Trichophyton* spp. and *Gliocladium* spp. overgrew and suppressed *C. gloeosporioides.*

Potential agents for biocontrol activity are rhizosphere-competent fungi and bacteria which, in addition to their antagonistic activity are capable of inducing growth responses by either controlling minor pathogens or by producing growth-stimulating factors (Kumar *et al.*, 2005). The growing interest in biocontrol with micro-organisms is also a response to the new tools of biotechnology using which plants and micro-organisms can now be manipulated to deliver the mechanism of biological control by gene transfer. Micro-organisms with inhibitory activity against plant pathogens are potential sources of genes for disease resistance.

7.2.1. Bio-control of soil borne diseases

Chemical control of soil borne plant diseases is frequently ineffective because of the physical and chemical heterogeneity of the soil, which may prevent effective concentrations of the chemical from reaching the pathogen. Biological control agents colonize the rhizosphere, the site requiring protection and leave no toxic residues, as opposed to chemicals (Berg, *et al.*,1994).

Microorganisms have been used extensively for the biological control of soilborne plant diseases as well as for promoting plant growth. Fluorescent *Pseudomonas* are the most frequently used bacteria for biological control and plant growth promotion (Bagnasco *et al.*, 1998). *Bacillus* and *Streptomyces* species have also been commonly used.

Trichoderma, Gliocadium, and *Coniothyrium* are the most commonly used fungal biocontrol agents. All these microbes have antagonistic properties against the pathogen. Cross protection using a related species of the pathogen is also sometimes attempted as in the case of *Agrobacterium radiobactor* strain K84, used against crown gall disease caused by *A. tumefaciens* (Pesenti-Barili *et al.*, 1991).

Competition for the ecological niches or for nutrients have been used as a mechanism of biological control for soil borne plant pathogens as well as the pathogens on the phylloplane. Naturally occuring, nonpathogenic strains of *Fusarium oxysporium* have been used to control wilt diseases caused by pathogenic *Fusarium* spp. (Mandeel and Baker, 1991).

Chitin and β - (1, 3) - glucan are the two major structural components of many plant pathogenic fungi, except by Oomycetes, which contain cellulose in their cell wall and no appreciable levels of chitin. Biological control of some soil-borne fungal diseases has been correlated with chitinase production. Bacteria producing chitinases or glucanases exhibit antagonism *in vitro* against fungi (Gay *et al.,* 1992).

Spraying on *Sclerotium rolfsii* with partially purified chitnase produced by a cloned gene effected rapid and extensive bursting of the hyphal tips. This chitinase preparation was found to be effective in reducing the disease incidence caused by *S. rolfsii* in beans and *Rhizoctonia solani* in cotton. (Shapira *et al.*, 1989).

Attempts at utilizing properties of microorganisms colonizing roots and soil for improvement of plant growth (Mishustin and Naumova, 1962; Barea and Brown, 1974) or for protection of plants against pathogenic organisms residing in the soil (Cook, 1993) have been reported. Inoculation of potato seed stock with selected isolates of rhizobacteria caused an increase in tuber yield (Vrany and Fiker, 1984).

Molecular techniques have also facilitated the introduction of beneficial traits into rhizosphere competent organisms to produce potential biocontrol agents. It is considered that mycoparasitism is one of the main mechanisms involved in the antagonism of *Trichoderma* as a biocontrol agent. The process apparently includes 1) chemotropic growth of *Trichoderma*, 2) recognition of the host by the mycoparasite 3) secretion of extracellular enzymes, 4) hyphae penetration, and 5) lysis of the host.

7.2.2. Bio-control of air borne diseases

Many naturally occurring microorganisms have been used to control diseases on the aerial surfaces of plants. The most common bacterial species that have been used for the control of diseases in the phyllosphere include *Pseudomonas syringae*, *P. fluorescens, P. cepacia, Erwinia herbicola* and *Bacillus subtilis.* Fungal genera that have been used for the control of air borne diseases include *Trichoderma, Ampelomyces* and the yeasts, *Tilletiopsis* and *Sporobolomyces* (Dennis and Webster, 1971; Kleifeld and Chet, 1992; Altomare *et al.,* 1999).

Antibiosis has been proposed as the mechanism of control of several bacterial & fungal diseases in the phyllosphere. (Wilson and Lindow, 1993). Molecular biology techniques could be used to enhance the efficacy of biocontrol agents that use antibiosis as a mode of action (Nakkeeran *et al.*, 2005).

Effect of antagonistic fungi, *Trichoderma viride, T. koningi,* and *T. harzianum* against *Phytophthora meadii* causing Abnormal leaf fall of rubber trees was studied *in vitro* by Vanitha *et al.* (1994). All the antagonists inhibited the growth of the pathogen. They penetrated the oospores of the pathogen and caused their lysis.

Biocontrol potential of phylloplane fungi against *Colletotrichum* leaf disease of rubber, *Hevea brasiliensis* Muell. Arg. has been demonstrated (Evueh and Ogbebor, 2008). *Aspergillus* spp. lysed the cytoplasm

of *Colletotrichum gloeosporioides* on Potato Dextrose Agar. *Trichophyton sp.* and *Gliocladium* spp. antagonized C. *gloeosporioides* by overgrowing on it. Other phylloplane organisms such as *Botrytis* spp., *Pleurothecium* spp. and *Staphylotrichum* spp. exhibited weak antagonism on the pathogen while *Gonatorrhodiella* spp.and *Syncephalastrum* spp. showed different levels of zones of inhibition with the pathogen. Metabolites produced by *Gonatorrhodiella* spp. and *Syncephalastrum* spp. affected the pathogen by antibiosis.

7.2.3. Mycoparasitism

Mycoparasitism occurs when one fungus exists in intimate association with another from which it derives some or all its nutrients while conferring no benefit in return. Biotrophic mycoparasites have a persistent contact with or occupation of living cells, whereas necrotrophic mycoparasites kill the host cells, often in advance of contact and penetration. The most common example of mycoparasitism is that of *Trichoderma* spp. which attack a variety of phytopathogenic fungi.

Antagonistic potentiality of eight isolates of *Trichoderma* and an isolate of *Gliocladium virens* was tested in vitro against *Colletotrichum gloeosporioides* and *Pestalotiopsis disseminata*, by Philip *et al.* (1996). *T. harzianum*-1 and 2 and *T. viride*-2 have shown high antagonism against *C. gloeosporioides*, and *P. disseminata* inhibiting their mycelial growth, spore production and spore germination. Similarly the culture filtrates of these antagonists have inhibited the mycelial growth of the pathogens by about 84% and spore production and spore germination by above 70%. The mechanisms of antagonism in above biocontrol agents were found to be hyperparasitism and antibiosis.

7.2.4. Actinomycetes against pathogens

Actinomycetes are known to produce metabolites that are inhibitory to plant pathogens. The actinomycete *Streptomyces lydicus* WYEC108 showed strong *in vitro* antagonism against various fungal plant pathogens

in plate assays by producing extracellular antifungal metabolites(Yuan and Crawford, 1995).

Streptomyces griseus produces an antibiotic substance against *Colletotrichum lindemuthianum* (Tu, 2008). In dual culture on potato dextrose agar, a large inhibitory zone was formed between the colonies of *S. griseus* and *C. lindemuthianum*. *S. griseus* was observed to sporulate profusely when it came in contact with a colony of *C. lindemuthianum* indicating that *S. griseus* might require some essential nutrients from *C. lindemuthianum*. Scanning electron microscopy showed that hyphae of S.griseus in contact with *C. lindemuthianum* produced appressorium-like swellings or simply grew on the hyphal surface of *C. lindemuthianum*. Internal parasitism was evidenced by the presence of hyphae and conidia of the mycoparasite inside the host hyphae.

7.2.5. Enzymes and volatile compunds produced by antagonists

Scanning electron microscopic studies on the antagonistic fungus *Dicyma pulvinata* and the pathogen, *Fusicladium macrosporum* on *Hevea* rubber plant by Mello *et al.* (2008) have shown that the antagonist produces hydrolytic enzymes whuich are involved in control of the pathogen.

Plant-pathogenic fungi produce an array of extracellular hydrolytic enzymes that enable them to penetrate and infect the host tissue; these enzymes are collectively called cell wall-degrading enzymes (CWDE). They may contribute to pathogenesis by degrading wax, cuticle and cell walls, thus aiding tissue invasion and pathogen dissemination. Furthermore, they can act as elicitors of host defense reaction. Microorganisms produce enzymes which are involved primarily in the degradation of macro-molecules to units capable of being taken into the living cell (Fogarty and Kelly, 1979).

Bio-control studies of plant pathogens focus on a multitude of factors related to the behaviour of the antagonistic bacteria and fungi. The

method by which the antagonists affect the pathogen is important. Handelsman and Stabb (1996) suggested that most bio-control agents suppress disease *via* more than one mechanism and that resistance to multiple antagonistic traits should occur only at a very low frequency. Also, antagonistic microorganisms are thought to exert only limited selection pressure since they operate in micro-size on the plant surface where only a fraction of the pathogen population is exposed during a short period of its life cycle. In the case of anti-biosis, only minute amounts of the compound(s) are produced by the bio-control agent as opposed to the inundated application of chemical pesticides.

Microorganisms produce volatile compounds such as alcohols, aldehydes, aromatics, sulphides and ketones. Several studies have indicated that volatile organic compounds are produced in soil and plant-associated environments. Wheatley (2002) referred to volatile organic compunds as ideal "infochemicals" in microbial interactions because of their ability to be effective over a wide range of spatial scales. Several volatiles produced by bacterial and fungal genera exert deleterious effects on the *in vitro* growth of diverse fungi. Mackie and Wheatley (1999) reported that volatile organic compounds produced by a diversity of root-colonizing bacteria inhibit growth of many plant pathogenic fungi.

The antifungal effects of volatile compounds produced by *Bacillus* strains against *Penicillium* were investigated *in vitro* and *in vivo* by Arrebola *et al.* (2010).

Hydrogen cyanide [HCN] is one of the best known examples of a volatile compound involved in biocontrol. Many antagonistic strains like *Pseudomonas fluorescens* produce HCN.

In search of efficient plant growth promoting rhizobacteria (PGPR) strains bacterial isolates have been isolated from the rhizospheric soil and screened *in vitro* for their plant growth promoting traits like production of HCN, siderophore, phosphate solubilization and antifungal activity (Ahmad *et al.*, 2006).

Phosphate solubilising bacteria isolated from the rhizosphere soil has been found to possess the ability to solubilize inorganic phosphates (Stephen and Jisha, 2008). Some fluorescent Pseudomonads isolated from banana rhizosphere were found to solubilize tri-calcium phosphate and some to possess antifungal activity towards phytopathogenic fungi (Naik *et al.*, 2008).

7.3. Materials and methods

7.3.1. Antagonism studies

Five known pathogens causing diseases in rubber namely, *Corynespora cassiicola*, *Phytophthora meadii*, *Phellinus noxius*, *Corticium salmonicolor* and *Colletotrichum acutatum* were grown on PDA plates.

A circular bit from periphery of a four day old culture of pathogenic fungi was cut and was inoculated at the middle of a fresh plate with PDA or NA depending on the antagonist to be screened. Four different fungal or actinomycete isolates (bits of mycelia) were inoculated at the four corners of the PDA plates. Bacteria were inoculated as small streaks on the media on either side of the pathogen. The inoculated plates were incubated at 28 ± 2^0 C for five days to study the antagonistic activity. The plates were observed regularly and the isolates showing antagonism were selected for further studies, using the Dual Culture Technique.

Dual Culture Technique: Antagonistic activity of the selected isolates was examined by inoculating the candidate organism along with the pathogen inoculated on the opposite side of a Petri plate containing medium. The plates were incubated for seven days and the width of the clear zone formed in between the growth of the test organism and the pathogen was measured in each case. Control plates which consisted of the pathogen alone were maintained in each case in order to measure the growth of the pathogen, in the absence of the antagonist.

7.3.2. HCN Production

Qualitative cyanide determination was done using Lorck method (Lorck, 1948) modified by Alstrom (Alstrom and Burns, 1989). Bacterial isolates were grown in Petriplates with King's B medium. Filter paper discs were sterilized and soaked in picric acid solution (2.5 picric acid, 12.5 g of Na_2CO_3, 1 L distilled water). These discs were placed in the lid of each petri dish. The dishes were sealed with parafilm and incubated at 28^0 C for 48 hours. A change of colour of the discs from yellow to light brown, brown or reddish brown were recorded respectively as an indication of weak, moderate or strong in the production of HCN.

7.3.3. Siderophore Tests

Bacterial isolates were cultured in King's B medium. Two tests were conducted to determine siderophore production by the isolates.

1. $FeCl_3$ test
2. Spectrophotometer assay

$FeCl_3$ test

Cell free cultures(0.5 ml) of supernatant was added to 0.5 ml of 2 % aqueous $FeCl_3$ solution. Appearance of orange or red brown colour indicated the presence of siderophore.

Spectrophotometer assay

Cell free cultural supernatants were examined for their absorption maximum in Schimadzer UV – Visible 160A spectrophotometer. A peak at or near 405 nm indicated the presence of siderophore.

7.3.4. Volatile compounds

Sterilized base plates of Petriplates (double the number of organisms) were taken. Half the number was poured with TSA and the other half with PDA. Antagonistic bacteria were inoculated in the plates with TSA and pathogenic fungi on the plates with PDA. The plate inoculated with the antagonist was inverted over the plate with the

pathogen. These base plates were sealed with parafilm. Control plates with the pathogens at the base on PDA and plain TSA plate without antagonist were examined in the case of each test.

Measured the percentage reduction in the growth of the pathogenic fungi using the following formula:

$$\text{Reduction in growth (\%)} = \frac{\text{Growth in control} - \text{Growth in antagonist inoculated plate}}{\text{Control}} \times 100$$

7.3.5. Phosphate solubilization

The phosphate solubilizaton was assessed by culture plate technique.

Culture plate technique: Pure cultures of rhizosphere phosphobacteria were streaked at the middle (single streak) of precipitated Apetite Agar. Plates were incubated for 4 to 5 days and the extent of the clear zone around the colony was measured. The presence of the halo confirms their phosphate solubilizing activity and the difference in the extent of the clear zones give a qualitative measure of their relative efficiency.

7.4. Observation and results

The major pathogens of rubber trees used for antagonism studies were from the culture collection of the Rubber Research Institute of India, coded as indicated in the following table:

Code	Pathogen
CC04	*Corynespora cassiicola*
PM59	*Phytophthora meadii*
Phel	*Phellinus noxius*
Cs	*Corticium salmonicolor*
C4	*Colletotrichum acutatum*

Table 7.4: Codes of pathogens used for antagonism studies

Pure cultures of the five pathogens cultured in potato dextrose agar showed specific pattern of growth as can be seen in Fig.7.4.1, Fig.7.4.2 and Fig. 7.4.3. Pure cultures that were maintained and were allowed to sporulate were used to prepare plugs for inoculation in the dual cultures. The plugs were cut from the periphery of actively growing cultures of the respective fungi.

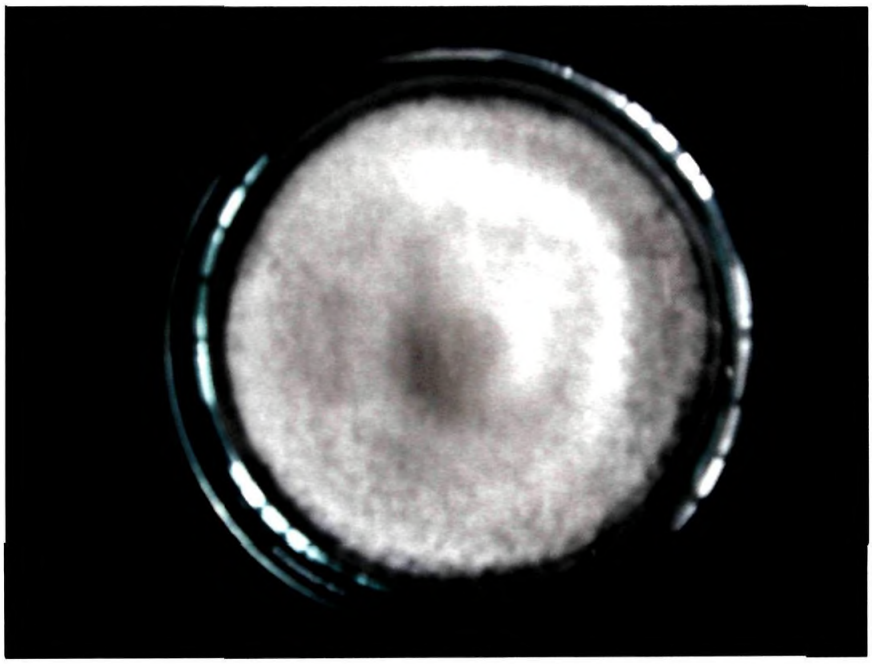

Fig. 7.4.1: *Corynespora cassiicola*

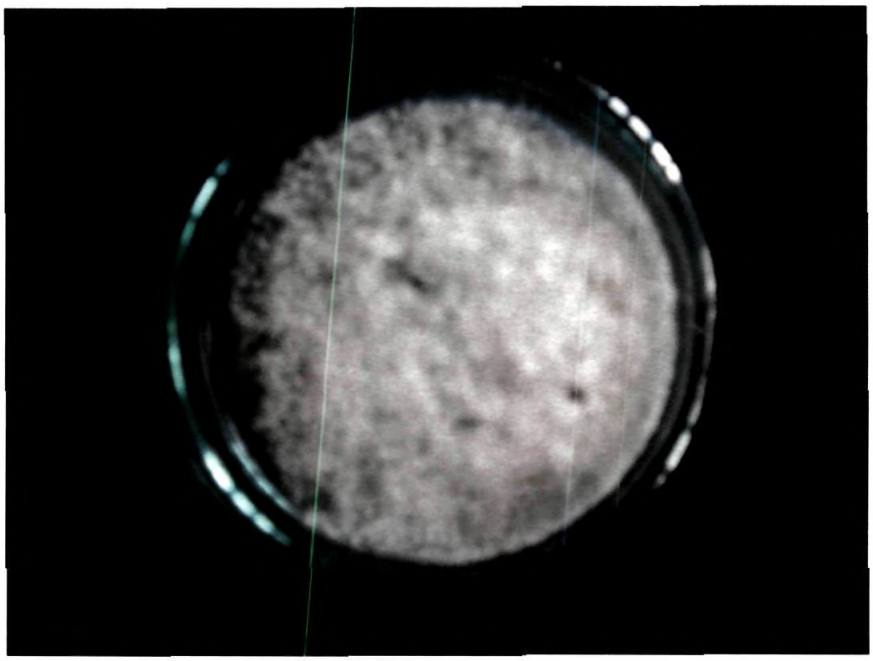

Fig. 7.4.2: ***Phytophthora meadii***

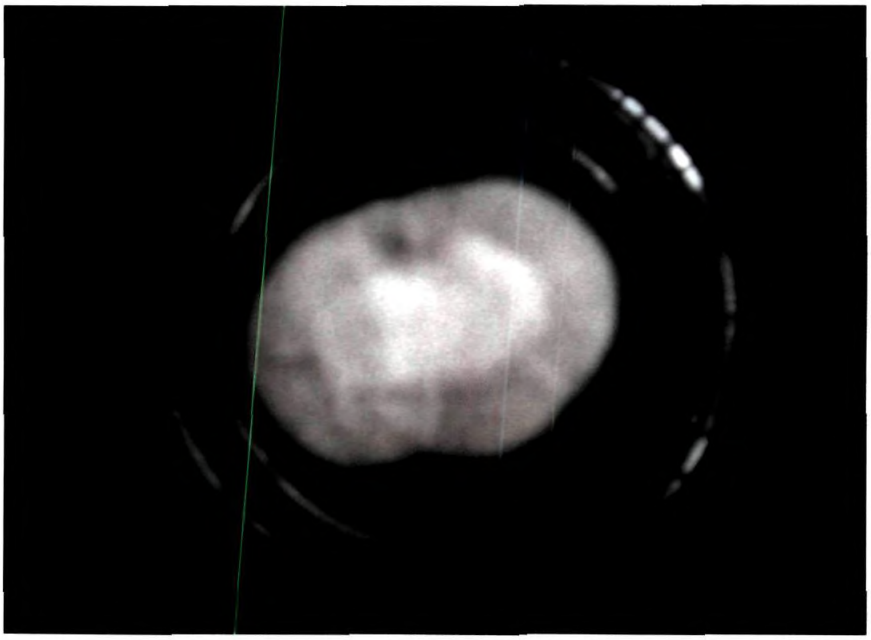

Fig. 7.4.3: ***Corticium salmonicolor***

7.4.1. Bacterial antagonism against five pathogens of rubber

Radial Growth of Pathogen, Bacteria and Zone of Antagonism (c m)

Isolate no.	CC04			PM59			Phel			Cs			C4		
Bacterium	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z
B1	3.0	2.4	1.4	1.3	0.4	3.3	3.1	2.8	1.4	1.6	1.6	1.7	1.0	0.6	3.0
B2	3.0	2.5	1.7	1.3	0.8	2.4	7.0	3.0	2.5	N/E			N/E		
B3	4.5	2.9	2.0	4.5	2.1	0.5	7.0	3.5	1.3	1.2	0.6	1.2	1.0	0.6	2.1
B4	4.5	3.0	1.0	4.5	1.9	1.6	3.1	1.5	2.4	1.0	0.4	3.5	1.0	0.7	2.4
B5	3.0	1.0	3.5	1.3	0.7	2.5	3.1	1.7	0.8	1.6	1.3	0.7	1.0	0.7	1.5
B6	3.0	2.3	1.7	1.3	0.8	0.7	7.0	2.8	1.8	1.0	0.4	3.8	1.0	0.5	0.7
B7	3.0	2.5	2.3	N/E			7.0	2.3	2.3	1.0	0.4	3.0	1.0	0.6	2.2
B8	N/E			N/E			N/E			N/E			N/E		
B9	N/E			N/E			N/E			N/E			N/E		
B10	3.0	1.8	2.5	N/E			7.0	2.0	1.8	1.0	0.4	2.0	1.0	0.5	3.1
B11	N/E			2.6	2.6		1.6	1.1	0.5	N/E			N/E		
B12	3.0	1.5	3.5	1.3	0.4	2.8	3.1	2.7	1.7	N/E			2.5	1.4	1.2
B13	3.0	2.1	2.7	N/E			7.0	3.5	2.0	N/E			N/E		
B14	N/E			N/E			N/E			N/E			2.5	1.1	0.7
B15	3.2	3.8	0.7	N/E			N/E			N/E			N/E		
B16	2.3	1.2	1.5	N/E			4.5	2.5	1.5	1.0	0.4	3.0	2.5	1.5	1.1
B17	N/E			4.5	2.4	0.7	N/E			N/E			N/E		
B18	3.2	2.7	0.7	4.5	3.5		4.5	2.5	1.4	1.0	0.4	2.0	1.0	0.5	2.5
B19	N/E			N/E			5.0	2.8	1.4	N/E			N/E		
B20	N/E			4.5	2.5	0.9	7.0	2.5	1.5	N/E			N/E		
B21	3.0	2.3	2.0	1.3	0.7	0.8	7.0	3.2	1.0	1.0	0.6	3.0	2.5	2.0	1.0
B22	N/E			N/E			7.0	2.8	1.0	N/E			2.5	1.2	1.0
B23	3.2	1.9	0.9	4.5	2.4	0.5	7.0	2.5	1.7	1.6	1.3	0.7	1.0	0.6	2.1
B24	3.0	2.4	1.5	1.3	0.5	3.4	1.2	0.5	3.5	1.2	0.5	3.5	1.0	0.4	1.8
B25	3.0	2.4	0.5	N/E			7.0	2.5	1.5	1.0	0.5	3.0	1.0	0.8	1.7
B26	4.5	3.0	0.6	4.5	2.1	1.5	7.0	2.1	1.0	1.0	0.5	2.3	1.0	0.6	2.3
B27	N/E			4.5	2.1	0.7	N/E			N/E			N/E		
B28	N/E			N/E			N/E			N/E			N/E		
B29	N/E			1.3	0.6	3.6	3.8	2.5	0.8	N/E			N/E		
B30	N/E			N/E			N/E			N/E			1.0	0.7	3.5
B31	3	1	3.5	1.3	0.7	2.6	N/E			N/E			N/E		
B32	3.0	2.0	2.7	N/E			N/E			1.0	0.4	2.0	1.0	0.6	2.7
B33	N/E			N/E			N/E			N/E			N/E		
B34	N/E			N/E			N/E			N/E			N/E		
B35	N/E			N/E			N/E			N/E			N/E		
B36	4.5	2.2	1.5	4.5	2.3	1.1	N/E			N/E			4.0	2.4	1.2
B37	N/E			N/E			N/E			N/E			N/E		
B38	N/E			4.5	3.3	0.3	N/E			4.0	1.6	0.5	N/E		
B39	3.0	2.4	2.2	N/E			7.0	2.5	1.5	1.0	0.5	3.5	N/E		

r_1 - radius of pathogen to where there is no antagonist
r_2 -radius of pathogen to where there is an antagonist
z- zone of inhibition
N/E - No Effect

Table 7.4.1.1: Bacterial antagonism against five pathogens of rubber [contd/-]

Radial Growth of Pathogen, Bacteria and Zone of Antagonism (c m)

Isolate no.	CC04			PM59			Phel			Cs			C4		
Bacterium	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z
B40	1.7	1.5	1.8	1.3	0.7	2.9	4.7	2.8	0.8	N/E			N/E		
B41	1.7	1.4	0.6	4.5	2.1	1.5	N/E			N/E			N/E		
B42	2.8	2.0	2.2	1.3	0.7	2.5	N/E			N/E			N/E		
B43	2.8	1.6	0.3	N/E			N/E			N/E			N/E		
B44	3.0	0.6	0.4	N/E			N/E			N/E			N/E		
B45	3.0	1.3	0.5	N/E			N/E			N/E			N/E		
B46	3.0	2.4	2.6	3.4	2.1	2.0	N/E			N/E			N/E		
B47	2.4	1.4	4.0	4.0	3.0	2.0	N/E			N/E			N/E		
B48	N/E			N/E			N/E			N/E			N/E		
B49	4.0	0.8	5.0	N/E			N/E			N/E			N/E		
B50	4.0	1.4	5.0	N/E			N/E			N/E			N/E		
B51	4.0	1.7	4.0	4.0	3.2	3.0	N/E			N/E			N/E		
B52	N/E			N/E			N/E			N/E			N/E		
B53	4.0	1.9	3.8	4.0	3.5	2.5	N/E			N/E			N/E		
B54	4.0	1.6	5.0	4.0	3.2	3.0	7.0	2.7	1.7	4.0	3.2	3.0	4.0	1.6	5.0
B55	4.0	1.1		1.3	0.7	3.7	1.1	1.0	1.3	0.7	0.6	2.5	N/E		
B56	N/E			N/E			N/E			N/E			N/E		
B57	1.2	0.5	0.8	3.2	2.1	2.2	2.8	1.3	0.5	N/E			N/E		
B58	1.2	0.7		1.3	0.7	3.3	2.8	3.0	3.0	N/E			N/E		
B59	N/E			N/E			N/E			N/E			N/E		
B60	1.2	0.6	1.2	N/E			N/E			N/E			N/E		
B61	1.2	0.7	4.0	1.4	1.2	3.1	2.8	2.5	2.8	1.3	0.5	2.0	4.0	2.5	2.4
B62	N/E			1.4	0.7	0.4	2.8	0.5	0.6	N/E			N/E		
B63	1.2	0.5	0.3	N/E			N/E			N/E			N/E		
B64	3.0	2.3	1.7	N/E			3.1	2.8	1.1	N/E			N/E		
B65	N/E			N/E			N/E			N/E			N/E		
B66	3.0	2.1	2.0	1.3	0.8	2.7	N/E			N/E			N/E		
B67	N/E			N/E			N/E			N/E			N/E		
B68	N/E			N/E			N/E			N/E			N/E		
B69	3.0	1.5		N/E			N/E			N/E			2.5	1.9	0.4
B70	3.5	1.5	1.5	1.3	0.4	4.1	3.1	2.8	1.0	N/E			N/E		
B71	3.0	1.0	3.5	N/E			3.1	2.2	1.0	N/E			N/E		
B72	3.0	2.7	2.0	1.3	0.9	2.5	N/E			N/E			N/E		
B73	3.0	1.7	1.5	1.3	1.1	3.7	N/E			N/E			2.5	2.1	2.0
B74	1.7	1.3	2.7	1.3	0.7	3.0	3.1	3.1	1.2	N/E			N/E		
B75	N/E			N/E			N/E			N/E			N/E		
B76	N/E			N/E			N/E			N/E			N/E		
B77	N/E			N/E			N/E			N/E			N/E		
B78	3.0	2.5	1.5	N/E			N/E			N/E			4.0	2.7	1.0
B79	3.0	2.8	1.4	N/E			3.1	2.5	1.5	N/E			2.5	2.1	0.8

r_1 - radius of pathogen to where there is no antagonist
r_2 -radius of pathogen to where there is an antagonist
z- zone of inhibition
N/E - No Effect

Table 7.4.1.1: Bacterial antagonism against five pathogens of rubber [contd/-]

Radial Growth of Pathogen, Bacteria and Zone of Antagonism (c m)																
Isolate no.	CC04			PM59			Phel			Cs			C4			
Bacterium	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z	
B80	N/E			N/E			N/E			N/E			N/E			
B81	N/E			N/E			N/E			N/E			N/E			
B82	N/E			1.3	0.6	1.9	N/E			N/E			N/E			
B83	2.9	1.8	2.0	1.8	2.0	1.8	N/E			N/E			2.5	1.4	0.7	
B84	3.5	1.3	1.0	N/E			N/E			N/E			4.0	2.1	0.6	
B85	N/E			N/E			3.8	2.2	0.8	N/E			N/E			
B86	2.5	1.5	0.7	1.8	1.7	0.8	3.8	1.5	0.7	N/E			2.5	1.3	0.7	
B87	3.5	2.3	0.6	N/E			N/E			N/E			N/E			
B88	3.0	1.8	2.0	N/E			3.8	2.7	1.9	N/E			N/E			
B89	N/E			N/E			N/E			N/E			N/E			
B90	3.4	2.5	0.8	N/E			N/E			N/E			4.0	2.4	0.7	
B91	3.0	1.7	1.8	N/E			N/E			N/E			N/E			
B92	2.9	1.8	1.7	N/E			3.8	2.0	1.8	1.0	0.4	2.2	2.4	1.4	1.5	
B93	2.8	2.0	0.8	4.2	2.7	0.7	N/E			3.6	2.1	0.5	2.5	1.2	0.9	
B94	N/E			N/E			N/E			N/E			N/E			
B95	3.5	2.2	1.0	4.2	2.7	0.5	N/E			N/E			N/E			
B96	3.5	2.4	1.2	N/E			N/E			N/E			N/E			
B97	N/E			N/E			N/E			N/E			N/E			
B98	N/E			N/E			N/E			N/E			N/E			
B99	N/E			2.0	0.4	1.0	N/E			N/E			N/E			
B100	N/E			N/E			N/E			N/E			N/E			
B101	N/E			N/E			N/E			N/E			N/E			
B102	N/E			N/E			N/E			N/E			N/E			
B103	N/E			N/E			N/E			N/E			N/E			
B104	N/E			N/E			N/E			N/E			N/E			
B105	N/E			N/E			N/E			N/E			N/E			
B106	N/E			N/E			N/E			N/E			N/E			
B107	N/E			N/E			N/E			N/E			N/E			
B108	2.0	1.3	2.0	N/E			N/E			N/E			N/E			
B109	N/E			N/E			N/E			N/E			N/E			
B110	2.0	1.2	1.5	N/E			N/E			N/E			N/E			
B111	N/E			N/E			N/E			N/E			N/E			
B112	N/E			N/E			N/E			N/E			N/E			
B113	2.0	1.3	1.5	2.0	0.4	2.5	N/E			N/E			N/E			
B114	N/E			N/E			N/E			N/E			N/E			
B115	N/E			N/E			N/E			N/E			N/E			
B116	2.0	1.5	2.7	N/E			N/E			N/E			N/E			
B117	2.0	1.5	2.7	N/E			N/E			N/E			N/E			
B118	2.0	1.5	2.9	N/E			N/E			N/E			N/E			
							r_1 - radius of pathogen to where there is no antagonist r_2 -radius of pathogen to where there is an antagonist z- zone of inhibition N/E - No Effect									

Table 7.4.1.1: Bacterial antagonism against five pathogens of rubber

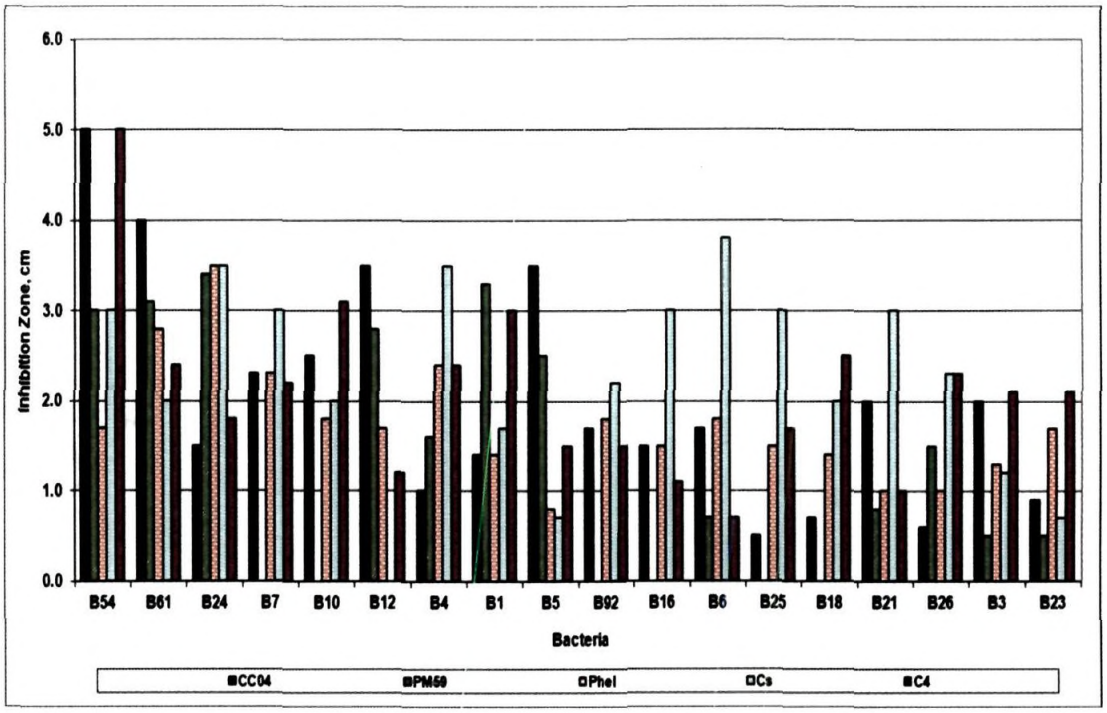

Fig.7.4.1.1 – Effect of antagonistic bacteria against pathogens

The extent of antagonism of a bacterium against the different pathogens was not the same. Out of the 118 bacterial isolates that were tested, eighteen isolates were selected, that exhibited antagonism against at least four of the five pathogens and with at least one pathogen with inhibition zone exceeding 2 cm. The readings of these eighteen bacterial isolates are plotted in Fig. 7.4.1.1

The zones of antagonism produced by the selected bacterial isolates are listed in Table 7.4.1.2.

Bacterial isolates / pathogens and inhibition zone (cm)						
Bacterium	**CC04**	**PM59**	**Phel**	**Cs**	**C4**	Mean
B54	**5.0**	**3.0**	**1.7**	**3.0**	**5.0**	**3.5**
B61	**4.0**	**3.1**	**2.8**	**2.0**	**2.4**	**2.9**
B24	**1.5**	**3.4**	**3.5**	**3.5**	**1.8**	**2.7**
B7	2.3		2.3	3.0	2.2	2.5
B10	2.5		1.8	2.0	3.1	2.4
B12	3.5	2.8	1.7		1.2	2.3
B4	1.0	1.6	2.4	3.5	2.4	2.2
B1	1.4	3.3	1.4	1.7	3.0	2.2
B5	3.5	2.5	0.8	0.7	1.5	1.8
B92	1.7		1.8	2.2	1.5	1.8
B16	1.5		1.5	3.0	1.1	1.8
B6	1.7	0.7	1.8	3.8	0.7	1.7
B25	0.5		1.5	3.0	1.7	1.7
B18	0.7		1.4	2.0	2.5	1.7
B21	2.0	0.8	1.0	3.0	1.0	1.6
B26	0.6	1.5	1.0	2.3	2.3	1.5
B3	2.0	0.5	1.3	1.2	2.1	1.4
B23	0.9	0.5	1.7	0.7	2.1	1.2

Table 7.4.1.2: Inhibition zone values of bacterial isolates that exhibited antagonism against at least four pathogens

It was observed that one of the bacterial isolates, B_{54} showed high level of antagonism against *C. cassiicola* and *C. acutatum*. However, isolate B_{61} showed overall effectiveness against all the five pathogens. When antagonism to *P. meadii, P. noxius and C. salmonicolor* alone are considered, isolate B_{24} also was effective. Isolates B_4, B_1, B_5, B_6 and B_{21} also showed specific antagonistic properties against one or two pathogens tested [Table 7.4.1.2]. While Isolate B_{26} indicated an inhibition zone of more than 2 cms against *C. salmonicolor,* Isolate B_3 showed the same against *C. cassiicola* and *C. acutatum.* Isolates B_7, B_{10}, B_{12}, B_{16} and B_{25} showed high level of antagonism [more than 3 cm] against one of the five pathogens. However, they were effective only against four of the tested pathogens.

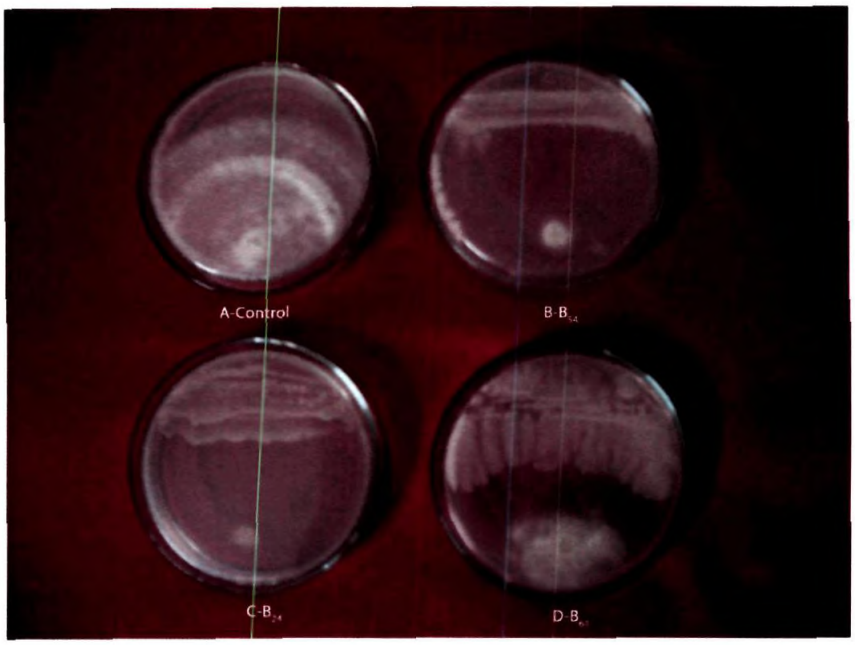

Fig. 7.4.1.2: Antagonism of selected bacteria against *Phytophthora meadii*

A – Control B – Isolate B_{54} C – Isolate B_{24} D – Isolate B_{61}

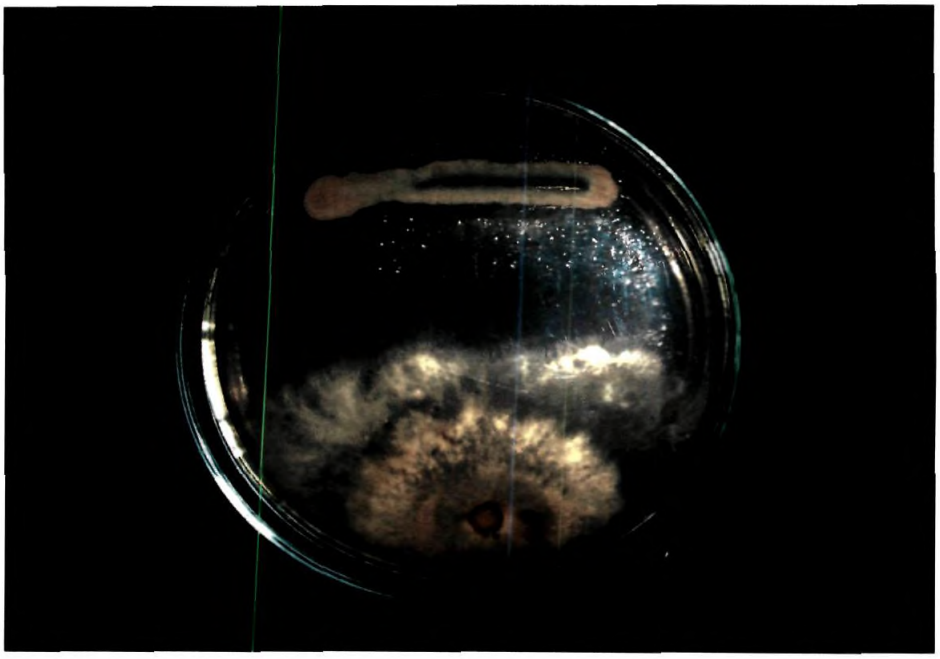

Fig 7.4.1.3: Antagonism against *Phellinus noxius* by B_{24}

Fig 7.4.1.4: Antagonism against *Phellinus noxius* by B_{61}

Fig 7.4.1.5: Antagonism against *Colletotrichum acutatum* by B_{61}

7.4.2. Fungal antagonism against five pathogens of rubber

Radial Growth of Pathogen, Fungi and Zone of Antagonism (c m)

Isolate No.	CC04			PM59			Phel			Cs			C4		
Fungus	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z
F1	3.4	1	0.5	N/E			3.8	2.1	2	4.2	1.7	1.8	4.5	2	0.8
F2	N/E			4.5	2.5	1.5	N/E			N/E			N/E		
F3	2.8	2.3	2..2	4.5	2.5	2	4.5	3.4	0.7	4.5	3.5	1.5	4.5	2.4	1.6
F4	N/E			N/E			N/E			N/E			N/E		
F5	N/E			N/E			N/E			N/E			N/E		
F6	3.4	2.3	1.6	1.7	1.4	0.5	2.9	2	0.3	4.5	2.1	1.8	N/E		
F7	N/E			N/E			N/E			N/E			N/E		
F8	N/E			N/E			N/E			N/E			N/E		
F9	N/E			N/E			N/E			N/E			N/E		
F10	N/E			N/E			N/E			N/E			N/E		
F11	N/E			N/E			N/E			N/E			1.5	1.3	0.6
F12	N/E			N/E			N/E			N/E			N/E		
F13	N/E			N/E			N/E			N/E			N/E		
F14	2.9	2.2	2	4.5	3.2	1.8	4.5	2.3	1.9	4.6	2.8	0.8	4.2	1.8	1.8
F15	N/E			N/E			N/E			N/E			N/E		
F16	2	1.8	1.2	4.5	2.1	1.8	4.5	3.5	0.6	3.6	2.4	1.2	3.1	2	0.8
F17	3.5	3	1	4.5	3	1.6	3.2	3	2	N/E			2.3	1.8	1.7
F18	Predator			Predator			Predator			Predator			Predator		
F19	N/E			N/E			N/E			N/E			N/E		
F20	N/E			N/E			N/E			N/E			N/E		
F21	3.4	2.8	1.7	1.7	1	2.1	N/E			N/E			4	2	1.5
F22	Predator			Predator			Predator			Predator			Predator		
F23	N/E			N/E			N/E			N/E			N/E		
F24	N/E			N/E			N/E			N/E			N/E		
F25	N/E			N/E			N/E			N/E			N/E		
F26	N/E			N/E			Predator			N/E			N/E		
F27	N/E			N/E			N/E			N/E			N/E		
F28	N/E			N/E			N/E			N/E			N/E		
F29	N/E			N/E			N/E			N/E			N/E		
F30	Predator			N/E			N/E			N/E			Predator		
F31	N/E			N/E			N/E			N/E			N/E		
F32	N/E			N/E			N/E			N/E			N/E		
F33	N/E			N/E			N/E			N/E			N/E		
F34	2.8	1.6	1.3	N/E			N/E			4.3	2.8	0.5	N/E		
F35	N/E			N/E			N/E			N/E			N/E		
F36	N/E			4.5	4	0.2	P/P			N/E			N/E		
F37	4.5	2	1	N/E			Predator			N/E			N/E		
F38	N/E			N/E			N/E			N/E			N/E		
F39	N/E			N/E			N/E			N/E			N/E		
F40	N/E			N/E			N/E			N/E			N/E		
F41	3	2.5	1.1	4	3.5	0.5	N/E			5	3	1	2.3	2	2.5
F42	N/E			N/E			N/E			N/E			N/E		
F43	N/E			N/E			N/E			N/E			N/E		
F44		2.3	1.5	4.5	2.8	0.8	4.5	4.1	0.6	5	2.8	1.8	2.5	1.5	2.4
F45	4.5	2.1	1.8	4.5	2.1	1.5	4.5	2.5	1.5	4.5	2.8	1.5	N/E		
F46	N/E			N/E			N/E			N/E			N/E		
F47	N/E			N/E			N/E			N/E			N/E		
F48	2.3	1.3	1.8	4.5	3.5	0.3	N/E			Predator			N/E		

Notes:

1. r_1 - radius of pathogen to where there is no antagonist
2. r_2 - radius of pathogen to where there is an antagonist
3. z - zone of inhibition
4. N/E - No Effect
5. P/P - Parasitism/Predation

Table 7.4.2.1: Fungal antagonism against five pathogens of rubber [contd/-]

Radial Growth of Pathogen, Fungi and Zone of Antagonism (c m)															
Isolate No.	CC04			PM59			Phel			Cs			C4		
Fungus	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z
F49	N/E			N/E			Predator			N/E			N/E		
F50	N/E			N/E			N/E			N/E			N/E		
F51	N/E			N/E			N/E			N/E			N/E		
F52	N/E			N/E			N/E			N/E			N/E		
F53	N/E			N/E			N/E			N/E			N/E		
F54	2.3	1.8	1.5	N/E			N/E			N/E			N/E		
F55	N/E			N/E			N/E			N/E			N/E		
F56	N/E			N/E			N/E			N/E			N/E		
F57		1.8	1	N/E			N/E			N/E			N/E		
F58	N/E			N/E			N/E			N/E			N/E		
F59	N/E			N/E			N/E			N/E			N/E		
F60	3.7	2.4	1.7	4.7	3.3	1.4	4.5	3.3	1.2	4.5	3.6	1.2	2.8	2.7	2.1
F61	3.7	2.4	2.1	4.7	2.1	1.4	4.7	2.7	0.8	4.5	2.5	1.9	2.8	1.8	1.5
F62	Predator			N/E			Predator			N/E			Predator		
F63	N/E			N/E			N/E			N/E			N/E		
F64	N/E			N/E			N/E			N/E			N/E		
F65	3.4	2.5	2.1	4.4	3	1.7	4.5	4	0.7	5	3	2	2.3	1.7	3
F66	N/E			N/E			N/E			N/E			N/E		
F67	Predator			Predator			N/E			Predator			N/E		
F68	Predator			Predator			N/E			Predator			P/P		
F69	N/E			N/E			N/E			N/E			N/E		
F70	N/E			N/E			N/E			N/E			N/E		
F71	3.1	2	0.3	4.5	2	0.7	N/E			N/E			1.5	1	1
F72	N/E			N/E			N/E			N/E			N/E		
F73	N/E			N/E			N/E			N/E			N/E		
F74	P/P			Predator			4.2	2.8	1.6	Predator			2.8	2.7	1
F75	N/E			N/E			N/E			N/E			N/E		
F76	N/E			N/E			N/E			N/E			N/E		
F77	N/E			N/E			N/E			N/E			N/E		
F78	N/E			N/E			N/E			N/E			N/E		
F79	N/E			N/E			N/E			N/E			P/P		
F80	N/E			N/E			N/E			N/E			N/E		
F81	N/E			N/E			N/E			N/E			N/E		
F82	3.4	1.5	2.5	4.7	3.7	0.8	3.2	2.5	1.3	5	1.5	1.3	2.8	2.6	1.6
F83	N/E			N/E			N/E			N/E			Predator		
F84	N/E			N/E			N/E			N/E			N/E		
F85	N/E			N/E			N/E			N/E			N/E		
F86	N/E			N/E			N/E			N/E			N/E		
F87	N/E			N/E			N/E			N/E			N/E		
F88	N/E			N/E			N/E			N/E			N/E		
F89	N/E			N/E			N/E			N/E			N/E		
F90	2.8	1.5	2.8	4.9	2.8	1.2	4.5	2.6	2.5	4.5	2.2	2.1	2.8	2.2	2
F91	N/E			N/E			N/E			4.4	3	1.7	N/E		
F92	N/E			N/E			N/E			N/E			N/E		
F93	N/E			N/E			N/E			N/E			N/E		
F94	N/E			N/E			N/E			N/E			N/E		
F95	N/E			N/E			N/E			N/E			Predator		
F96	N/E			N/E			N/E			N/E			N/E		

Notes:

1. r_1 - radius of pathogen to where there is no antagonist
2. r_2 - radius of pathogen to where there is an antagonist
3. z - zone of inhibition
4. N/E - No Effect
5. P/P - Parasitism/Predation

Table 7.4.2.1: Fungal antagonism against five pathogens of rubber [contd/-]

Radial Growth of Pathogen, Fungi and Zone of Antagonism (c m)															
Isolate No.	CC04			PM59			Phel			Cs			C4		
Fungus	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z	r1	r2	z
F97	4.5	1.5	2.5	4	2.8	2.2	4.5	2.5	2.5	4.5	3	2.2	2.5	1.7	0.8
F98	N/E			Predator			N/E			N/E			N/E		
F99	N/E			Predator			N/E			N/E			N/E		
F100	N/E			N/E			N/E			N/E			Predator		
F101	N/E			N/E			N/E			N/E			N/E		
F102	N/E			N/E			N/E			N/E			N/E		
F103	N/E			N/E			N/E			N/E			2.7	1.3	0.4
F104	N/E			N/E			Predator			Predator			Predator		
F105	N/E			N/E			N/E			N/E			N/E		
F106	N/E			N/E			N/E			Predator			Predator		
F107	N/E			N/E			N/E			N/E			N/E		
F108	N/E			N/E			N/E			Predator			Predator		
F109	N/E			N/E			N/E			N/E			N/E		
F110	N/E			N/E			N/E			N/E			N/E		
F111	N/E			N/E			N/E			Predator			N/E		
F112	N/E			N/E			N/E			N/E			N/E		
F113	N/E			N/E			4.5	2.1	1	N/E			2.8	2.3	1.2
F114	N/E			N/E			N/E			N/E			N/E		
F115	N/E			N/E			N/E			N/E			N/E		
F116	N/E			N/E			N/E			N/E			N/E		
F117	N/E			N/E			N/E			N/E			N/E		
F118	N/E			N/E			3.8	3.5	1.2	N/E			N/E		
F119	N/E			2.9	2.1	0.6	N/E			N/E			2.3	1.5	1.3
F120	N/E			N/E			N/E			N/E			N/E		
F121	2.3	2	1	4.5	3.3	0.6	4.5	2.8	1.4	4.5	2.7	1.5	1.6	1.3	1.5
F122	N/E			N/E			N/E			N/E			N/E		
F123	N/E			N/E			N/E			N/E			N/E		
F124	N/E			4.5	3.1		4.5	4	0.2	N/E			N/E		
F125	N/E			N/E			N/E			N/E			N/E		
F126	N/E			N/E			N/E			N/E			N/E		
F127	2.8	2.5	1.1	N/E			N/E			N/E			N/E		
F128	N/E			N/E			N/E			N/E			N/E		
F129	N/E			N/E			N/E			N/E			N/E		
F130	N/E			N/E			N/E			N/E			N/E		
F131	N/E			N/E			N/E			N/E			N/E		
F132	Predator			Predator			N/E			Predator			N/E		
F133	Predator			N/E			N/E			N/E			N/E		
F134	4.5	2.2	2.1	4.7	3.3	0.5	N/E			N/E			2.8	2	0.4
F135	N/E			4.5	3.5	1.6	4.5	4	0.5	5	3.5	1.3	2.7	2	2.5
F136	N/E			N/E			N/E			N/E			N/E		
F137	N/E			N/E			N/E			N/E			N/E		
F138	N/E			N/E			N/E			N/E			N/E		
F139	2.5	1.7	0.8	3.4	1.4	0.8	3.8	1.4	1.3	5	3	1	2.7	1.6	1.7
F140	N/E			N/E			N/E			N/E			N/E		
F141	N/E			N/E			N/E			N/E			Predator		
F142	N/E			N/E			Predator			N/E			N/E		
F143	N/E			N/E			N/E			N/E			N/E		
F144	N/E			N/E			N/E			N/E			N/E		

Notes:

1. r_1 - radius of pathogen to where there is no antagonist
2. r_2 - radius of pathogen to where there is an antagonist
3. z - zone of inhibition
4. N/E - No Effect
5. P/P - Parasitism/Predation

Table 7.4.2.1: Fungal antagonism against five pathogens of rubber

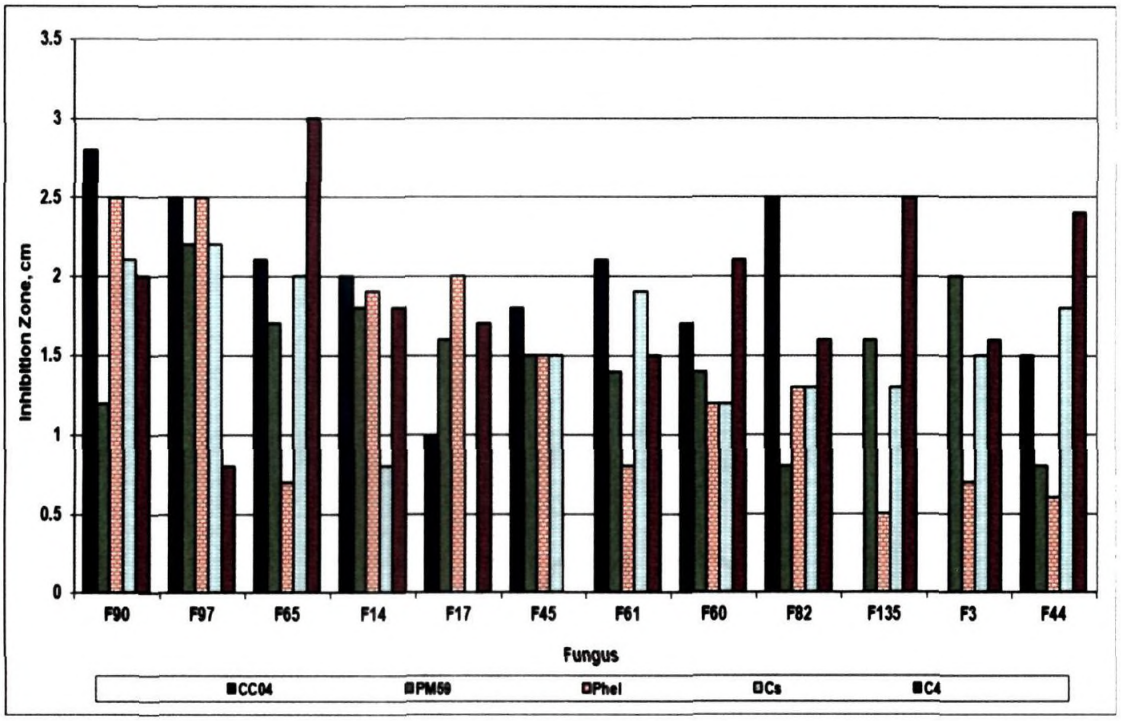

Fig.7.4.2.1: Effect of antagonistic fungi against pathogens

Several fungal isolates were found to show antagonism against the major pathogens of rubber trees (Fig. 7.4.2.1). Out of the 144 fungal isolates that were tested, twelve isolates were selected, that exhibited antagonism against at least four out of the five pathogens, and, with a mean inhibition zone value exceeding 1.4 cm.

The observations from the studies on antagonism by the isolated fungi are shown in Table 7.4.2.1.

Fungal isolates / pathogens and inhibition zone (cm)						
Fungus	**CC04**	**PM59**	**Phel**	**Cs**	**C4**	Mean
F90	**2.8**	**1.2**	**2.5**	**2.1**	**2.0**	**2.12**
F97	**2.5**	**2.2**	**2.5**	**2.2**	**0.8**	**2.04**
F65	**2.1**	**1.7**	**0.7**	**2.0**	**3.0**	**1.90**
F14	2.0	1.8	1.9	0.8	1.8	1.66
F17	1.0	1.6	2.0		1.7	1.58
F45	1.8	1.5	1.5	1.5		1.58
F61	2.1	1.4	0.8	1.9	1.5	1.54
F60	1.7	1.4	1.2	1.2	2.1	1.52
F82	2.5	0.8	1.3	1.3	1.6	1.50
F135		1.6	0.5	1.3	2.5	1.48
F3	2..2	2	0.7	1.5	1.6	1.45
F44	1.5	0.8	0.6	1.8	2.4	1.42

Table 7.4.2.2: Inhibition zone values of fungal isolates that exhibited antagonism against at least four pathogens

The fungal isolates which produce more effective zone of inhibition were sorted out and are presented in Table 7.4.2.2. It can be observed from Table 7.4.2.2, that nine fungal isolates (F_{90}, F_{97}, F_{65}, F_{14}, F_{61}, F_{60}, F_{82}, F_3, and F_{44}) showed antagonism against all the five major pathogens of rubber. Among these, isolate F_{65} was most effective against *C. acutatum*. However, overall effectiveness against all the five pathogens was shown by isolate F_{90}. Though F_{97} was effective against all the five pathogens, it was less effective against *C. acutatum*. In the case of F_{65} least effectiveness was shown against *P. noxius*.

Fig.7.4.2.2: Antagonism of F_{97} against *Corynespora cassiicola*

Fig.7.4.2.3: Antagonism of F_{65} against *Colletotrichum acutatum*

7.4.3. HCN Production

The results obtained on testing the production of HCN by the bacterial antagonists are shown in Table 7.4.3

HCN PRODUCTION OF DIFFERENT ANTAGONISTIC BACTERIA

Isolate No.	Low	Medium	High
B1	1		
B2	1		
B3	1		
B4	1		
B5	1		
B6		2	
B7			3
B8	1		
B9	1		
B10	1		
B11	1		
B12			3
B13		2	
B14			3
B15	1		
B16			3
B17	1		
B18	1		
B19		2	
B20		2	
B21		2	
B22		2	
B23	1		
B24			3
B25	1		
B26	1		
B27	1		
B28	1		
B29	1		
B30	1		
B31	1		
B32	1		
B34	1		
B36	1		
B38	1		
B39	1		
B40	1		

Table 7.4.3: HCN Production [contd/-]

HCN PRODUCTION OF DIFFERENT ANTAGONISTIC BACTERIA			
Isolate No.	Low	Medium	High
B41	1		
B42	1		
B43	1		
B44	1		
B45			3
B46	1		
B47	1		
B49	1		
B50	1		
B51	1		
B52	1		
B53	1		
B54	1		
B55			3
B57	1		
B58	1		
B60	1		
B61	1		
B62	1		
B63	1		
B64		2	
B66			3
B67	1		
B69	1		
B70	1		
B71	1		
B72		2	
B73	1		
B74	1		
B78		2	
B79	1		
B80		2	
B82	1		
B83		2	
B84	1		
B85	1		
B86	1		
B87	1		
B88			3
B90	1		
B91	1		
B92			3
B93	1		
B94	1		
B95		2	
B96			3

Table 7.4.3: HCN Production

Among the 96 bacterial isolates that were tested, only 82 isolates indicated for Hydrogen Cyanide production.

Fig 7.4.3.1: Rating of HCN production by bacteria

7.4.4. Siderophore tests

Observations recorded in Spectrophotometer assay and $FeCl_3$ test		
Sample	**Absorbance (OD)**	**$FeCl_3$**
B01	1.033	++
B02	0.685	+
B03	0.0981	+
B04	1.827	+
B05	0.754	+
B06	0.93	+
B07	0.803	+
B08	0.743	+
B09	0.942	+
B10	0.973	+
B11	0.89	+++
B12	0.633	+
B13	1.26	++
B14	0.832	+
B15	0.719	+
B16	0.937	+
B17	0.72	+
B18	0.889	+
B19	0.624	+
B20	1.087	+
B21	0.893	+
B22	0.838	+
B23	0.937	++
B24	2.844	++
B25	0.483	++
B26	1.05	+
B27	0.926	+
B28	0.706	+
B29	0.465	++
B30	1.084	+
B31	1.436	++
B32	0.806	+
B34	0.731	+
B36	0.833	+
B38	0.513	++
B39	1.856	++
B40	1.014	+

Table 7.4.4: Siderophore production by bacteria [contd/-]

Observations recorded in Spectrophotometer assay and $FeCl_3$ test		
Sample	**Absorbance (OD)**	**$FeCl_3$**
B41	0.86	+
B42	0.72	+
B43	0.43	+
B44	0.473	+
B45	0.519	++
B46	0.859	+
B47	1.538	+
B49	1.686	++
B50	0.647	++
B51	0.581	+
B52	0.746	+++
B53	0.812	++
B54	3.364	+++
B55	1.535	++
B57	1.104	++
B58	0.683	++
B60	0.897	++
B61	2.661	+++
B62	0.846	+
B63	1.222	+
B64	0.824	++
B66	0.668	+
B70	1.709	+
B71	1.134	+++
B72	1.649	++
B73	1.352	+
B74	1.043	++
B78	0.619	+
B79	1.443	+++
B82	0.838	+
B86	0.728	++
B87	1.231	+++
B88	0.665	+++
B90	1.041	+
B91	0.846	++
B92	0.775	+++
B93	0.767	+++
B94	0.831	++
B95	0.715	+++
B96	0.769	+++

Table 7.4.4: Siderophore production by bacteria

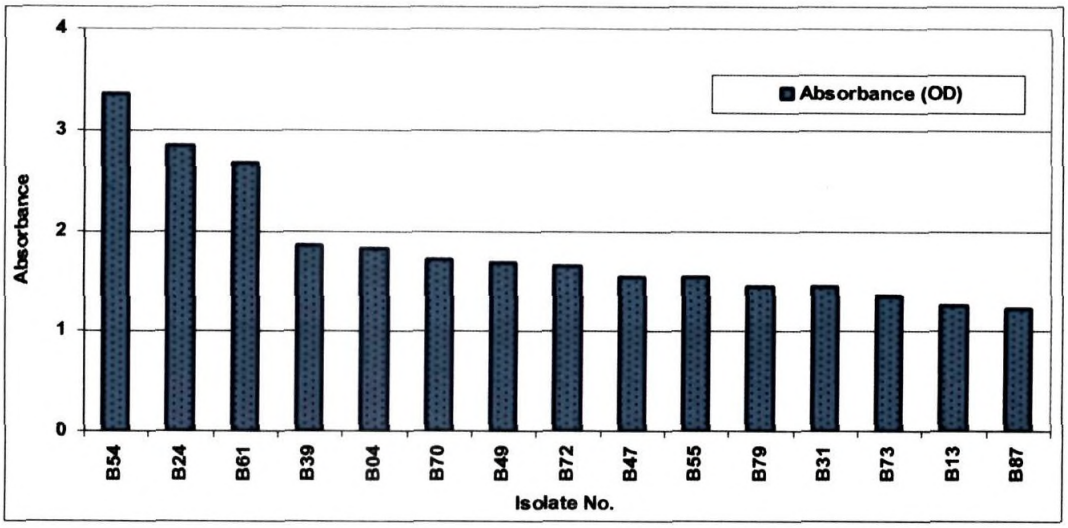

Fig.7.4.4: Siderophore test [Spectrophotometer assay]

Siderophore tests were conducted on 77 bacterial isolates using spectrophotometer assay and $FeCl_3$ test. The results are tabulated in Table 7.4.4. The isolates which showed more siderophore production in the spectrophotometer assay were sorted and plotted in descending order (Fig.7.4.4). The highest siderophore production was recorded by isolate B_{54}. The isolates B_{24} and B_{61} were the next prominent ones. $FeCl_3$ tests indicate high values for several isolates out of which again, B_{54} and B_{61} were prominent. However, isolate B_{24} showed lower value.

7.4.5. Volatile compounds test

Pathogen / Bacterial isolate	Volatile Compounds Production				
	CC04	PM59	Phel	Cs	C4
B01	57.1	52.5	46.66	47.5	40.0
B24	66.7	60.0	81.1	77.5	43.3
B25	59.60	51.3	4.4	47.5	57.8
B54	81.00	55.8	58.9	52.5	50.0
B61	71.90	59.3	77.8	50.0	44.4

Table 7.4.5: Percentage reduction in growth of pathogens by bacterial isolates

Volatile compound tests were perfomed on bacterial isolates, and the percentage inhibition in growth of pathogens was measured. The results are tabulated in Table 7.4.5. Bacterial isolate B_{54} produced a volatile

compound which caused higher inhibition of *Corynespora cassiicola* and *Colletotrichum acutatum*. Bacterial isolate B_{24} produced a volatile compound which caused higher inhibition of *Phytophthora meadii*, *Phellinus noxius* and *Corticium salmonicolor*.

7.4.6. Phosphate solubilization

Phosphate solubilizing bacteria which were present in the soil samples were detected by the presence of a clear halo around each colony on apetite agar plates. These bacteria were isolated from the dilution plates. Since the colonies were crowded in the petri plate, all such isolates were grown on separate plates and their activity was confirmed by measuring the colony growth and extent of halo formed by them.

Isolate no.	Diameter of bacterial colony, including clearing zone [mm]	Extent of halo, (mm)
PB_1	7	2
PB_2	8	2
PB_3	9	2
PB_4	9	2
PB_5	9	1
PB_6	9	2
PB_7	11	3
PB_8	13	5
PB_9	11	5
PB_{10}	9	2
PB_{11}	8	2
PB_{12}	9	1

Table 7.4.6: Growth and phosphate solubilization capability of phosphobacterial isolates

Among the twelve isolates of phosphobacteria, isolates PB_8 and PB_9 showed most effective halo around the colony (5mm), whereas the isolate PB_5 and PB_{12} showed the least halo size,1 mm (Table 7.4.6).

8. STUDIES ON FUNGAL ENZYMES

8.1. Introduction

In order to correlate enzyme production and antagonism of fungi against the five pathogens, the fungal antagonists were selected and their cellulase enzymes were assayed by employing their appropriate substrates.

8.2. Review of literature

Of the various proteins induced in response to infection, chitinases and β-1,3-glucanases have been the focus of particular attention due to their believed antimicrobial activity through the hydrolysis of the main fungal wall components, chitin and β-1,3-glucans. (Benhamou, 2005)

8.2.1. Cellulase

Cellulases are a group of enzymes that catalyze the degradation of cellulose, a polysaccharide built of β-1, 4 linked glucose units. This group consists of endo-1,4-glucanase, exo-1,4-glucanase and β-d-glucosidase. The products of cellulose degradation are glucose, cellobiose, and higher molecular weight oligosaccharides

Cellulases are enzymatic proteins which hydrolyze cellulose polymers to smaller oligosaccharides, cellobiose and glucose. They are present in three major types of enzymes: endoglucanases, cellobiohydrolases and β-glucosidases.

Miller(1959) assessed cellulase production using Czapek-dox medium amended with 3% w/v concentration of carboxy methyl cellulose (CMC) as sole carbon source. Cellulase activity was monitored by the estimation of reducing sugar using dinitro salicylic method. The absorbance of reducing sugar was measured at 575 nm. One unit of endoglucanase activity was the amount of enzyme which liberates 1µm reducing sugar from 1% CMC in the assay condition.

8.2.2. Endo-β-1,4 glucanase

Endo (β-1,4 glucanases) have not been widely studied for biocontrol purposes, although cellulose is abundant in Oomycetes. Endo-β-1,4 glucanase attacks the 1,4-β-glucosidic linkages of cellulose molecules at random, releasing reducing sugars which can be measured with DNS reagent.

8.3. Materials and methods

8.3.1. Preparation of cellulase of antagonistic fungi

Prepared Czapek's medium (Composition is listed in Appendix 13.10). Inoculated 100ml of Czapek's medium containing 0.2% carboxymethylcellulase (CMC, pH 6.5) with antagonistic fungi. Incubated the conical flask at room temperature for 7-10 days. Filtered through a funnel using Whatman No.1 filter paper and centrifuged at 2000 rpm for 40 minutes. Collected the clear supernatant and dialysed in cellophane dialysis tubings against distilled water at 2-4^{0}C for 24 hours changing the water at 8 hour interval. The contents of the dialysis tube was centrifuged at 2000 rpm and the supernatant collected. A few drops of toluene were added. This was used as enzyme source. Stored the filtrate at 2-4^{0}C.

Estimation of cellulase activity

Cellulase (Cx Endoglucanase) activity was measured by loss in viscosity of cellulosic substrate.

Dissolved 0.5 g of CMC in 100 ml of sodium acetate - acetic acid buffer at pH 5.2 at 50-60^{0}C kept in a blender. Blended for 3-5 min at low speed and again blended at high speed for 3-5 min. Filtered through Whatman No.1 filter paper. Added 2 ml of 1% aqueous merthiolate solution as preservative.

Pipetted out 4 ml of CMC, 1 ml of buffer and 2 ml of enzyme into the viscometer kept in a water bath at 30±1^{0}C. Mixed the contents by drawing air gently through the large arm of the viscometer. Applied suction to the small arm and determined the efflux time of the mixture at prefixed

intervals. Calculated the % loss in viscosity of CMC by employing the formula:

$V = (T_0-T / T_0-T_{H_2O}) X 100$

V = % loss in viscosity

T_0 = Flow time in seconds at 0 time

T = Flow time of the reaction mixture at time T

T_{H_2O} = Flow time of distilled water

8.3.2. Preparation of endo-β-1,4 glucanase (carboxy methyl cellulase)

β-1,4 glucanase activity was assayed by the protocol of Miller (1959). Enzyme activity was measured as µm equivalents of glucose released/ml of culture filtrate/min/mg protein.

CMC (0.5%) was dissolved in acetate buffer with pH 5.2. Sodium acetate-acetic acid buffer was prepared with pH 5.2. Pipetted out 4 ml of CMC into a test tube followed by 1 ml of buffer and 2 ml of the enzyme extract. Incubated the test tubes in a water bath 30±1^{0}C. Withdrew aliquots of 1 ml from each tube at intervals of 2, 5, 10, 15, 20, 25 and 30 min and determined the amount of reducing sugars released using dinitrosalicylic acid method.

Estimation of endo-β-1,4 glucanase activity

Pipetted out 3 ml of the extract into a test tube and 3 ml of DNS reagent. Heated the mixture for 5 min in a boiling water bath. After the colour has developed, added 1 ml of 40% Rochelle salt when the contents of the tubes were still warm. Cooled the tubes under running water. Measured the absorbance at 575 nm. Calculated the amount of reducing sugar using a standard prepared from glucose.

8.4. Observation and results

The percentage loss in viscosity caused by the enzymes produced by three major antagonists, namely, F_{90}, F_{97} and F_{65} are shown in Figures 8.4.1.1 and 8.4.1.2.

8.4.1. Measurement of cellulase activity

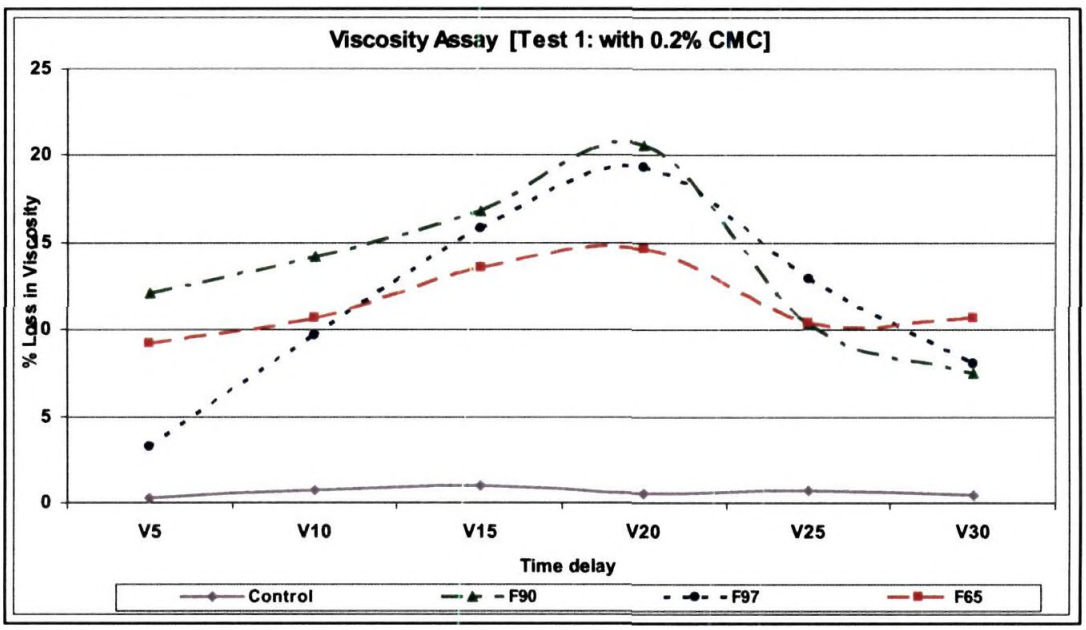

Fig. 8.4.1.1: Viscosity assay test with 0.2% CMC

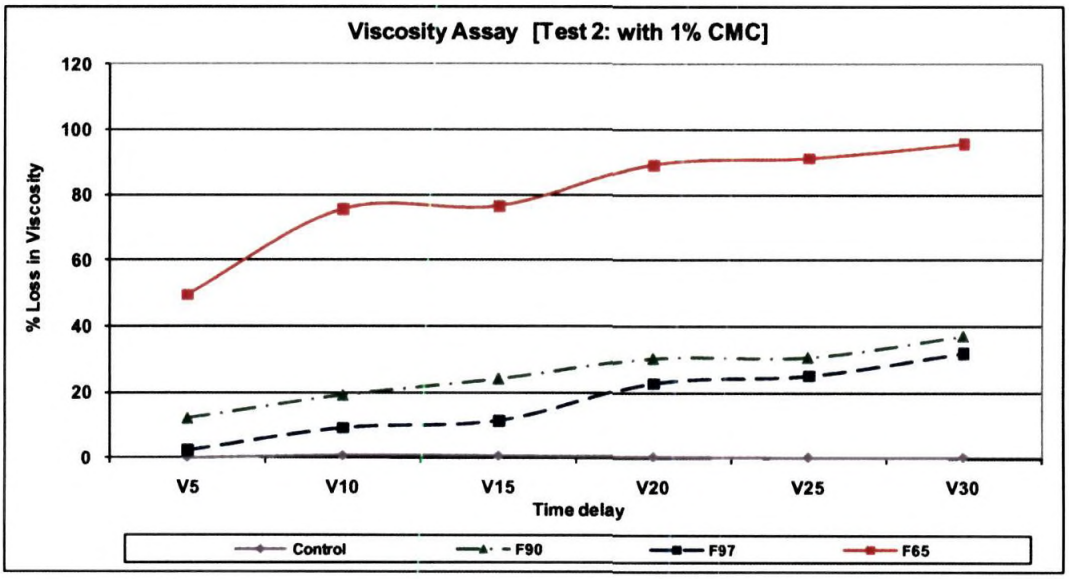

Fig. 8.4.1.2: Viscosity assay test with 1% CMC

The three fungal antagonists selected, namely, F_{65}, F_{90} and F_{97} showed high level of cellulose activity, as indicated by the percentage loss in viscosity in comparison to control. When 0.2 % CMC was used as substrate, all the three antagonists showed significant enzyme activity which peaked at 20 minutes [Fig. 8.4.1.1] while, when the substrate concentration was 1%, isolate F_{65} showed higher enzyme activity as indicated by the percentage loss in viscosity which showed a rising trend upto 30 minutes.

8.4.2. Measurement of endo β -1, 4 glucanase [CMC] using DNS reagent

The absorbance measured and the corresponding reducing sugar content are given in Table 8.4.2. These values are plotted in Figures 8.4.2.1, 8.4.2.2 and 8.4.2.3:

Time (min)	Absorbance					
	F90: non-enzyme	F90: with CMC	F97: non-enzyme	F97: with CMC	F65: non-enzyme	F65: with CMC
2	0.006	0.383	0.002	0.553	0.004	0.653
5	0.028	0.445	0.010	0.479	0.004	0.722
10	0.020	0.325	0.009	0.429	0.014	0.735
20	0.010	0.387	0.008	0.414	0.012	0.714
30	0.022	0.489	0.006	0.405	0.005	0.549
40	0.019	0.447	0.004	0.430	0.011	0.412

Time (min)	Reducing sugar content					
	F90: non-enzyme	F90: with CMC	F97: non-enzyme	F97: with CMC	F65: non-enzyme	F65: with CMC
2	1.01	4.15	0.98	5.56	1.00	6.39
5	1.20	4.66	1.05	4.95	1.00	6.97
10	1.13	3.67	1.04	4.53	1.08	7.07
20	1.05	4.18	1.03	4.41	1.06	6.90
30	1.15	5.03	1.01	4.33	1.01	5.53
40	1.12	4.68	1.00	4.54	1.06	4.39

Table 8.4.2: Absorbance and reducing sugar content of F_{90}, F_{97} and F_{65}

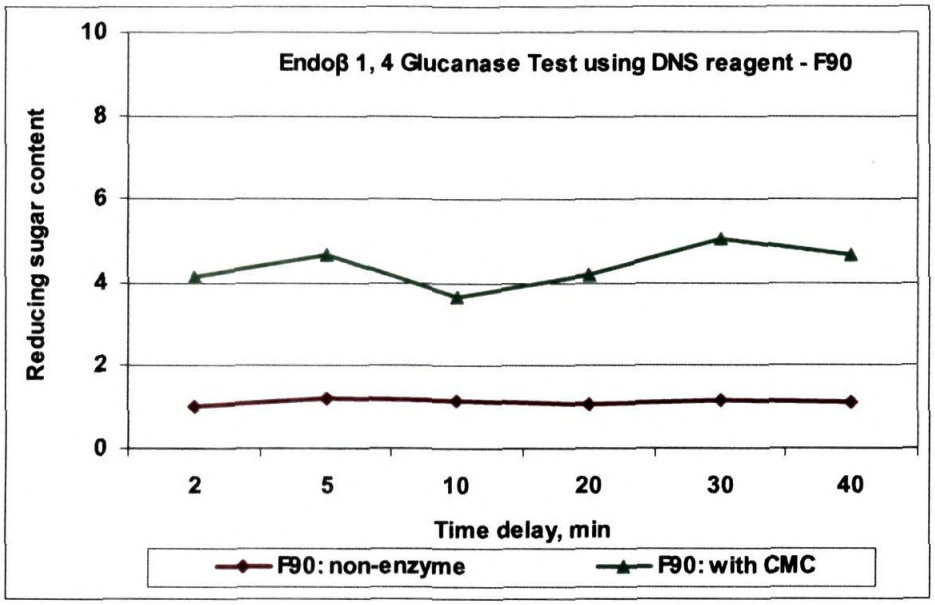

Fig.8.4.2.1: Reducing sugar content for fungal isolate F_{90}

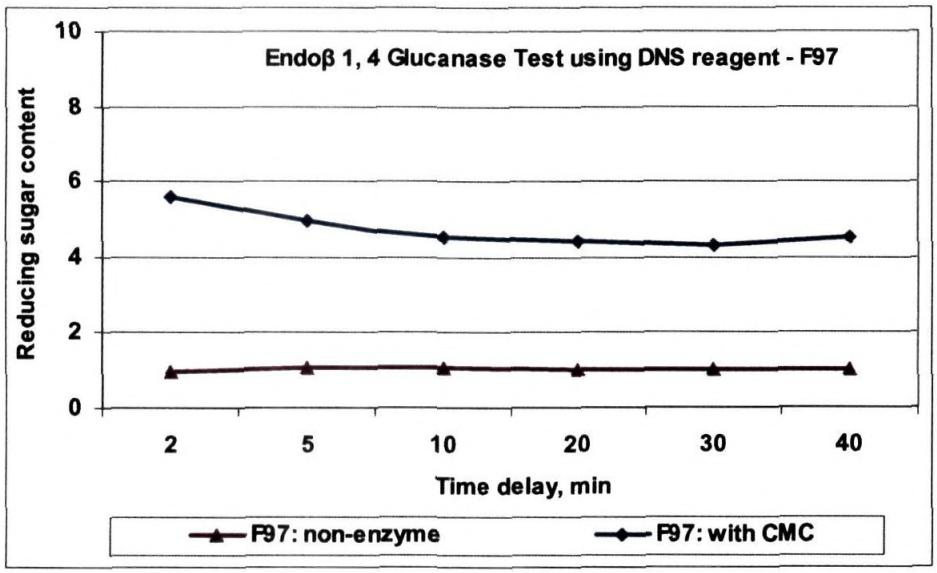

Fig.8.4.2.2: Reducing sugar content for fungal isolate F_{97}

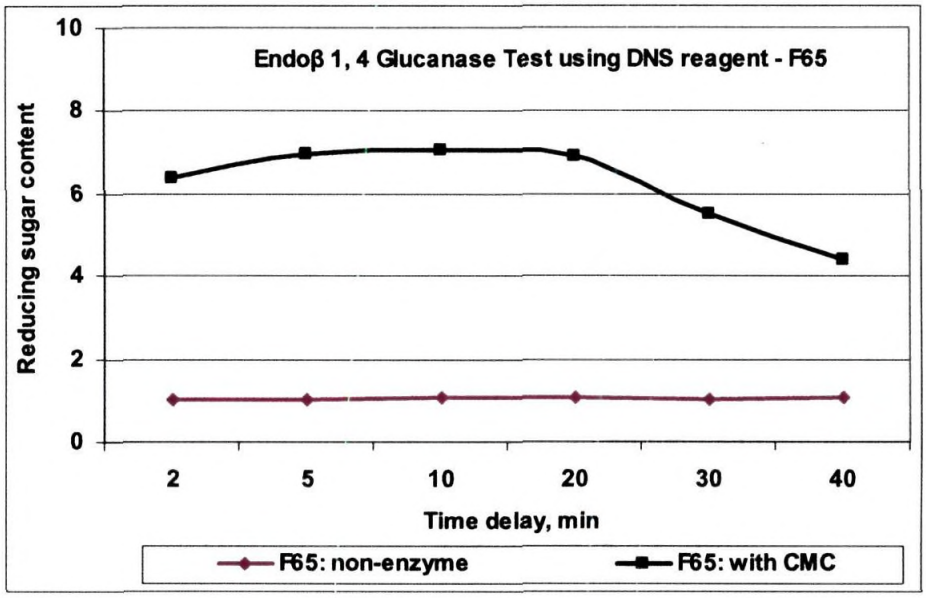

Fig.8.4.2.3: Reducing sugar content for fungal isolate F_{65}

The isolates F_{90}, F_{97} and F_{65} produced higher quantity of Endo β-1, 4 glucanase. The isolate F_{65} showed more enzyme activity as is evident from the release of more reducing sugar which was measured using DNS reagent. Negligible quantity of Endo β 1, 4 glucanase was produced by these isolates in the non-enzyme filtrates.

9. *IN VIVO* STUDIES

9.1. Introduction

The principal objective of these *in vivo* studies was to determine whether the bacterial and fungal antagonists that were observed to be effective against the pathogens of *Hevea brasiliensis* under laboratory conditions would be effective when applied on the plant. *Corynespora cassiicola* and *Colletotrichum acutatum* were the pathogens selected for *in vivo* experiments.

9.2. Review of literature

Trichoderma and *Aspergillus* species, isolated from rubber plantations in Nigeria, were found to be antagonistic to *Ganoderma psuedoferreum*, the pathogen causing red root disease when applied in the root zone (Ogbebor *et al.*, 2010).

9.3. Materials and methods

For the *in vivo* studies, young healthy plants of rubber clone RRII 105 grown in polythene bags were selected.

Pure cultures of *Corynespora cassiicola* and *Colletotrichum acutatum* were cultured on potato dextrose agar plates. Confirmed the sporulation and viability of the spores of these pathogens under the microscope using slide culture technique.

9.3.1. Bacterial antagonists

To assess the potential of bacterial antagonists, the selected bacterial isolates, B_{24}, B_{54} and B_{61} were grown on Nutrient Agar in Petri plates. Each bacterium was collected separately into distilled water so that the inoculum potential was 100 cells / ml. Around 10 ml of this bacterial suspension was sprayed on to the leaves of each plant using a plastic atomizer. Control plants were sprayed with sterile water.

The experiment included the following treatments in three replicates.

1. Uninoculated seedlings[Control-uninoculated]
2. Seedlings inoculated with pathogen [Control-inoculated]
3. Seedlings inoculated with pathogen first, and after 24 hours, with bacterial antagonist
4. Seedlings inoculated with bacterial antagonist first and after 24 hours, with pathogen

After spraying the inocula the seedlings were covered with polythene cover and fastened with a twine in order to maintain humidity, to enhance the growth of the microbes.

Examined the leaves, after every 24 hours up to 7 days, when disease incidence was observed as lesions on leaves.

Disease assessment was carried out according to the Horsfall-Barratt rating scale (Horsfall and Barratt, 1945; Egel and Harmon, 2001).

Disease symptoms were observed on the leaves of rubber seedlings which were inoculated with the pathogens. Leaf fall was observed in severely infected plants. The control plants which were not inoculated were free of disease symptoms. The differences observed in the various treatments were assessed using the following rating scale:

- 0% leaf area affected = 1
- 1-10% leaf area affected = 2
- 11-25% leaf area affected = 3
- 26-50% leaf area affected = 4
- >50% or leaf fall = 5

Fig. 9.3.1.1: Seedling inoculated with *Corynespora cassiicola* (Control)

Fig. 9.3.1.2: Seedling inoculated with bacterial antagonist first followed by pathogens

9.3.2. Fungal antagonists

To assess the potential of fungal antagonists, selected F_{65}, F_{90} and F_{97} isolates which showed potential to control the pathogens. Each isolate was collected separately into distilled water so that the inoculum potential was 100 spores / ml. Around 10 ml of this spore suspension was sprayed on to the leaves of each plant using a plastic atomizer. Control plants were sprayed with sterile water.

The experiment included the following treatments in three replicates.

1. Uninoculated seedlings.[Control-uninoculated]
2. Seedlings inoculated with pathogen [Control-inoculated]
3. Seedlings inoculated with pathogen first, and after 24 hours, with fungal antagonist

4. Seedlings inoculated with fungal antagonist first and after 24 hours, with pathogen

Inoculation was carried out and observation recorded similarly as described for bacterial antagonists.

9.4. Observation and results

The disease incidence on the leaves was recorded. The data is presented in tables for bacteria (Table 9.4.1 and 9.4.2) and fungal (Table 9.4.3 and 9.4.4) antagonists.

Treatments	Replications			
	R1	R2	R3	Average
Control - uninoculated	1	1	1	1
Control -inoculated (CC04 alone)	5	3	5	4.33
CC04 + B_{24}	4	5	5	4.67
CC04 + B_{54}	4	3	3	3.33
CC04 + B_{61}	3	4	3	3.33
B_{24} + CC04	4	4	4	4
B_{54} + CC04	2	1	2	1.67
B_{61} + CC04	2	3	2	2.33

Table 9.4.1: Effect of bacterial antagonists on the growth of *Corynespora cassiicola* in rubber seedlings

Treatments	Replications			
	R1	R2	R3	Average
Control - uninoculated	1	1	1	1
Control -inoculated (C4 alone)	4	4	5	4.33
C4 + B_{24}	4	4	4	4
C4 + B_{54}	3	2	3	2.66
C4 + B_{61}	3	2	2	2.33
B_{24} + C4	4	3	3	3.33
B_{54} + C4	2	2	2	2
B_{61} + C4	3	3	2	2.66

Table 9.4.2: Effect of bacterial antagonists on the growth of *Colletotrichum acutatum* in rubber seedlings

Treatments	Replications			
	R1	R2	R3	Average
Control - uninoculated	1	1	1	1
Control -inoculated (CC04 alone)	5	4	5	4.66
CC04 + F_{65}	4	3	3	3.33
CC04 + F_{90}	4	3	4	3.66
CC04 + F_{97}	4	3	3	3.33
F_{65} + CC04	2	2	3	2.33
F_{90} + CC04	2	3	3	2.66
F_{97} + CC04	3	3	3	3

Table 9.4.3: Effect of fungal antagonists on the growth of *Corynespora cassiicola* in rubber seedlings

Treatments	Replications			
	R1	R2	R3	Average
Control - uninoculated	1	1	1	1
Control -inoculated (C4 alone)	5	5	5	5
C4 + F_{65}	4	3	3	3.33
C4 + F_{90}	4	4	4	4
C4 + F_{97}	4	4	3	3.66
F_{65} + C4	2	2	3	2.33
F_{90} + C4	3	2	3	2.66
F_{97} + C4	3	4	4	3.66

Table 9.4.4: Effect of fungal antagonists on the growth of *Colletotrichum acutatum* in rubber seedlings

The results showed that the bacterial and fungal antagonists found effective *in vitro* were effective *in vivo* also, particularly, when the antagonist was inoculated prior to the pathogen. Among the bacterial antagonists, B_{54} showed superiority over the other two antagonists for control of both the pathogens. Similarly among the fungal antagonists, F_{65} showed superiority over the other two antagonists.

Fig. 9.4.1: Uninoculated leaves (control)

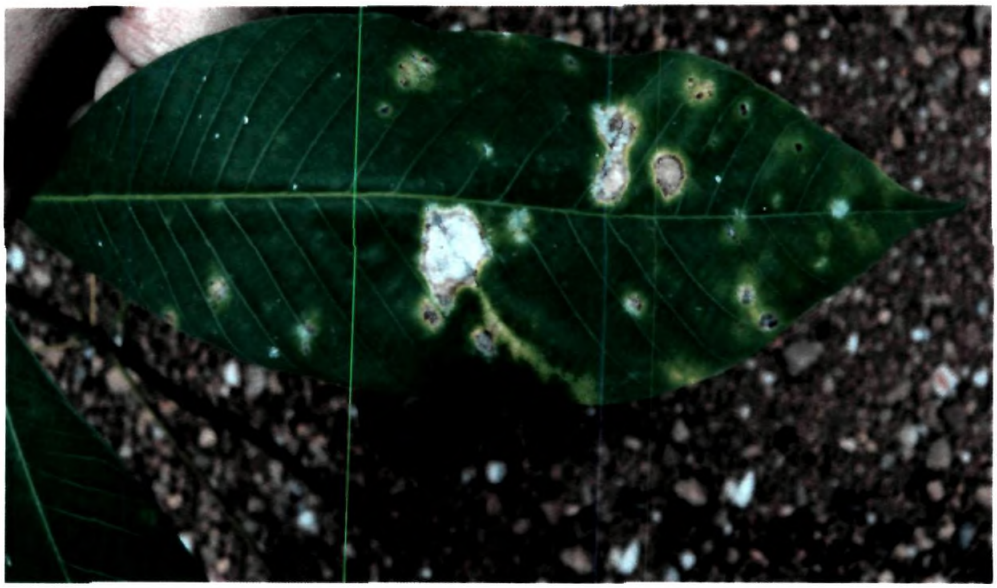

Fig. 9.4.2: Disease incidence in seedling inoculated with *Corynespora cassiicola*

10. DISCUSSION

The most important source of natural rubber, *Hevea brasiliensis* is constantly under threat from various pathogens that cause severe diseases like abnormal leaf fall, Corynespora leaf disease, Colletotrichum leaf disease, pink disease and brown root disease. Jacob *et al.* (1989), has reported the crop loss due to heavy defoliation caused by abnormal leaf fall disease. The severity of Corynespora Leaf Fall (CLF) has been reported by Manju *et al.*, (2001). The widespread incidence of Colletotrichum leaf disease also has attained attention (Manju *et al.*, 2001). Hilton, (1958), has reported the symptoms and severity of pink disease. Rajalakshmi and Jayarathnam(2000) has described the maladies of brown root disease.

Although control measures for these diseases have been developed, the dependance is mainly on fungicides. Chemical disesase control in large areas under rubber plantation may not be desirable for environmental health. Although biological control has been suggested as the alternative, it has not been developed on commercial scale. One of the constraints is the survival of introduced antagonist on the rubber trees to offer protection against pathogens. Study of the population dynamics of the natural flora associated with rubber trees and the search for antogonists among them is relevant in this context.

The aim of the present research was, to provide insight into the population dynamics of fungi, bacteria and other microbes associated with the two popular high yielding clones of rubber trees namely, RRII 105 and PB 260 cultivated in the selected locations namely Nettana, Padiyoor, Palappilly, Chethackal, Lahai and Vaikundam during the different seasons and to check whether the natural microflora residing in the phylloplane, cauloplane and rhizosphere can act as biocontrol agents against the major pathogens causing diseases of rubber trees.

The location selected for the study experiences variation in weather although all of them lie in the south western region of India. The northern

location Nettana experiences heavy South West monsoon rainfall which is followed by prolonged dry period of 6 to 8 months. Padiyoor also receives more of South West monsoon and only few North East monsoon rains. Palappilly, Chethackal and Lahai receives both South West and North East monsoon rains with rain free period ranging from 4 to 5 months. Vaikundam receives mainly the North East monsoon rains with scanty rains during South West monsoon season and the low rainfall period is about 9 months. While all other locations are at lower elevations (below 100m) Lahai is a high elevation (more than 400m) region with a cold period of about two months (Vijayakumar *et al.*, 2000).

In the present study, it was observed that all the phylloplane samples collected from the six regions harboured a variety of microorganisms. Bacteria and fungi were abundant in all the samples whereas the number of actinomycetes and yeasts were very low or absent in many cases. Phylloplane and cauloplane microorganisms were more during the post monsoon period and minimum during the summer season.

Bacterial population was very low in the phylloplane of both mature and immature rubber plants during the summer season and higher during the rainy season. It was most abundant during the post monsoon season. It is observed that the phylloplane bacterial population was higher in areas where the rainfall is confined to 3-4 months. Prolonged rainfall may wash away the bacteria from the leaf surfaces leading to lower population.

The phylloplane population of bacteria was in general higher in the immature plantations. The immature plantations are maintained under more frequent nutrient application regimes to support active growth. This results in higher physiological activities on the leaves and may result in more nutrient rich leaf exudates supporting a higher phylloplane population of bacteria. Nix-Stohr *et al.* (2008) observed that organic nitrogen supplementation of tall fescue turf grass (*Festuca arudinacea*) stimulates the population of yeasts on the phylloplane.

Between the two clones of rubber, RRII 105 harboured higher phylloplane bacterial population in the mature stage. Although PB 260 is reported as a vigorously growing clone (Licy, 1979) the yield of rubber is reported to be higher for clone RRII 105 (Nazeer *et al.*, 1986). It is probable that higher photosynthetic efficiency of the leaves may support more bacterial population on them.

The statistical analysis of seasonal variation in phylloplane bacterial population revelead significant variations in mature rubber trees of RRII 105 at Nettana, Padiyoor, Palappilly, Lahai and Vaikundam indicating that the bacterial activity on phylloplane is weather dependant for this clone. For the mature trees of clone PB 260 variation in bacterial population was not significant at Nettana and Lahai. Low significance was observed at Vaikundam while the population was significant at Palappilly and Chethackal. This indicates that the clone supports a steady bacterial population in spite of the extreme weather condition. Under moderate weather, the seasonal variation is expressed. The immature trees of both the clones, in general, showed population variation similar to that of the mature trees. Hence the influence of the age of trees on the phylloplane bacteria can be reckoned as minimal.

In general, the phylloplane bacterial population was significantly high during post monsoon season and low during the summer season irrespective of the clones RRII 105 and PB 260.

Like phylloplane bacteria, population of phylloplane fungi also was, in general, higher during post monsoon season across the locations. Between the mature and immature trees, the mature trees harboured higher population of phylloplane fungi. This could be due to the dense coverage of the mature tree canopy which increases humid environment within the plantation favouring fungal survival. The age of the leaves may have a positive influence as observed in the case of phylloplane fungi on sugarcane. Sharma (2004) isolated sixty-seven different fungi from young,

mature, senescent and dead leaves of sugarcane and the number of fungi increased with the age of leaves.

On mature rubber trees of clone PB 260 the occurrence of phylloplane fungi was uniform across locations during the summer season. The fungi might have been in quicent stage due to limitation in availability of nutrients and favourable environment for growth and multiplication. But the variation observed in trees of clone RRII 105 during summer shows that the trees of this clone may provide favourable conditions for phylloplane fungi at locations like Lahai where the temperature is relatively lower during summer. Waller and Masaba (2006) observed that phylloplane population of microflora varied with the altitude at which coffee plants are cultivated. The population was larger at higher altitudes.

Between the two clones, PB 260 harboured higher population of phylloplane fungi during immature phase while RRII 105 had higher population in the mature phase, the trend being similar to that of phylloplane bacteria. Although there is a general trend towards increase of fungal population from summer to rainy and finally post-monsoon seasons, the difference was more significant at two locations, namely Nettana and Chethackal.

Statistical analysis of phylloplane fungal population revealed significant variation for mature trees of clone RRII 105 at all the locations indicating influence of weather. Significant variation was observed in mature trees of PB 260 also except in two locations. The immature trees of both the clones showed similar trend. The results thus indicate that fungal population on the phylloplane is weather dependant although some variations may be observed at certain locations.

In general, the phylloplane fungal population was high during the post monsoon season and less during summer season irrespective of the clones. The mature trees showed higher population than immature trees. This agrees with the observations of Banik and Krishnamurthy (2004) who reported that the phylloplane fungal population increases with the leaf

maturity in black gram. They also observed variation in the composition of fungal species on the phylloplane with the maturity of leaves. Maturity of leaves may also alter the availability of niche for fungal survival. Girivasan and Suryanarayanan(2004) reported that the phylloplane fungal populations are niche specific and that they do not occupy the niche utilized by endophytes thus avoiding competition between them.

Seasonal variation in cauloplane bacteria was significant in clone RRII 105 at all locations except Nettana. For the mature trees of clone PB 260 the seasonal variation was significant except at Chethackal. Immature trees also had similar trend in cauloplane bacteria population. These observations clearly indicate that survival of bacteria on the cauloplane is weather dependant irrespective of rubber clones and location of plantations.

The cauloplane bacterial population was observed to be highest during the post monsoon season. Between the two clones of rubber trees, RRII 105 harboured higher population of cauloplane bacteria. The population was higher in mature trees. As the trees become mature the surface of the bark tends to be rough which provides niches for bacterial survival. This may be one of the reasons for the higher population. Prolonged presence of moisture in the crevices of the mature bark could be favouring bacterial survival.

Cauloplane fungi on mature trees of RRII 105 showed significant seasonal variation across locations except at Chethackal. The seasonal variation in cauloplane fungi in mature trees of PB 260 was low or absent in most locations except at Nettana and Lahai. This shows that the clone supports a more steady population across seasons. Immature plantation of both clones also showed similar trend with certain exceptions. With the exception of PB 260 at Palappilly both the clones had similar population at each location. Highest cauloplane fungal population was observed in clone RRII 105 at Nettana during the post monsoon season.

In general, cauloplane fungi were highest in the post monsoon season. Higher cauloplane fungal population was reported in clone RRII 105 than in PB 260. The population was higher in mature trees. Besides the rough bark providing ecological niches for survival during mature stage the closed canopy of mature rubber trees also may prevent direct hit of solar radiations on the surface of the main trunk of the trees. It also protects the tree trunk from wind and rain lashes which may affect the microbial population adversely.

Studies on fungi inhabiting healthy stems and branches of American beech by Chapela (1989) have shown that very high water content in the wood prevented fungal growth and fast drying of wood resulted in poor development of the fungus.

Analysis of seasonal variation on rhizosphere bacteria indicates that RRII 105 mature trees had significant variation at Nettana, Padiyoor, Palappilly, Chethackal, Lahai as well as Vaikundam. Immature RRII 105 trees also had significant variation at all the above regions. Rhizosphere bacteria on PB 260 mature trees had significant seasonal variation at Nettana, Chethackal, Lahai and Vaikundam. But at Palappilly the variation was not significant. In the case of immature PB 260 trees, there was significant variation at Palappilly, Chethackal and Vaikundam.

Rhizosphere bacterial count was observed to be low during the rainy season. Highest rhizosphere bacterial count was found during the post monsoon season.This is in agreement with the observation on American beech. In the rhizosphere of American beech, microbes were present in lesser numbers during the rainy season (Chapela, 1989). The low rhizosphere population might be due to the washing off of the spores of microbes during heavy rain.

The rhizosphere bacterial population of mature trees was generally higher in clone PB 260 than in RRII 105. In general the population was higher in mature plantation probably due to the higher organic matter content of soil through more litter addition. The annual addition of litter in

mature rubber plantation is estimated to be 4824 kg per hectare (Philip *et al.*, 2003).

Some bacteria are specifically adapted to the rhizosphere of perennial plants. Pandey and Palni, (1997) observed that *Bacillus subtilis* is specifically adapted to well established tea bushes and are capable of survival during unfavourable seasons. It can be inferred that in mature plantations the bacterial population gets adapted to the rhizosphere.

Seasonal variation in rhizosphere fungi was significant in all the samples from Nettana, Padiyoor, Palappilly, Chethackal and Lahai. Significant variation was observed at Vaikundam in mature and immature RRII 105 and immature PB 260 while mature PB 260 had no significant variation.

In the soil, microbial population is higher in the surface layer, as observed in general, due to the higher organic matter status in those layers. The microbial population is also influenced by fertilizer placements. In the rubber growing soils the organic carbon status is very high in the surface layers. Rubber plantation, being a closed ecosystem with deciduous trees recycles enormous biomass through litter decomposition which takes place rapidly thus producing considerable organic carbon in surface layers (Krishnakumar *et al.,* 1991).

Recycling of crop residues is reported to improve the population of bacteria, fungi, P solubilizers, azotobacter and actinomycetes in the rhizosphere of sorghum in a permanent experiment plot which received the treatment continuously for twenty years (Bhakare *et al.*, 2008).

Chaverri and Gazis (2010) observed that *Perisporiopsis lateritia,* a fungus found in association with rubber trees play a role in the degradation of rubber tree litter in the Amazon basin.

Seasonal changes in rhizosphere microbial communities associated with *Brassica napus* has been reported by Kari and James (2003). Studies of Obire and Wemedo (2002) on the seasonal effect of bacterial and fungal

population of an oilfield wastewater-polluted soil in Nigeria have shown that different seasons selectively favour the growth of certain microbial types. The drier seasons supported large active microbial populations and the wetter seasons had smaller populations. Seasonal influence was more pronounced on the fungi than on the bacteria. Seasonal shifts in rhizosphere microbial population on pea, wheat and sugar beet showed that shifts in the diversity of fungal and bacterial communities were more pronounced in maturing pea and sugar beet plants (Houlden *et al.*, 2008). Pandey *et al.,* 2001, observed that though *Penicillium* spp. and *Trichoderma* spp. were dominant in the rhizosphere of tea, the latter showed less variation in population across seasons. The population of these fungi was also observed to be inversely correlated to the population of bacterial species, *Bacillus subtilis* and *B. mycoides* as these had antagonistic effect against the fungi.

The summer season samples in the present study were devoid of actinomycetes while all the samples in the post monsoon and all the samples except one in the rainy season possessed actinomycetes. In general, among the mature trees of the two clones, PB 260 harboured more rhizosphere actinomycetes than RRII 105. However, at Vaikundam and Lahai RRII 105 mature and immature plantation respectively harboured high population of actinomycetes.

Phosphobacterial colonies on mature RRII 105 and PB 260 clones of rubber trees showed significant variation at all the locations. Post monsoon season was more favourable. Highest phosphobacterial count was observed in samples from Chethackal and Nettana. In general, the azotobacter population was higher in the rhizosphere of clone PB 260 with the exception of samples from Vaikundam. The high rate of growth and girthing observed in PB 260 (76 cm) could be related to higher availability of nitrogen to the plants mediated through microbial action. Mature RRII 105 at Nettana, also had high population.

In the tea soils of Assam populations of bacteria, fungi, phosphate solubilizers, ammonifiers and nitrifiers were observed to decrease significantly with depth. Seasonal variation was significant in case of bacteria, fungi, ammonifiers and *Nitrosomonas*. Except for nitrifiers, other microbes attained population peak in monsoon season (Gogoi *et al.,* 2003).

The seasonal variation of VAM incidence on the roots of rubber trees was highly significant at all the six regions with regard to all the clones belonging to the two age groups. The incidence in general was low during summer and it was upto 100 percent during post monsoon season. The VAM incidence was higher at Vaikundam in both the clones RRII 105 and PB 260.

It was observed that in all the six regions, the VAM spore count was highly significant in mature and immature RRII 105, PB 260 plantations as well as in the virgin soils. The spore count was consistently high during summer season and low during rainy season.

The post monsoon season with optimum soil moisture and other environmental conditions favourable for VAM establishment in roots promotes the growth of these fungi. When the environment becomes drier the tendency is towards production of spores that help the fungus to perennate adverse season and hence the higher spore count during the summer season.

Jayaratnae (1982), observed that for the endomycorrhizal fungi in rubber growing soils of Sri Lanka the spore numbers varied considerably from site to site. Ikram and Mahmud (1984) reported that *Hevea* feeder roots showed mycorrhizal colonization ranging from 0 to 50 percent of the root length infected.

The soil from the six locations in the present study had a pH value below 6.29 and a minimum of 3.61. Even in such acidic pH, fungi, actinomycetes, azotobacter and VAM growth was not significantly affected.

Only the bacterial population was observed to be influenced by the pH of the soil.

The soil moisture is season dependant with high moisture content during rainy and low during summer. The microbial population in the rubber plantation had no correlation with the soil moisture content except that the azotobacter population was lower during rainy season. In the studies on seasonal variations in microbial population by Gogoi *et al.* (2003) in a tea ecosystem of Assam the population was observed to be influenced both by season and the soil factors. Microbial population showed positive correlation in premonsoon and monsoon seasons with soil parameters.

Highest rhizosphere bacterial population was recorded during the post monsoon season from Chethackal PB 260 mature plantation soil with pH 5.19. Rhizosphere phosphobacterial colonies were highest in RRII 105 mature plantation soil with pH 4.38.

The concentration of bacteria found around the roots of plants is generally much greater than in the surrounding soil. The rhizosphere supports higher microbial growth rates and activities as compared to the bulk soil (Soderberg and Baath, 1998). The increased availability of soluble organic compounds that results from plant exudation are the main factor that supports the higher growth rate.The composition and quality of root exudates varies depending on plant samples (Smith, 1976) and abiotic conditions such as water content and temperature (Martin and Kemp, 1980).

Phenol estimation in the present study has revealed that when the phenol and sugar content in the leaves are more, the population of phylloplane microbes is higher. A proportionate increase in phylloplane fungi was observed with increasing phenol and sugar content in all leaf samples from all the regions except Lahai where fungal count was high even when the sugar content was low. Pridham (1965) has reported accumulation of phenols in diseased plants which may be the consequence of release of phenols from their glucosides by β-glucosidase of either host or pathogen. Influence of non pathogenic phylloplane microorganisms on the leaf phenol content has not

been reported. Role of phenolics and oxidative enzymes particularly the peroxidase in defence against Alternaria blight disease of cluster bean has been suggested earlier. Sankpal and Nimbalkar (1979) and Dhumal and Nimbalkar (1982) have reported an increase in reducing sugar content in leaves of infected plants. The results reveal that rubber clones with high phenol and sugar content may harbour higher population of phylloplane microflora and hence may contribute in the defense of the plant against diseases.

Osbourn (1996) reported that a range of plant pathogenic fungi can tolerate high levels of HCN. In the present study, it was found that some of the plant pathogenic fungi were not affected by HCN production of certain antagonistic bacterial isolates.

The phylloplane, cauloplane and rhizosphere population of bacteria and that in the soil was significantly high in areas where intercrop of banana with rubber was practised. The phylloplane, cauloplane and rhizosphere fungal population and the population of fungi and azotobacter in soil were significantly high in areas where intercrop of coffee with rubber was done. In non-intercrop rubber, rhizosphere population of actinomycetes was higher. In virgin soil actinomycete, phosphobacteria and azotobacter populations were high.The bacterial and fungal population was higher in areas with intercrop during the rainy season.

Among the 118 bacteria isolated from the phylloplane, cauloplane and rhizosphere of rubber trees from the selected locations, eighteen isolates were selected in the initial screening, based on their antagonistic properties against the five selected pathogens of rubber. Further screening by dual culture technique revealed the superiority of isolate B_{24}, B_{54} and B_{61} which were highly effective antagonists of more than two pathogens *in vitro*. Twelve bacterial isolates were found to be phosphate solubilisers of which PB_8 and PB_9 were more efficient, and hence can be used in phosphate deficient soils after detailed field evaluation.

Effect of thirteen phylloplane fungi in biocontrol of leafspot caused by *Corynespora cassiicola on* black gram was observed by Banik and Krishnamurthy (2004). Among these *Penicillium funiculosum* was the most effective antagonist. Control of late blight of tomato caused by *Phytophthora infestans* using two phylloplane bacteria namely, *Novosphingobium capsulatum* and *Bacillus cereus* has been reported (Vieira *et al.*, 2008). Reduction in disease intensity ranged between 55 to 62 percent. Waller and Masaba (2006) observed that unsprayed coffee plants had very low levels of *Colletotrichum kahawae* and low incidence of coffee berry disease as the natural microflora prevented the spread of the pathogen.

The mechanism of antagonism in the 96 bacterial isolates tested was studied by evaluating HCN production and 77 isolates by siderophore production. Eighty two isolates showed HCN production at varying intensities. The highest siderophore production was observed in isolate B_{54} and B_{61} while B_{24} showed low production. Production of volatile compounds that cause pathogen inhibition was also observed for bacterial isolate B_{54}. Inhibition of growth of pathogenic fungi by antifungal volatiles produced by four effective antagonistic Bacillus strains isolated from the rhizosphere of cucumber has been reported (Liu WeiWei *et al.*, 2008).

Among the 144 fungal isolates tested for antagonistic properties, twelve were selected for further screening using dual culture technique. Nine fungal isolates showed varying degrees of antagonistic properties against all the five pathogens. Of these isolates F_{90} and F_{97} were highly effective antagonists against all the five pathogens while isolate F_{65} specifically showed high antagonism against *C. acutatum.* This observation is significant in the context that the effectiveness of F_{90} and F_{97} can be used against multiple target pathogens of rubber. If a multitarget antagonist population can be maintained on the rubber tree it may lead to reduction in disease incidence.

Colletotrichum acutatum has been reported as the more significant pathogen species causing Colletotrichum leaf disease of rubber in India (Saha *et al.* 2002) and Sri Lanka (Jayasinghe *et al.* 1997). The observation that

isolate F_{65} is highly antagonistic to *C. acutatum* assumes significance in formulating biocontrol strategy against this pathogen.

The isolates F_{90}, F_{97} and F_{65} produced higher quantity of Endo β-1, 4 glucanase, when compared to the other isolates under study. The isolate F_{65} showed more enzyme activity as is evident from the release of more reducing sugar. Negligible quantity of Endo β-1, 4 glucanase was produced by these isolates in the non-enzyme filtrates.

Several other antagonistic bacteria and fungi have earlier been screened *in vitro* against pathogens of rubber. However, none of these organisms have been reported as effective *in vivo.* The survival of introduced antagonist in the plant vicinity is essential for effective protection against invading pathogens.

There are several reports on use of bacterial isolates as antagonists against leaf and root pathogens of trees. *Bacillus subtilis* isolated from *Pinus roxburghii*, exhibited strong antagonistic activity against *Macrophomina phaseolina, Fusarium oxysporum* and *Rhizoctonia solani* (Neetu *et al.*, 2008).

Although fungal antagonists like *Ampelomyces quisqualis* (Kiss *et al.*, 2003), *Chaetomium globosum* (Cummings, 1954) have been reported as effective, they have not been widely used in crop protection of perennial plants. *Dicyma pulvinata* has been reported to be antagonistic to *Fuslcladium macrosporum,* the pathogen that causes South American leaf blight of rubber trees from Brazil (Mello *et al.*, 2008).

Evueh and Ogbebor (2008) screened *in vitro* several phylloplane fungi from rubber against *Colletotrichum gleosporioides* and observed that *Trichophyton* sp. and *Gliocladium* sp. were antagonistic to the pathogen. Metabolites produced by *Gonatorrhodiella* and *Syncephalastrum* also affected the pathogen by antibiosis. Evueh *et al.* (2011) also observed that *Trichoderma viride* and *Aspergillus niger* showed antagonism against *Corynespora cassiicola* from among ten phylloplane fungi of rubber screened for biocontrol potential.

The antagonistic efficiency of *Trichoderma harzianum* against *Rigidoporus microporus,* the pathogen causing white root disease of rubber was demonstrated by Jayasuriya and Thennakoon (2007). They also reported that the antagonist when grown on a medium consisting of rice bran and farmyard manure sporulated well and remained active for four months. Sudirman *et al.* (1992) observed that *Lentinus squarrosulus* a wood inhabiting basidiomycete fungus on *Hevea brasiliensis* could invade the zone previously colonized by *Rigidoporus lignosus* causing *white* root disease of rubber and kill the pathogen.

Antagonistic effect of *Trichoderma hamatum, T. harzianum* and *Aspergillus* spp. against *Ganoderma pseudoferreum* the pathogen causing red root disease of rubber trees in Nigeria has also been reported (Ogbebor *et al.,* 2010).

Romero *et al.* (2004) evaluated antagonistic bacteria against the cucurbit powdery mildew fungus *Podosphaera fusca* which proved to be efficient in the control on detached leaves and seedling bio-control assays, where reductions of disease severity of up to 80% were observed.

Cook and Baker (1983), Anandaraj and Sarma(1995) reported that various antagonistic microorganisms are efficient against many soil borne pathogen including *Phytophthora* species. Several rhizobacterial isolates were found inhibitory to rot pathogens such as *Phytophthora meadii, Fusarium oxysporum* f. spp. *vanillae,* and *Colletotrichum vanillae* (Bhai and Kumar, 2008) on vanilla.

Grosch *et al.* (2006) studied the potential of fungal antagonists against the soil borne pathogen *Rhizoctonia solani* using *in vitro* and *in vivo* assays. Antagonistic *Trichoderma* strains were active under field conditions also. Velmurugan *et al.* (2009), demonstrated the antifungal activity of bacterial strains, *Bacillus subtilis* and *B. licheniformis* against sapstaining fungal cultures, *Ophiostoma* spp. in both *in vitro* and *in vivo* conditions. Fluorescent pseudomonads, showed antagonistic activity against *Macrophomina*

phaseolina which causes charcoal rot in a number of plants (Arora *et al.* 2008).

Bacteria and fungi isolated from the phyllosphere, rhizosphere, endorhiza and endosphere of sugar beet plants exhibited antagonism towards *Aphanomyces cochlioides, Phoma betae, Pythium ultimum* and *Rhizoctonia solani* (Zachow *et al.*, 2008).

The antagonistic effect of *Trichoderma viride, T. koningi and T. harzianum* against *Phytophthora meadii* which causes Abnormal leaf fall disease in rubber trees has been reported (Vanitha *et al.*, 1994).

In vivo studies attempted here indicated that the isolates that showed antagonism *in vitro* were also effective *in vivo* when introduced prophylactically. The bacterial isolate B_{54} and fungal isolate F_{65} were superior in affording protection against *C. cassiicola* and *C. acutatum* in nursery plants of clone RRII 105.

The protection given by the selected isolates in the limited *in vivo* assay is indicative of its potential for use, in larger scale. However, utilization of these observations on rubber trees in the main field requires further detailed experimentation and perfection of delivery systems for the antagonist. One of the constraints in perennial crops is the survival of antagonists across the seasons. The present study reveals that there is significant seasonal variation in the survival of bacteria and fungi in the vicinity of rubber trees. Hence timing of application of antagonists to plants and the maintenance of a high population through repeated application may assure significance. Although antagonists may be preferred to other control measures from environmental considerations, commercial agriculture demands economic viability of crop protection recommendations. The sustainability of crop protection depends on striking an acceptable balance between the economic and environmental considerations.

11. SUMMARY AND CONCLUSION

Production and productivity of natural rubber from *Hevea brasiliensis* are influenced adversely by diseases like Abnormal Leaf fall of Rubber, Corynespora leaf disease, Brown root disease, Pink disease and Colletotrichum leaf disease of rubber trees. The pathogens causing the diseases are being controlled by application of fungicides. The application of such chemicals may have adverse effects on the beneficial microorganisms and the environment in general. Hence the use of biocontrol agents against the diseases attains significance.

In an attempt to study the seasonal fluctuations in microflora associated with rubber trees and to recognize the natural microflora which may be used as biocontrol agents, samples were collected from six major rubber growing zones in south western India viz. South Kerala, Central Kerala, North Kerala, South West Karnataka and Tropical High Altitudes. Trees belonging to two different clones of different age groups were selected and marked for sampling in six rubber plantations at Nettana, Padiyoor, Palappilly, Chetheckal, Lahai and Vaikundam. The sampling, isolation and enumeration of microorganisms were done during three seasons viz. summer, monsoon and post monsoon. Soil samples were collected from the virgin forests in the representative areas near to the rubber plantations to enumerate the soil microflora and compare it with the microbial flora from rubber plantations.The moisture content and soil pH were recorded to observe their influence on the soil microflora.Levels of sugar and phenols in the plant parts were studied in order to study their role in the variation in population of microorganisms.

Root samples of the different clones were processed, stained and examined under the microscope to observe mycorrhizal association. The percentage incidence of vesicular arbuscular mycorrhizae (VAM) in rubber roots was calculated. Spores of VAM were collected by wet sieving and decantation and the spore count was recorded, microphotographs were

taken to visualize the presence of vesicles and arbuscular nature of the fungi within the roots.

Studies on antagonism were conducted using the isolated bacteria and fungi against five major pathogens of rubber trees namely, *Corynespora cassiicola* causing Corynespora leaf disease, *Phytophthora meadii* causing abnormal leaf fall, *Phellinus noxius* causing brown root disease, *Corticium salmonicolor* causing pink disease and *Colletotrichum acutatum* causing Colletotrichum leaf disease.

Out of 118 bacterial isolates and 144 fungal isolates, the major bacterial and fungal antagonists were selected and cultured in order to study their role in biological protection against the pathogens.

Studies on HCN production, siderophore production and volatile compound production by the antagonistic bacteria were conducted to correlate these properties with the antagonism shown by the bacteria. Enzyme assays were conducted to measure the activity of the enzyme cellulose in relation to antagonistic activity of the selected fungi.

The effect of cultural practices on microbial population was studied using samples collected from inter-cropping systems at Central Experimental Station of Rubber Research Institute of India at Chethackal.

The following conclusions were drawn from the observation recorded:

- Studies on seasonal variation in phylloplane microorganisms have shown that the population of bacteria and fungi were highest during the post-monsoon season, irrespective of the clones. The microbial number in summer was much less than that in the rainy season. The mature trees harboured higher population of phylloplane fungi than immature trees. The dense coverage of the mature tree canopy which increases the humidity of the environment within the plantation, may favour fungal growth.

- Cauloplane bacteria and fungi also showed remarkable variation during the three seasons. Highest population of cauloplane bacteria and fungi were observed during the post monsoon season. RRII 105 mature trees harboured higher population of cauloplane bacteria. The rough surface of the bark which provides niches for bacterial survival may be one of the reasons for such high population.
- Rhizosphere bacterial count was low during the rainy season and highest during the post monsoon season. The spores of microbes may be washed off during heavy rain. The rhizosphere bacterial population of mature trees was generally higher for clone PB 260 than RRII 105. This is probably due to the higher organic matter content of soil through more litter addition. In mature plantations, the bacterial population gets adapted to the rhizosphere. There was significant seasonal variation in rhizosphere fungi in all the samples from Nettana, Padiyoor, Palappilly, Chethackal and Lahai.
- Actinomycetes were present in all the samples collected in the post monsoon season and all the samples except one in the rainy season but were not present in the summer season samples.
- At all the locations, phosphobacterial colonies on mature RRII 105 and PB 260 clones showed significant seasonal variation.
- In general, population of azotobacter was higher in the rhizosphere of clone PB 260.
- VAM incidence on the roots of rubber trees showed highly significant seasonal variation at all the six regions for both the clones belonging to two age groups. The soil moisture and other environmental conditions during the post monsoon season favoured VAM establishment, leading to high incidence, even upto 100%, in the roots, during the post monsoon season.
- VAM spore count was consistently high during summer season and low during rainy season. In all the six regions, the summer season

VAM spore count was highly significant in mature and immature RRII 105, PB 260 plantations as well as in the virgin soils. The dry environmental conditions during the summer season, probably led to increased production of spores to tide over the unfavourable conditions.

- Evaluation of the pH of soil showed that even in acidic soils, fungi, actinomycetes, azotobacter and VAM growth was not significantly affected.
- There was no correlation between microbial population in the rubber plantation and soil moisture except that the azotobacter population was lower during rainy season.
- The biochemical studies indicated that an increase in sugar and phenol content increased the number of phylloplane microflora.
- Inter-cropping was more favourable to the growth of beneficial microbes, in comparison with sole rubber crop plantations.
- Several microorganisms which were isolated from the phylloplane, cauloplane and rhizosphere proved to be antagonistic to the five known major pathogens of rubber trees. Isolate B_{24}, B_{54} and B_{61} were highly effective antagonists of more than two pathogens *in vitro.*
- Twelve bacterial isolates were found to be phosphate solubilisers of which PB_8 and PB_9 were more efficient, and hence can be used in phosphate deficient soils after detailed field evaluation.
- Siderophore, HCN and volatile compound production by antagonistic bacteria indicate their role in inhibiting pathogens.
- Enzyme assays have suggested the presence of cellulase and Endo β-1, 4 glucanase in the selected fungi, which play a key role in the antagonistic property of these fungi. The isolates F_{90}, F_{97} and F_{65}

produced higher quantity of Endo β-1, 4 glucanase, when compared to the other isolates under study.

- *In vivo* studies indicated that the isolates that showed antagonism *in vitro* were also effective *in vivo* when introduced prophylactically. The bacterial isolate B_{54} and fungal isolate F_{65} were superior in affording protection against *C. cassiicola* and *C. acutatum* in nursery plants of clone RRII 105.

12. REFERENCES

Ahmad, F., Ahmad, I. and Khan, M. S. (2006). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research*, **163** (2): 173-181.

Alstrom, S. and Burns, R. G. (1989). Cyanide production by rhizobacteria as possible mechanism of plant growth inhibition. *Biology and Fertility of Soils*, **7**: 232-238.

Altomare, C., Norvell, W. A., Bjorkman, T. and Harman, G. E. (1999). Solubilization of phosphates and micronutrients by the plant-growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai. *Applied and Environmental Microbiology,* **65**: 2926-2933.

Anandaraj, M. and Sarma, Y. R. (1995). Disease of black pepper (*Piper nigrum* L.) and their management. *Journal of Spices and Aromatic Crops*, **41**: 17–23.

Arora, N. K., Khare, E., Verma, A. and Sahu, R. K. (2008). *In vivo* control of *Macrophomina phaseolina* by a chitinase and beta-1, 3-glucanase producing pseudomonad NDNI. *Journal Symbiosis (Rehovot)*, **46**(3): 129-135.

Arrebola, E., Sivakumar, D. and Korsten, L. (2010). Effect of volatile compounds produced by *Bacillus* strains on postharvest decay in citrus. *Biological Control*, **53** (1): 122-128.

Bagnasco, P., De-la-Fuente, L., Gualtieri, G., Noya, F. and Arias, A. (1998). Fluorescent *Pseudomonas* sp. as biocontrol agents against forage legume root pathogenic fungi. *Soil Biology and Biochemistry,* **30**: 1317-1322.

Bagyaraj, D. J. (1995). *Mycorrhizae* association in crop plants and their utilization in agriculture. In: M.C. Nair and S. Balakrishnan (eds.).

Beneficial Fungi and their Utilization. *Scientific Publishers. Jodhpur. India.* pp. 61-71.

Banik, S. and Krishnamurthy, K. V. M. (2004). Effects of phylloplane mycoflora of black gram on the growth and conidial germination of *Corynespora cassiicola* (Berk. and Curt) Wei. *Journal of Interacademicia*, **8**(1): 1-6.

Barea, J. M. and Brown, M. E. (1974). Effect on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. *Journal of Applied Bacteriology*, **37**: 583-593.

Basuki, S. (1992). Colletotrichum leaf disease of rubber in Indonesia. International Natural Rubber Conference. Abstracts, 5-8, February 1992, Bangalore, India, p. 35.

Benhamou, N. (2005). Immunocytochemistry of plant defense mechanisms induced upon microbial attack. *Microscopy Research and Technique,* **31**: 63-78.

Berg, G., Knaape, C., Ballin, G. and Seidel, D. (1994). Biological control of *Verticillium dahlia* kleb. by natural occurring rhizosphere bacteria. *Archives of Phytopathology and Plant Protection*, **29**(3): 249-262.

Berger, P. (2007). Phenolic compounds of rubber leaves and their role in resistance to *Colletotrichum gloeosporioides* and *Microcyclus ulei.* Published by SETAC and Allen Press Publishing Services.

Bhai, R. S. and Kumar, A. (2008). Effect of rhizobacteria on *Phytophthora meadii, Fusarium oxysporum* f. sp. *vanillae* and *Colletotrichum vanillae* infecting vanilla. *Journal of Biological control*, **22**(1): 33-41.

Bhakare, B. D., Shelke, S. R. and Kadam, J. R. (2008). Influence of organic inputs on the yield and economics of rabi sorghum, soil

moisture and microbial status of soil under rainfed agriculture. *Asian Journal of Soil Science*, **3**(1): 102-105.

Bier, J. E. (1963). Further effect of bark saprophytes on *Hypoxylon* canker. *Forest Science,* **9**: 263 - 269.

Bier, J. E. and Rowat, M. H., (1962). The relation of bark moisture to the development of canker diseases caused by native, facultative parasites. VII. Some effects of the saprophytes on the bark of Poplar and Willow on the incidence of Hypoxylon canker. *Canadian Journal of Botany*, **40**: 61-69.

Breton, F., Sanier, C. and d'Auzac, J. (2000). Role of cassiicolin, a host - selective toxin, in pathogenecity of *Corynespora cassiicola* causal agent of a leaf fall disease of *Hevea. Journal of Natural Rubber Research,* **3**: 115-128.

Chang, T. T. (1996). Survival of *Phellinus noxius in* soil and in the roots of dead host plants. *Phytopathology,* **86**: 272-276.

Chang, T. T. and Yang, W. W. (1998). *Phellinus noxius in* Taiwan: distribution, host plants and the pH and texture of the rhizosphere soils of infected hosts. *Mycological Research,* **102**: 1085-1088.

Chapela, I. H. (1989). Fungi in healthy stems and branches of American beech and aspen: a comparative study. *New Phytologist,* **113**: 65-75.

Chaverri, P. and Gazis, R. (2010). *Persporiopsis lateritia,* a new species on decaying leaves of *Hevea* spp. from the Amazon basin in Peru. *Mycotaxon*, **118**: 163-169.

Chee, K. H. (1988). Studies on sporulation, pathogenecity and epidemiology of *Corynespora cassiicola* on *Hevea* rubber. *Journal of Natural Rubber Research,* **3**(1): 21-29.

Chiariello, N., Hickman, J. C., and Mooney, H. A., (1988). Endomycorrhizal role in interspecific transfer of phosphorus in a community of annual plants. *Science*, **217**: 941 – 943.

Cleveland, C. C., Townsend, A. R., Schmidt, S. K. and Briana, C. C.(2003). Soil Microbial Dynamics and Biogeochemistry in Tropical Forests and Pastures, Southwestern Costa Rica. *Ecological Applications,* **13**(2): 314-326.

Coakley, S. M. and Scherm, H. (1996). Plant diseases in a changing global environment. In: Implications of "Global Environment change" for crops in Europe, Cambridge, UK (Eds. Froud Williams, R. J., Harrington, R., Hocking, J. J., Smith, H. G., Thomas, T.H.). *Aspects of Applied Biology*, **45**: 227-258.

Cook, R. J. (1993). Making greater use of introduced microorganisms for biological control of plant pathogens. *Phytopathology,* **31**: 53-80.

Cook, R. J. and Baker, K. F. (1983). The nature and practice of biological control of plant pathogens. *American Phytopathological Society,* St. Paul, Minnesota. 539pp.

Corner, E. J. H. (1932). The identification of the brown-root fungus. *The Garden Bulletin, Straits Settlement,* **5**: 317-350.

Cotter, H. T. and Blanchard, R. O. (1982). The Fungal Flora of Bark of *Fagus grandifolia. Mycologia,* **74**(5): 836-843.

Cummings, J. (1954). Antagonistic Activity of Chaetomium globosum against Fungi. *Mycologia,* **46**(3): 289-292.

Deka, H. K. and Mishra, R. R., (1984). Distribution of soil microflora in Jhum fallows in North-east India. *Acta Botanica Indica,* **12**(2): 180-184.

Deka, H. K., Philip, V., Vinod, K. K. and Krishnakumar, A. K. (1998). Spatial distribution of soil microflora in a five year old rubber plantation in Tripura. *Indian Journal of Natural Rubber Research*, **11**(1&2): 88-93.

Deka, H. K., Thapliyal, A. P., Mondal, G. C., Sinha, R. R. and Sethuraj, M. R. (1996). Occurrence of *Gleosporium alborubrum* on rubber in Meghalaya. *Indian Journal of Natural Rubber Research*, **9**(1): 55-57.

Deka, H. K., Thapaliyal, A. P., Sinha, R. R., Potty, S. N. and Sethuraj, M. R. (1992). Seasonal variation in microflora of rubber growing soils of Meghalaya. *Indian Journal of Natural Rubber Research,* **5**(1&2): 151-154.

Del Río, J. A., Báidez, A. G., Botía, J. M., and Ortuño, A. (2003). Enhancement of phenolic compounds in olive plants (*Olea europaea* L.) and their influence on resistance against *Phytophthora* sp. *Food Chemistry*, **83**(1), 75-78.

Dennis, C. and Webster, J. (1971). Antagonistic properties of species groups of *Trichoderma* I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society*, **57**: 25-29.

Dennis, C. and Webster, J. (1971). Antagonistic properties of species groups of *Trichoderma* II. Production of volatile antibiotics. *Transactions of the British Mycological Society*, **57**: 41-48.

Dhumal, K. N. and Nimbalkar, J. D. (1982). Physiological studies on grassy shoot disease infected sugarcane cultivars. *Indian Phytopathology*, **35**: 341-342.

Edathii, T. T., Jacob, C. K. and Joseph, A. (2000). Leaf diseases. *In: Natural Rubber: Agromanagement and Crop Processing.* (Eds. P. J.

George and C. Kuruvilla Jacob). *Rubber Research Institute of India, Kottayam, India,* pp. 273-296.

Egel, D. S. and Harmon, P. (2001). Effect of nozzle type and spray pressure on control of Alternaria leaf blight of musk melon with chlorothalonil. *Plant Disease,* **85**: 1081-1084.

Ellis, M. B. (1957). Some species of *Corynespora. Mycological Papers,* **65**: 1-15.

Evueh, G. A. and Ogbebor, N. O. (2008). Use of phylloplane fungi as biocontrol agent against *Colletotrichum* leaf disease of rubber (*Hevea brasiliensis* Muell. Arg.). *African Journal of Biotechnology,* **7** (15): 2569-2572.

Evueh, G. A., Okhuoya, J. A., Osemwegie, O.O., Atiitalla, I. H. and Ogebebor, O. N. (2011). Evaluation of Phylloplane Fungi as Biocontrol Agent of Corynespora Leaf Fall Disease of Rubber (*Hevea brasiliensis* Muell. Arg.). *World Journal of Fungal and Plant Biology,* **2**(1): 01-05.

Fanxiang, K. and Min, Z. (1996). Effects of copper, manganese and pH on the growth and several enzyme activities of mycorrhizal fungus *Cenococcum geophilum* Fr. *Journal of Environmental Sciences,* **8**(4): 419-425.

Fernando, T. H. P. S., Jayasinghe, C. K. and Wijesundera, R. L. C. (2000). Factors affecting spore production, germination and viability of *Colletotrichum acutatum* isolates from *Hevea brasiliensis. Mycological Research*, **104**: 681-685.

Fogarty, W. M. and Kelly, C. T. (1979). Developments in microbial extracellular enzymes. *In: Alan Wiseman (ed.), Topics in Enzyme and Fermentation Biotechnology*. Ellis Horwood Ltd., Publishers, England, **3:** 289.

Foley, M. F. and Deacon, J. W. (1986). Susceptibility of *Pythium* spp. and other fungi to antagonism by the mycoparasite *Pythium oligandrum. Soil Biology and Biochemistry,* **18**: 91-95.

Frankland, Le (1982). Biomass and nutrient recycling by decomposer basidiomycetes. In: *Decomposer basidiomycetes* (eds. I.C. Frankland, I.N. Hedger and M.I. Swift). 241-261.

Gay, P. A., Saikumar, K. V., Cleveland, T. E. and Tuzun, S. (1992). Antagonistic effect of chitinolytic bacteria against toxin producing fungi. *Phytopathology*, **82:** 1074.

Geiger, J. P., Nicole, M. and Nandris, D. (1985). The aggression of *Hevea brasiliensis* by *Rigidoporus lignosus* and *Phellinus noxius. European Journal for Pathology,* **15:** 293-300.

George, C. K. (1999). Studies on phyllosphere microflora of two clones of *Hevea brasiliensis* and their interaction with *Phytophthora meadii.* Microbiology M.Sc. Thesis submitted at University of Madras.

Gerdemann, J. W. and Nicolson, T. H. (1963). Spores of Mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, **46**: 235-244.

Giovannetti, M. (1985). Seasonal variations of vesicular-arbuscular and endogonaceous spores in maritime sand dunes. *Transactions of the British Mycological Society,* **84**: 677-684.

Girivasan, K. P. and Suryanarayanan, T. S. (2004). Intact leaves as substrate for fungi: distribution of endophytes and phylloplane fungi in rattan palms. *Czechoslovakian Mycology,* **56**(1/2): 33-43.

Gogoi, S., Bhuyan, M. K. and Karmakar, R. M. (2003). Dynamics of microbial population in tea ecosystem. *Journal of the Indian Society of Soil Science,* **51(**3): 252-257.

Grosch, R., Scherwinski, K. Lottmann, J. and Berg, G. (2006). Fungal antagonists of the plant pathogen *Rhizoctonia solani*: selection, control, efficacy and influence on the indigenous microbial community. *Mycological Research,* **110**(12): 1464-1474.

Guadarrama, P. and Alvarez-Sanchez, J. (1999). Abundance of arbuscular mycorrhizal spores in different environments in a tropical rain forest, Veracruz, Mexico. *Mycorrhiza,* **8**: 267-272.

Guleria, S. Paul, B. and Bajaj, K. L. (1997). Biochemical changes in powdery mildew (*Erysiphe polygoni* D. C.) resistant and susceptible cultivars of pea (*Pisum sativum* L.). *Plant Disease Research,***12**: 185-188.

Haas, D. and Keel, C. (2003). Regulation of antibiotic production in root colonizing *Pseudomonas* spp.and relevance for biological control of plant disease. *Annual Review of Phytopathology,* **41**: 117-153.

Handelsman, J. and Stabb, E. V. (1996). Biocontrol of soil-borne plant pathogens. *Plant Cell,* **8**: 1855-1869.

Harinikumar, K. M. and Bagyaraj, D. J. (1988). The effect of season on VA mycorrhizae of lencaena and mango in a semiarid tropic. *Arid Land Research and Management,* **2** (2): 139-143.

Hilton, R. N. (1958). Pink disease of *Hevea* caused by *Corticium salmonicolor* Berk. et Br. *Journal of the Rubber Research Institute of Malaya,* **15**: 275-292.

Hodges, C. S. (1964). The effect of competition by *Peniophora gigantea* on the growth of *Fomes annosus* in stumps and roots. *Phytopathology,* **54**: 623.

Horsfall, J. G. and Barratt, R. W. (1945). An improved grading system foı measuring plant disease. *Phytopathology,* **35**: 655.

Houlden, A., Timms Wilson, T. M., Day, M. J. and Bailey, M. J. (200 Influence of plant developmental stage on microbial community structu and activity in the rhizosphere of three field crops. *FEMS-Microbiolog Ecology,* **65**(2)**:** 193-201.

Howell, C. R. and Stipanovic, R. D. (1980). Suppression of *Pythium ultimu* induced damping-off of cotton seedlings by *Pseudomonas fluoresce* and its antibiotic pyoluteorin. *Phytopathology*, **70**: 712-715.

Ikram, A. (1989). Beneficial effects of a plant growth promoting rhizobacterium on the early growth of *Pueraria phaseoloides*. *Journal of Natural Rubber Research*, **4**(8): 219-222.

Ikram, A., Jensen, E. S., Jacobsen, I. (1994). No significant transfer of N and P from *Pueraria phaseoloides* to *Hevea brasiliensis* via hyphal links of arbuscular mycorrhiza. *Soil Biology and Biochemistry*, **26**: 1541-1547.

Ikram, A. and Mahmud, A. W. (1984). Endomycorrhizal fungi in soils under rubber. *Journal of Rubber Research Institute of Malaysia*, **32**:198-206.

Ikram, A. and Yusoff, M. (1999). Effects of compost and effective microorganisms on rubber seedling growth. *Journal of Natural Rubber Research*, **2**(1): 40-49.

Jacob, C. K. (2006a). Corynespora leaf disease of *Hevea brasiliensis*: A threat to natural rubber production. *In:Corynespora leaf disease of Hevea brasiliensis: Strategies for Management.* (Ed. C. Kuruvilla Jacob). Rubber Research Institute of India, Kottayam, India, pp. 9-16.

Jacob, C. K. (2006b). Symptoms of Corynespora leaf disease on rubber (*Hevea brasiliensis*). *In: Corynespora leaf disease of Hevea brasiliensis: Strategies for Management.* (Ed. C. Kuruvilla Jacob). Rubber Research Institute of India, Kottayam, India, pp. 17-25.

Jacob, C. K. and Edathil, T. T. (1986). New approaches of pink disease management in *Hevea*. *The Planter,* **62**: 463-467.

Jacob, C. K., Edathil, T. T., Idicula, S. P., Jayarathnam, K. and Sethuraj, M. R. (1989). Effect of abnormal leaf fall disease caused by *Phytophthora* spp. on the yield of rubber tree. *Indian Journal of Natural Rubber Research,* **2**(2): 77-80.

Jacob, C. K., Prem, E. E., Manju, M. J., Idicula, S. P. and Edathil, T. T. (2006). Crop loss due to abnormal leaf fall disease of rubber (*Hevea brasiliensis)* caused by *Phytophthora* spp. *Natural Rubber Research,* **19**(1&2): 1-8.

Jayaratnae, A. H. R. (1982). Endomycorrhiza of rubber growing soils of Sri Lanka. *Journal of the Rubber Research Institute of Sri Lanka*, **60**: 47-57.

Jayasinghe, C. K., Fernando, T. H. and Priyanka, U. M. (1997). *Colletotrichum acutatum* is the main cause of Colletotrichum leaf disease of rubber in Sri Lanka. *Mycopathologia,* **137**(1): 53-56.

Jayasuriya, K. E. and Thennakoon, B. I. (2007). Biological control of *Rigidoporus microporus,* the cause of white root disease in rubber. *Ceylon Journal of Science(Biological Sciences)*, **36**(1): 9-16.

Jayasuriya, K. E., Wijesundera, R. L. C., Jayasinghe, C. K. and Thennakoon, B. I. (1999). A comparative study of *Phytophthora meadii* isolates from rubber (*Hevea brasiliensis*) plantations in Sri Lanka. *Mycopathologia*, **147**: 125-132.

Joseph, A. (1998). Investigations on certain biochemical changes and phyllosphere microflora of *Hevea brasiliensis* as influenced by nitrogenous fertilizer application and *Corynespora cassiicola* inoculation. Ph. D. Thesis, Mahatma Gandhi University, Kottayam, India. 188 p.

Joseph, A. (2006). Toxins in pathogenesis and toxin-based screening of *Hevea brasiliensis* clones against *Corynespora cassiicola. In:Corynespora leaf disease of Hevea brasiliensis: Strategies for Management.* (Ed. C. Kuruvilla Jacob). Rubber Research Institute of India, Kottayam, India, pp. 73-82.

Joseph, K., Kothandaraman, R. and Mathew, J. (1998). Actinomycetes population of rubber growing soils and its antagonistic activity against *Phytophthora meadii. Indian Journal of Natural Rubber Research,* **1**(1) : 27-30.

Kari, E. D. and James, J. G. (2003). Seasonal Changes in Rhizosphere microbial Communities Associated with Field-Grown genetically Modified Canola (*Brassica napus*). *Applied Environmental Microbiology*, **69**(12): 7310 – 7318.

Kaur, R., Kaur, J., Singh, R. S. and Albouvette, C. (2007). Biological control of *Fusarium oxysporum* f.sp. *cicero* by nonpathogenic *Fusarium* and fluorescent *Pseudomonas. International Journal of Botany*, **3**: 114-117.

Khade, S. W. and Rodrigues, B. F. (2004). Populations of arbuscular mycorrhizal fungi associated with rhizosphere of banana (*Musa* sp.) as influenced by seasons. *Mycorrhiza News,* **16**: 11-13.

Kiss, L. (2003). A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. *Pest Management Science,* **59**: 475-483.

Kjoller, A. H., Struwe, S. (1982). Microfungi in ecosystems, fungal occurrence and activity in litter and soil. *Oikos*, **39**: 389-422.

Kleifeld, O. and Chet, I. (1992). *Trichoderma harzianum* interactions with plants and effect on growth response. *Plant and Soil*, **144** (2): 267-272.

Kothandaraman, R. (1984). Studies on certain biochemical and physiological changes and phyllosphere microflora of rice as influenced by potassium fertilization and *Pyricularia oryzae* inoculation. *Doctoral thesis submitted to Annamalai University, Tamil Nadu , India.*

Kothandaraman, R., Mathew, J., Krishnakumar, A. K., Joseph, K. and Sethuraj, M. R. (1989). Comparative efficiency of *Mucuna bracteata* D. C. and *Pueraria phaseoloides* Benth. on soil nutrient enrichment, microbial population and growth of *Hevea. Indian Journal of Natural Rubber Research*, **2**(2): 147-150.

Krebs, B., Hoding, B., Kubart, S. M., Workie, A., Junge, H., Schmiedeknecht, G., Grosch, R., Bochow, H. and Hevesi, M. (1998). Use of *Bacillus subtilis* as biocontrol agent. 1. Activities and characterization of *Bacillus subtilis* strain. *Journal of Plant Disease Protection,* **105**: 181-197.

Krishnakumar, A. K., Gupta, C., Sinha, R. R., Sethuraj, M. R., Potty, S. N., Eapen, T. and Das, K. (1991). Ecological impact of rubber (*Hevea brasiliensis*) plantation North East India. 2. Soil properties and biomass recycling. *Indian Journal of Natural Rubber Research,* **4**(2): 134 -141.

Kumar, G. S. (2002). Seasonal variations in the biodiversity of Arbuscular Mycorrizal fungi in forest ecosystems. *Journal of Ecobiology,* **14**: 35-38.

Kumar, B., Dubey, R. C. and Maheshwari, D. K. (2005). Biocontrol of *Macrophomina phaseolina*: Prospects and Constraints, in Microbial diversity: Current perspectives and Potential applications. Ed. T. Sathyanarayana and B. N. Johri. pp.471-492.

Laiho, O. (1970). *Paxillus involutus* as a mycorrhizal symbiont of forest trees. *Acta Forestalia Fennica,* **106***:* 1-73.

Lattanzio, V., Lattanzio, V. M. T. and Cardinali, A. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: *Phytochemistry: Advances in Research :* (ed. F. Imperato), pp.23-67.

Leben, C. (1969). Colonization of soyabean by bacteria: Observation with SEM. *Canadian Journal of Microbiology,* **15**: 319-320.

Lekshman, H. C., Inchal, R. F. and Mulla, F. I. (2006). Seasonal fluctuations of arbuscular mycorrhizal fungi on some commonly cultivated crops of Dharwad. *In: Mycorrhiza*, (Eds.) Anil Prakash and V. S. Mehrotra. Scientific Publishers (India), Jodhpur, pp. 11-19.

Licy, J. (1979). Variability, correlations and heterosis for yield and yield components in certain hybrid clones of the para rubber tree, *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. Ph. D. Thesis, University of Kerala, India, 158 p.

Liu WeiWei., Mu Wei., Zhu Bing Yu., Du You Chen and Liu Feng (2008). Antagonistic activities of volatiles from four strains of *Bacillus* spp.and *Paenibacillus* spp. against soil-borne plant pathogens. *Agricultural Sciences in China*, **7**(9): 1104-1114.

Liyanage, N. I. S. and Wheeler, B. E. J. (1989). Comparative morphology of *Phytophthora* species on rubber. *Plant Pathology*, **38**(4): 592-597.

Longa, C. M. O., Pertot, I. and Tosi, S. (2008). Ecophysiological requirements and survival of a *Trichoderma atroviride* isolate with biocontrol potential. *Journal of Basic Microbiology*, **48**: 269-277.

Lorck, H. (1948). Production of hydrocyanic acid by bacteria. *Physilogia Plantarum*, **1:** 142-146.

Loyter, A., Rosenbluh, J., Zakai, N., Li, J., Kozlovsky, S. V., Tzfira, T. and Citovsky, V. (2005). The plant Vir E_2 Interacting Protein 1. A Molecular Link between the Agrobacterium T- complex and the Host Cell Chromatin? *Plant Physiology,* **138**: 1318-1321.

Mackie, A. E. and Wheatley, R. E. (1999). Effects and incidence of volatile organic compound interactions between soil bacterial and fungal isolates. *Soil Bilogical Biochemistry*, **31**: 375-85.

Mandeel, Q. and Baker, R. (1991). Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum. Phytopathology,* **81**: 462-469.

Manju, M. J., Idicula, S. P., Jacob, C. K., Vinod, K. K., Prem, E. E., Suryakumar, M. and Kothandaraman, R. (2001). Incidence and severity of Corynespora Leaf Fall (CLF) disease of rubber in coastal Karnataka and north Malabar region of Kerala. *Indian Journal of Natural Rubber Research*, **14** (2): 137-141.

Manju, M. J., Idicula, S. P., Joseph, A., Joy, M. and Kothandaraman, R. (1999). Incidence and severity of *Gleosporium* leaf disease of rubber in South India. *Indian Journal of Natural Rubber Research*, **12**(1 &2): 34 -38.

Martin, J. K. and Kemp, J. R. (1980). Carbon loss from roots of wheat cultivars. *Soil Biology and Biochemistry*, **12:** 551-554.

Mathew, T. and Jacob, C. K. (2004). Microflora associated with phylloplane, cauloplane and rhizosphere of two popular rubber clones at two locations in India. *Natural Rubber Research*, **17**(1): 47-52.

Mc Rae, W. (1918). *Phytophthora meadii* n. sp. on *Hevea brasiliensis Memoirs of the Department of Agriculture in India, Botanical Series,* **9**: 219-273.

Mello, S. C. M., Estevanato, C. E., Brauna, L. M., Capdeville, G. de, Queiroz, P. R. and Lima, L. H. C. (2008). Antagonistic process of *Dicyma pulvinata* against *Fusicladium macrosporium* on rubber tree. *Tropical Plant Pathology*, **33**(1): 5-11.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry,* **31**: 426-428.

Mishustin, E. N. and Naumova, A. N. (1962). Bacterial fertilizers, their effectiveness and mode of action. *Microbiology*, **31**: 442-452.

Mohankumar, V., Ragupathy, S., Nirmala, C. B. and Mahadevan, A. (1988). Distribution of vesicular arbuscular mycorrhizae (VAM) in the sandy beach soils of Madras Coast. *Current Science,* **57**: 367 – 368.

Morgan-Jones, G. (1988). Notes on hyphomycetes. LX. *Corynespora matuszakii*, an undescribed species with narrow cylindrical, catenate conidia and highly reduced conidial cell lumina. *Mycotaxon,* **33**: 483-487.

Mugnai, L., Bertelli, E., Surico, G. and Bruna, E. (1997). Effect of some phenolic compounds on the growth of wood-inhabiting fungi associated with "esca disease" of grape wine. *Petria*, **7**: 35-46.

Mukerji, K. G. and Subba Rao, N. S. (1982). Plant surface microflora and plant nutrition. In: *Advances in Agricultural Microbiology* (Ed. N. S. Subba Rao), pp. 111 – 138.

Naik, P. R., Sahoo, N., Goswami, D., Ayyadurai, N. and Sakthivel, N. (2008). Genetic and functional diversity among fluorescent pseudomonads isolated from the rhizosphere of banana. *Microbial Ecology*, **56**(3): 492-504.

Nakkeeran, S., Dilantha Fernando, W. G. and Siddiqui, Z. A. (2005). Chapter 10. Plant growth promoting rhizobacteria formulations and

its scope in commercialization for the management of Pests and diseases, in *PGPR: Biocontrol and Biofertilization* ed. Z. A. Siddiqui. Springer, Dordrecht, The Netherlands. pp. 257-296.

Nandris, D., Nicole, M. and Geiger, J.P. (1983). Artificial infections of *Hevea brasiliensis* seedlings by *Rigidoporus lignosus* and *Phellinus noxius. European Journal for Pathology*, **13:** 65-76.

Nandris, D., Nicole, M. and Geiger, J.P. (1987). Root rot diseases of rubber trees. *Plant disease*, **71:** 298-306.

Nazeer, M. A., Markose, V. C., George, P. J. and Panikkar, A. O. N. (1986). Performance of a few Hevea clones from RRII 100 series in large scale trial. *Journal of Plantation Crops,* **14:** 99-104.

Neetu, S., Pandey, P., Dubey, R. C. and Maheshwari, D. K. (2008). Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1. *World Journal of Microbiology and Biotechnology,* **24**(9)**:** 1669-1679.

Nicole, M., Geiger, J. P. and Nadris, D. (1987). Ultrastructural aspects of rubber tree root rot diseases. *European Journal of Forest Pathology*, **17:** 1-11.

Nix-Stohr, S., Burpee, L. L. and Buck, J. W. (2008). The influence of exogenous nutrients on the abundance of yeasts on the phylloplane of turf grass. *Microbial Ecology*, **55**(1): 15-20.

Obire, O. and Wemedo, S. A. (2002). Seasonal Effect on the Bacterial and Fungal population of an oil field, waste water- polluted soil in Nigeria. *Journal of Applied Sciences and Environmental Management,* **6**(2): 17-21.

Ogbebor, N. O., Adekunle, A. T., Eghafona, N. O. and Ogboghodo, A. I. (2010). *Ganoderma psuedoferreum*: Biological control possibilities with microorganisms isolated from soils of rubber plantations in Nigeria. *African Journal of General Agriculture*, **6** (4): 301-305.

Oke, O. A. (1988). Changes in Chemical Constituents of Tobacco leaves infected with *Corynespora cassiicola* and *Colletotrichum nicotianae*. *Journal of Phytopathology,* **122**: (181-185).

Onesirosan, P., Arny, D. C. and Durbin, R. D. (1975). Increasing sporulation of *Corynespora cassiicola*. *Mycopathologia*, **55**: 121-123.

Osbourn, A. E. (1996). Preformed antimicrobial compounds and plant defense against fungal attack. *Plant Cell*, **I8**:1821-1831.

Pandey, A., Paini, L. M. (1997). *Bacillus* species: the dominant bacteria of the rhizosphere of established tea bushes. *Microbiological Research*, **152**(4): 359-365.

Pandey, A., Palni, L. M. and Bisht, D. (2001). Dominant fungi in the rhizosphere of established tea bushes and their interaction with the dominant bacteria under in situ conditions. *Microbiological Research*, **156**(4): 377-382.

Parupus, E. V. (1967). Effect of Various Plant Phenols on Protein synthesis in excised plant tissues. *Biochemistry and Cell Biology,* **45**(4): 427-434.

Pathiratna, L. S. S., Perera, M. K. P. and Balasooriya, C. K. (2007). Cinnamon (*Cinnamomum verum* J. Pres) intercropped under different inter row spacings of rubber (*Hevea brasiliensis* Muell. Arg.). Performance after rubber reached maturity. *Journal of the Rubber Research Institute of Sri Lanka*, **88**: 30-46.

Pendleton, R. L. and Smith, B. N. (1983). Vesicular- arbuscular mycorrhizae of weedy and colonizer plant species at distributed sites in Utah. *Oecologia,* **59**: 296 – 301.

Persiani, A. M., Maggi, O., Casado, M. A. and Pineda, F. D. (1998). Diversity and variability in soil fungi from a disturbed tropical rain forest. *Mycologia,* **90**(2): 206-214.

Pesenti-Barili, B., Ferdani, E., Mosti, M. and Degli-Innocenti, F. (1991). Survival of *Agrobacterium radiobacter* K84 on Various Carriers for Crown gall Control. *Applied and Environmental Microbiology,* **57**(7): 2047-2051.

Philip, A., Philip, V., George, E. S., Punnoose, K. I. and Mathew, M. (2003). Leaf litter decomposition and nutrient release in a fifteen year old rubber plantation. *Indian Journal of Natural Rubber Research,* **16**(1& 2): 81-84.

Philip, T., Sharma, D. D. and Shivapratap, H. R. (1996). Environmental Toxicology and Chemistry Antagonism against Colletotrichum. Central Sericultural Research and Training Institute, Mysore, 570 008, *INDE Article*: pp. 2455–2460.

Pridham, J. B. (1965). Low Molecular Weight Phenols in Higher Plants. *Annual Review of Plant Physiology,* **16**: 13-36.

Ragupathy, S. and Mahadevan, A. (1993). Distribution of vesicular-arbuscular mycorrhizae in the plants and rhizosphere soils of the tropical plains. Tamil Nadu, India. *Mycorrhiza*, **3**: 123-136.

Rajalakshmi, V. K. and Jayarathnam, K. (2000). Root disease and non microbial maladies. *In: Natural Rubber :Agromanagement and Crop Processing.*(Eds. P. J. George and C. Kuruvilla). Rubber Research Institute of India, Kottayam, India. pp. 309-324.

Rajalakshmi, V. K. and Pillay, P. N. R. (1975). Nectar stage of *Corticium salmonicolor (Pellicularia salmonicolor)* in culture. *Indian Phytopathology*, **28:** 112-113.

Ramakrishnan, T. S. and Pillay, P. N. R. (1962). Secondary leaf fall caused by *Glomarella cingulate* (Stonem) S & VS *Gleosporiium alborubrum* petoh). *Rubber Board Bulletin,* **6** (2): 72-78.

Ramakrishnan, T. S. and Pillay, P. N. R. (1962). Pink disease of rubber caused by *Pellicularia salmonicolor* (Berk and Br.) Dastur (*Corticium salmonicolor* Berk and Br.). *Rubber Board Bulletin,* **5** (3): 120-126.

Rao, P. B. and Mullaiah, K. V. (1988). Effect of phylloplane fungi on the leaf spot pathogen *Cercospora canasens. Indian Journal of Microbiology*, **2**(1 &2): 103 – 107.

Rinzo, S. and Kenji, K. (1980). Occurrence of Soybean Root Rot caused by *Corynespora cassiicola* (Berk. &Curt.) Wei in Hokkaido. *Annals of Phytopathological Society of Japan,* **46** : 193-199

Romero, D., Perez-Garcia, A., Rivera, M. E., Cazorla, F. M. and de Vicente, A. (2004). Isolation and evaluation of antagonistic bacteria towards the cucurbit powdery mildew fungus *Podosphaera fusca. Applied Microbiology and Biotechnology*, **64**: 2.

Ruinen, J. (1956). Occurrence of *Beijerinckia* spp. in the Phyllosphere. *Nature*, **177**: 220 -221.

Saha, T., Kumar, A., Ravindran, M., Jacob, C. K., Roy, C. B and Nazeer, M. A. (2002). Identification of *Colletotrichum acutatum* using random amplified polymorphic DNAs and ribosomal DNA polymorphisms. *Mycological Research*, **106**(2): 215-221.

Sankpal, S. D. and Nimbalkar, J. D. (1979). Effect of smut infection on sucrose metabolism in sugarcane. *Indian Phytopathology,* **32**: 306-307.

Senevirathna, A. M. W. K., Stirling, C. M., Rodrigo, V. H. L., Pathirana, P. D. and Karunathilake, P. K. W. (2010). High density banana/rubber intercrops have no negative effects on component crops under smallholder conditions. *Journal of the Rubber Research Institute of Sri Lanka*, **90**: 1-17.

Shapira, R., Ordentlich, A., Chet, I. and Oppenheim, A. B. (1989). Control of plant diseases by chitinases expressed from cloned DNA in *Escherichia coli. Phytopathology*, **79:** 1246 – 1249.

Sharma, I. (2004). Phylloplane microfungi of sugarcane. *Indian Journal of Microbiology*, **44**(2): 113-115.

Sharma, J. K., Mohanan, C. and Maria Florence, E. J. (1984). Outbreak of pink disease caused by *Corticium salmonicolor* in *Eucalyptus grandis* in Kerala, India. *International Journal of Pest Management,* **30**(3): 253-255.

Singh, J., Singh, D. K. (2005). Bacterial, Azotobacter, Actinomycetes, and Fungal Population in Soil after Diazinon, Imidacloprid, and Lindane Treatments in Groundnut (*Arachis hypogaea* L.) Fields. *Journal of Environmental Science and Health*, **40** (5): 785 – 800.

Singh, K. and Verma, A. (1981). Endogonaceous spores associated with xerophytic plants in northern India. *Transactions of the British Mycological Society,* **77**: 655-658.

Smith , W. H. (1976). Character and Significance of Forest Tree Root Exudates. *Ecology*, **57**: 324-331.

Soderberg, K. H. and Baath, E. (1998). Bacterial activity along a young barley root measured by the thymedicine and leucine incorporation techniques. *Soil Biology and Biochemistry*, **30**: 1259-1268.

Sripathi Rao, B. (1975). Maladies of *Hevea* in Malaysia. Kuala Lumpur, Rubber Research Institute of Malaysia, 108p.

Stavely, J. R. and Chaplin, J. F. (1972). Effect of *Cercospora nicotianae* on four major chemical constituents in cured tobacco leaves. *Phytopathology*, **7:**1392-1397.

Stephen, J. and Jisha, M. S. (2008). Buffering Reduces Phosphate Solubilizing Ability of Selected Strains of Bacteria. *American-Eurasian Journal of Agriculture and Environmental Sciences,* **4** (1): 110-112

Sudirman, L., Iraqi Housseini, A. I., Le Febvre, G., Kiffer, E. and Botton, B. (1992) Screening of some basidiomycetes for biocontrol of *Rigidoporus lignosus,* a parasite of the rubber tree *Hevea brasiliensis. Mycological Research,* **96**(8): 621-625.

Sulton, B. C. and Pascoe, I. C., (1988). Some dematiaceon hyphomycetes from branches and phyllodes on Acacia in Australia. *Australian Systematic Botany*, **1:** 127-138.

Suzuki, A., Uchida, M. and Kita, Y. (2002). Experimental analyses of successive occurrence of ammonia fungi in the field. *In: Fungal Succession* (eds. K. D. Hyde and E. B. G. Jones). *Fungal Diversity*, **10**: 139-163.

Taber, R. A. and Trappe, J. M. (1982). Vesicular arbuscular mycorrhiza in rhizomes, scale- like leaves, roots and xylem of ginger. *Mycologia*, **74**: 156 – 161.

Trappe, J. M. (1987). Phylogenetic and ecological aspects of mycotrophy in the angiosperms from an evolutionary stand point. In; G.R.Safir (ed.).*Ecophysiology of VA Mycorrhizal plants.* CRC Press. Boca Raton. Florida. pp. 5 – 25.

Tu, J. C. (2008). Antibiosis of *Streptomyces grisieus* against *Colletotrichum lindemuthianum. Journal of Phytopathology,* **121**(2): 97-102.

Ubalua, A. O. and Oti, E. (2007). Antagonistic properties of *Trichoderma viride* on post harvest cassava root rot pathogens. *African Journal of Biotechnology*, **6**(21): 2447-2450.

Vanitha, S., Jacob, C. K., and Jayarathnam, K. (1994). *In vitro* studies on biological control of *Phytophthora meadii* using *Trichoderma* spp. *IRRDB Symposium on Diseases of Hevea,* 21-22 November 1994, Cochin, India, pp. 78-81.

Velmurugan, N., Choi, M. S., Han, S. S. and Lee, Y. S. (2009). Evaluation of antagonistic activities of *Bacillus subtilis* and *Bacillus licheniformis* against wood staining fungi: *in vitro* and *in vivo* experiments. *Journal of Microbiology*, **47**(4): 385-302.

Venkataram, C. S. (1960). Foliar application of nutrients and rhizosphere microflora of *Camellia sinensis. Nature,* **187**: 621-622.

Vieira, H. B. A., Romeiro, R. S., Mounteer, A., Mizubuti, E. S. G. (2008). Efficiency of phylloplane bacteria in controlling aerial tomato diseases under field conditions. *Summa Phytopathologica,* **34**(1): 86-87.

Vijayakumar S. K., Rajesh, K. M., Mendon, R. and Haricharan, V. (2000). Seasonal distribution and behavior of nutrients with reference to tidal rhythm in the Mulki estuary, South West Coast of India. *Journal of Marine Biological Association of India,* **42:** 21-31.

Vrany, J. and Fiker, A. (1984). Growth and yield of potato plants inoculated with rhizosphere bacteria. *Folia Microbiologica*, **29**(3): 248-253.

Waller, J. M. and Masaba, D. M. (2006). The microflora of coffee surfaces and relationships to coffee berry disease. *International Journal of Pest Management*, **52**(2): 89-96.

Wardle, D. A., Yeates, G. W., Nicholson, K. S., Bonner, K. I. and Watson, R. N. (1999). Response of soil microbial biomass dynamics, activity and plant litter decomposition to agricultural intensification over a seven-year period. *Soil Biology and Biochemistry,* **31**(12): 1707-1720.

Wastie, R. L. (1965). The occurrence of an Endogone type of endotrophic mycorrhiza in *Hevea brasiliensis*. *Transactions of the British Mycological Society,* **48**: 167-168.

Wei, C. T. (1950). Notes on *Corynespora. Mycological papers*, **34**: 1-10

Wheatley, R. E. (2002). The consequences of volatile organic compounds mediated bacterial and fungal interactions. *Antonie van Leeuwenhoek*, **81**: 357-364.

Wilson, M. and Lindow, S. E. (1993). Effect of phenotypic plasticity on epiphytic survival and colonization by Pseudomonas syringae. *Applied Environmental Microbiology*, **59**: 410-416.

Yang, S. S., Fan, H. Y., Yang, C. K. and Lin, I. C. (2003). Microbial Population of Spruce Soil in Tatachia Mountain of Taiwan. *Chemosphere*, **52**: 1489-1498.

Yuan, W. M. and Crawford, D. L. (1995). Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root

and seed rots. *Applied Environmental Microbiology,* **61**(8): 3119-3128.

Zachow, C., Tilcher R. and Berg, G. (2008). Sugar beet-associated bacterial and fungal communities show a high indigenous antagonistic potential against plant pathogens. *Microbial Ecology*, **55**(1): 119-129.

Zainol, E., Mahmud, A. W. and Sudin, M. N. (1993). Effects of Intercropping Systems on Surface Processes in an Acid Ultisol 2. Changes in Soil Chemical Properties and Their Influence on Crop Performance. *Journal of Natural Rubber Research*, **8**: 124-136.

Zheng, F. C. and Ward E. (1998). Variation Within and between *Phytophthora* species from Rubber and Citrus Trees in China, Determined by Polymerase Chain Reaction Using RAPDs. *Journal of Phytopathology*, **146** (2-3): 103-109.

Zhihe, C., Qingping, W., Lihong, X., Xiaoyan, Z. and Jumei, Z. (1998). Advance of biocontrol of *Trichoderma* and *Gliocladium. Journal of Microbiology*, **25**(5): 284-286.

13. APPENDICES

13.1. Leben's medium

The composition of the basal medium was as follows:

Peptone	-	10.0g
Casein hydrolysate	-	1.0g
Glucose	-	5.0g
Agar	-	20.0g
Distilled water :	-	1000ml
pH	-	6.5 - 6.8

13.2. Soil extract agar (SEA)

Soil extract	-	100 ml
K_2HPO_4	-	0.5g
Glucose	-	1.0g
Agar	-	15.0 g
Distilled water	-	900 ml
pH	-	7 - 7.2

Soil extract was prepared by autoclaving 1000g of soil in 1000ml water fo one hour and filtering it through a filter paper. 100ml of this was used in th preparation of SEA.

Rose Bengal agar (RBA)

Dextrose (Glucose)	-	10.0 g
Peptone	-	5.0 g
K_2HPO_4	-	1.0 g
$MgSO_4(7H_2O)$	-	0.5 g
Rose Bengal	-	30.0 mg
Streptomycin (1% sol)	-	0.3 ml/100ml (added just before use)
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	5.5

13.3. Apetite agar (AA)

Yeast extract	-	0.2 g
$(NH_4)_2 SO_4$	-	0.5g
$MgSO_4(7H_2O)$	-	0.1g
KCl	-	0.2g
Glucose	-	10.0g
Soil extract	-	200ml
K_2HPO_4 (10%)	-	6 ml/100ml
$CaCl_2$ (10%)	-	4ml/100ml
Distilled water	-	800 ml
Agar	-	15 gm

K_2HPO_4 and $CaCl_2$ were prepared and sterilized separately and added to the medium just prior to plating. Soil extract was prepared as explained for SEA.

13.4. Ken Knight agar (KA)

K_2HPO_4	-	1.0 g
$NaNO_3$	-	0.1 g
KCl	-	0.1 g
$MgSO_4$ ($7H_2O$)	-	0.1 g
Cellulose source		
(Filter paper strips)	-	10.0 mg
Distilled water	-	1000 ml
pH	-	7 to 7.2

13.5. Jensen's agar(JA)

Sucrose	-	20.0 g
$K_2H\ PO_4$	-	1.0 g
$MgSO_4$ ($7H_2O$)	-	0.5 g
$Fe\ SO_4$ ($7H_2O$)	-	0.1 g
$CaCO_3$	-	2.0 g
NaCl	-	0.5 g
Na_2MoO_4	-	0.005 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	6.5 - 7.0

13.6. King's B medium

Peptone	-	20g
$MgSO_4$	-	6g
K_2HPO_4	-	2.5g
Glycerol	-	15ml
Agar	-	18g
Distilled water	-	1000ml
pH	-	6.8 – 7

13.7. Potato dextrose agar (PDA)

Peeled potato	-	250 g
Glucose	-	20 g
Agar	-	15 g
Distilled water	-	Enough to make up to 1litre
pH	-	6 - 6.5

Peeled potatoes were cut into small pieces and boiled for half an hour. The extract was strained and collected and was made up to 1 litre with distilled water. Into this, agar and dextrose were added and heated again in a water bath for 15 to 20 min until the agar was fully melted. This was poured into conical flasks or into test tube and plugged with cotton and sterilized in autoclave at 121^0C for 30min. After taking out, the test tubes were kept in a slanting position until the medium solidified and were then stored.

13.8. Nutrient agar (NA)

Peptone	-	5.0 g
Glucose	-	5.0 g
Beaf extract	-	3.0 g
NaCl	-	0.5 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	6.8 to 7

All the components were dissolved one by one in distilled water. This was then sterilized in conical flasks or made into slants.

13.9. Czapek's medium

KH_2P0_4	-	1 g
$NaNO_3$	-	2 g
$MgS0_4.7H_2O$	-	0.5 g
KCl	-	0.5 g
$FeSO_4.7H_2O$	-	0.01 g
Carboxy Methyl Cellulose	-	10 g
Distilled water	-	1 L
pH	-	6.8 - 7