MICROBIOLOGICAL STUDIES IN RELATION TO HIGH DENSITY MULTI-SPECIES CROPPING SYSTEM IN COCONUT

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CERTIFICATE

I hereby certify that this thesis entitled "Microbiological studies in relation to high density multispecies cropping system in coconut" embodies the results of bonafide research work done by Shri B.M. Bopaiah for the degree of Doctor of Philosophy of the University of Mysore, under my guidance and direct supervision. I further certify that this thesis or part thereof has not previously been formed the basis for the award of any degree, diploma, associateship, fellowship or other similar award.

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DECLARATION

I do hereby declare that the thesis entitled "Microbiological studies in relation to high density multispecies cropping system in coconut" is the result of the work carried out by me at the Central Plantation Crops Research Institute, Regional Station, Vittal, under the guidance of Dr. N. Shekara Shetty, Chairman, Department of Applied Botany, University of Mysore. Further, I declare that this work has not formed the basis of the award of any degree or fellowship previously.

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(B.M. BOPAIAH)

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INTRODUCTION

INTRODUCTION

The coconut palm (*Cocos nucifera* L.) has through the years acquired many eulogistic names such as "The tree of the Heaven" (Kalpa Vriksha) indicating the usefulness of the crop. The area under coconut cultivation in India is about 1.2 million hectares with the production of 6887 million nuts*. This is essentially a crop of the small growers and about 98% of the holdings are below 2 ha (Thampan, 1976). In coconut, planting is done at 7.5m x 7.5m spacing, the lateral spread of roots are not beyond 2m from the bole and the vertical distribution is 0-120 cm (Kushwah *et al*., 1973). On surface area basis, the area occupied by the palm is 56.25 m^2 (7.5m x 7.5m), the area at active root zone is 12.57 m^2 (πr^2 where r=2m). Therefore, the fraction of total area effectively utilised by the palm is 22.24%. Thus, in pure stand of coconut about 78% of the total area is not being effectively utilised.

Taking an overall view of the growth habit of the palm, the pattern of its utilisation of soil and solar energy and of the interception of the light by its canopy, the analysis reveals that the coconut palm is amenable to intensive cropping at most periods of its life and great

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* Source: Directorate of Economics and Statistics, Government of India (1985-86).

possibilities do exist for increasing agricultural production. The intensive cropping systems involving coconut are essentially crop combinations which envisage the cultivation of other compatible crops in the interspaces between the palms. The crops chosen should create only minimum problem of soil exhaustion, pest build-up, while ensuring at the same time maximum production per unit input.

Several intercrops such as roots and tubers (cassava, elephant foot yam and sweet potato), rhizome species (turmeric and ginger), grain legumes (green gram, black gram, horse gram, cowpea and soybean), fodder crops (fodder legumes, napier and guinea grass), plantation crops (cacao, clove, nutmeg, cinnamon, pepper and Robusta coffee) and horticultural crops (Banana, sapota and citrus) could be successfully grown in coconut gardens (Nelliat and Shama Bhat, 1979). Cacao is a relatively new plantation crop introduced to India (Bhat and Bavappa, 1972). Planting of cacao in coconut plantation as mixed crop is being practiced in other coconut growing countries, such as Philippines (Rodrigo and Mangbat, 1964), Malaysia (Kin, 1968; Blencowe, 1969) and Papua New Guinea (Wood, 1975).

The term 'multistoreyed cropping' is used to refer to multispecies crop combinations involving both annual and perennials with an existing strand of perennials (Nelliat, et al. 1974; Nair, 1977). The crops having different status (canopy size) and rooting pattern are selected to have the compatible combinations. Mixed farming involves cultivation of fodder grasses in the interspaces of coconut palms, maintenance of milch animals and recycling the cattle manure in the coconut-fodder mixed stand.

The nature and the activity of microflora and fauna in a given environment depends upon the crops grown and the management practices followed. The nature of micro-organisms associated with perennials like tree crops are almost constant but the introduction of other annual or perennials could change the equilibrium and this in turn could be either beneficial or detrimental for the crop community. The activities of the rhizosphere and root surface microorganisms can affect the nutrient uptake (Bowen and Rovira, 1968) and wide range of organic compounds known to be present in the rhizosphere can influence the growth of the plant (Rovira, 1965a; Stevenson, 1967; Subba Rao, *et al*., 1961). The greater root volume of the crops per unit volume of soil adds more organic matter by way of dead roots. The fallen leaves provide the organic substrate for the growth of microorganisms.

The interaction between the neighbouring plants, however, need not always produce a competition but may also involve the action of biologically active plant exudates and the transfer of microbially fixed nitrogen. The biological interaction involving microorganisms, insect pests and plant pathogens are of considerable importance in crop combinations. Due to the enormous pressure on the better utilisation of land, in more recent years research work is directed towards identifying suitable crop combinations in the cropping system. Based on the growth and yield of multistoreyed cropping (coconut + black pepper + cacao + pineapple or coconut + black pepper + cinnamon + pineapple) and the mixed farming system (coconut + black pepper + fodder grass + napier grass + pusa gaint) it has been shown that these crops are compatible and increase the yield of coconut as compared to coconut monocropping (Appendix Ia and Ib).

The concepts of multistoreyed cropping has been experimented with regard to interspaces competition for moisture, nutrients, solar energy and soil fertility (Nelliat, *et al*., 1974; Varghese *et al*., 1978). In addition to the compatibility, the additional synergistic effect of increase in yield of palm is an excellent example of 'non-monetary' input in crop production. The possible components of non-monetary inputs are worth examining. Therefore, it is also essential to understand the long term effect of multistoreyed or mixed farming on the biological activities of the soil, as ultimately these parameters affect the overall fertility and productivity of the soil.

The soil microflora of coconut and coconut based mixed farming system has been studied by a few workers under root (wilt) disease conditions (Radha and Menon, 1954; Potty *et al*., 1977). Nair and Subba Rao (1977a) studied the microflora and specific biological attributes in the rhizosphere of coconut under mixed cropping with cacao. But no work has been attempted on the coconut based multistoreyed cropping system. With this in view, studies were envisaged to examine the effect of cropping system on the dynamics of rhizosphere and root region microorganisms, soil fertility and biological productivity. The present study includes the following aspects:

1. The influence of multistoreyed cropping and mixed farming in coconut on the dynamics of microorganisms and soil biological activities.
2. The effects of coconut based multistoreyed cropping system, mixed farming system and coconut monocropping on soil fertility parameters.
3. The occurrence and the activities of the rhizoplane and rhizosphere microorganisms of the coconut based multistoreyed cropping, mixed farming and coconut monocropping systems.
4. The antagonism of rhizosphere and rhizoplane microorganisms against *Phytophthora arecae* and *Phytophthora palmivora* *in vitro*.

5. Biochemical characterisation of root exudates of coconut and soils of different coconut based cropping systems.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The term "rhizosphere" was coined by the German Scientist Hiltner (1904) to denote the region of the soil which is subjected to the influence of plant root. Since then, the studies on rhizosphere have progressed along several important and interesting lines (Starkey, 1929a; 1929b; 1931; 1938; Lochhead, 1940, 1952; Katznelson, 1946, 1965; Katznelson et al., 1948; Clark, 1969; Alexander, 1961; Subba Rao, et al., 1961; Lakshmikumari, 1964; Games et al. 1969; Stotzky, 1972; Ayanaba and Omayali, 1975; Bowen and Rovira, 1976; Odunfa and Uso, 1979; Doran, 1980a, 1980b; Wahua, 1984; and Talukdar et al., 1985).

The nature and activity of microflora and fauna in a given environment depend upon the crops grown and the management practices (Clark, 1949). The nature of microorganisms associated with perennials such as tree crops appears to be almost constant, but the introduction of other annuals or perennials could change the equilibrium. This could be either beneficial or detrimental to the crop community. Biological interactions involving microorganisms, insect pest and plant pathogens are of considerable importance in crop combination. The rhizosphere is characterised by greater microbiological activities than the soil away from plant roots. The activity of rhizosphere and rhizoplane microorganisms can affect the nutrient uptake (Rovira, 1965a; Bowen and Rovira, 1968; Nair and Subba Rao, 1974; Tinker, 1984) and the wide range of organic compounds known to be present in the rhizosphere can influence the growth of the plant (Rovira, 1965b; Tinsley and Darbyshire, 1984).

Microorganisms grow where the supply of organic matter is adequate and environmental conditions are favourable and in the rhizosphere also, their growth on roots is controlled principally by the supply of organic matter. The sloughed off root caps and root hairs supply considerable substrates for the growth of microorganisms. The root exudates also provide substrate for the growth of microorganisms in the root region of the crops. Several factors such as soil type, soil moisture, soil pH, temperature, fertiliser application, mode of cultivation, environmental factors, age and condition of the plants are known to influence the rhizosphere microorganisms (Rovira, 1965b; Katznelson and Rouatt, 1957; Rodica *et al*., 1969; Raminath and Rangaswami, 1971; Shetty and Rangaswami, 1970; Subba Rao, 1977).

It is now clearly established that bacteria, fungi and actinomycetes are more in the rhizosphere than in non-rhizosphere soil (Lakshmikumari, 1964; Abdel-Nasser and Makawi, 1977; Nair and Subba Rao, 1977a; Mukerji and Chattopadhyay, 1979; Talukdar, *et al.*, 1988).

The metabolic activities of the rhizosphere micro-organisms are different from those of the non-rhizosphere soil microorganisms (Katznelson and Stevenson, 1956; Katznelson and Rouatt, 1957; Subba Rao, *et al.*, 1961).

The nature of the crops and its mode of cultivation, either as inter or mixed crop also influence considerably the rhizosphere microflora population. Rodica *et al.* (1969) reported that the microflora increased in the rhizosphere of corn as compared to wheat over a period of time. Domsch (1969) suggested that repeated cropping with the same plant results in an increase in plant residue in the soil, which may help in build up of specific dominance within the rhizosphere microflora. Mishra and Srivastava (1971) reported that the population of bacteria and fungi in the rhizosphere of *Hordeum vulgare* L., *Pisum sativum* L., *Linum usitatissimum* L. and *Brassica nigra* L. decreased with increase in soil depth.

Dudchenko *et al.* (1973) studied the species composition of microflora in rotation and continuous seeding of perennial grasses and clean cultivated crops. They found that microbial composition varied within the range of 35 to 40 species. *Pseudomonas* sp. prevailed under perennial grasses, while *Pseudomonas* sp., *Bacillus* sp. and *Chromobacterium* sp. were abundant in the rhizosphere of clean cultivated crops. The occurrence of certain physiological groups of microorganisms in the rhizosphere and rhizoplane of water melon, cucumber and cowpea were studied by Abdel-Nasser and Makawi (1977). The numbers of heterotrophs such as ammonifiers, *Azotobacter* and aerobic decomposers were generally more in the rhizosphere as compared to non-rhizosphere soil.

Further, Mukherji and Chattopadhyay (1979) reported the changes in population of denitrifying, nitrifying, nitrogen fixing and cellulose decomposing bacteria in rhizosphere and non-rhizosphere soil at three different depths and in three stages of growth. The counts of aerobic microorganisms, facultative anaerobes and denitrifiers in the surface (0-7.5 cm) of no-till soils were 1.14 to 1.58, 1.57 and 7.1 times higher respectively in surface ploughed soil (Doran, 1980a).

Studies on the rhizosphere population of selected physiological groups of bacteria have revealed the changes in microbial population demonstrating the specificity of plants (Neal *et al.*, 1973). Studies on the effect of organic matter on the rhizosphere microorganisms and root development of sorghum revealed that the application of compost stimulates the growth and increased the total aerobic bacteria (Pere *et al*., 1983).

Ayanaba and Omayali (1975) studied the microbial ecology of acid tropical soils and found that soil acidity had a great influence on the density of the microorganisms. The enumeration of frequency of occurrence of the predominant fungal species in the rhizosphere and rhizoplane of cowpea indicated that the rhizosphere inhabited *Aspergillus*, *Fusarium*, *Trichoderma* and *Penicillium* while *Fusarium* sp. were abundant in the rhizoplane (Odunfa and Oso, 1979).

Abdel-Nasser and Makawi (1977) reported the occurrence of certain physiological groups of microorganisms in the rhizosphere and rhizoplane of watermelon, cucumber and cowpea. Nitrifiers were less or absent in the rhizoplane but the heterotrophs occurred in large numbers. The frequency of occurrence of the predominant fungal species were recorded in the rhizosphere and rhizoplane of cowpea; the rhizosphere recorded *Aspergillus*, *Fusarium*, *Trichoderma* and *Penicillium* while *Fusarium* sp. were abundant in the rhizoplane.

Microflora in the rhizosphere of plantation and tree crops:

Literature on the rhizosphere microflora of plantation crops is scanty. Watanabe (1971) reported the occurrence of species of *Trichoderma*, *Mortierella*, *Gliocladium*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Chaetomium*, *Humicola*, *Diplodia* and *Pythium* in the rhizosphere of wilt affected pineapple plants. Species of *Fusarium* and *Mortierella* were recorded in higher proportion.

Studies on the rhizosphere microflora of healthy arecanut palm have revealed the occurrence of *Bacillus* spp. *Arthrobacter* spp., *Micrococcus* spp. and *Pseudomonas* spp. (Bopaiah, 1979). Among the bacterial flora, the gram +ve bacteria (70-80 per cent) were more as compared to gram -ve bacteria (20-30 per cent). The *Trichoderma* spp. (18-35%) and *Aspergillus* spp. (21-39%) were the predominant fungi in the root region. The other genera of fungi recorded were *Penicillium*, *Curvularia*, *Rhizoctonia*, *Rhizopus*, *Mucor*, *Fusarium*, *Cylindrocarpon*, *Cladosporium* and *Alternaria*. The actinomycetes were represented by *Streptomyces*, *Nocardia*, *Micromonospora* and *Actinomyces*.

Bopaiah and Koti Reddy (1982) studied the distribution of microflora in the root region of arecanut palm. The microflora (bacteria, fungi and actinomycetes) were more in 0-15 and 16-30 cm depths between the lateral distance of 30-60 cm from the base of the palms and the reduction in population was recorded in 31-45 and 46-60 cm soil depth.

The effect of continuous application of manures and fertilizers on the rhizosphere microflora in arecanut plantation showed that the application of organic manure increased the microbial population (Bopaiah and Bhat, 1981). The surface cultifation of soil increased the microbial population and the application of inorganic fertilizers increased the soil acidity.

Studies of root region of areca palm revealed the presence of various microorganisms and the application of arecanut husk and intercropping in arecanut garden have influenced soil microflora (Bopaiah, 1982a). The occurrence of asymbiotic nitrogen fixers, phosphate solubilizers and cellulolytic microorganisms were recorded in the root region of arecanut palm (Bopaiah, 1982b).

The effect of NPK fertilizers and season on the root region microflora in cashew was studied (Harishu Kumar *et al*., 1985). The bacterial and actinomycetes population build up was recorded in post-monsoon season. Fungal population and urease activity were more during pre-monsoon period. The phosphate solubilisers did not show conspicuous variations.

Talukdar *et al*. (1985) studied the distribution of fungi in the rhizosphere and non-rhizosphere soil of tea and their relation to pH, total N and available P and K. The number of fungi were more in the rhizosphere and with a predominance of *Aspergillus* and *Fusarium* spp. The number of fungi in the rhizosphere soil was negatively correlated with the pH. Radha and Menon (1954) reported greater number of microorganisms in the rhizosphere of healthy coconut palms as compared to 'root (wilt)' affected coconut palms.

Seasonal variation in rhizosphere microflora of coconut with special reference to fungi, showed a considerable reduction in soil microflora during the monsoon (Radha and Rawther, 1959).

Garcia and Valera (1963) reported that *Trichoderma*, *Aspergillus*, *Fusarium*, *Gliocladium*, *Verticillium* and *Pythium* as the commonly occurring fungi in coconut rhizosphere. Rawther and Radha (1963), Potty (1977) and Alice *et al.* (1980) have compared the rhizosphere microflora between the healthy and root (wilt) disease affected coconut palms. *Cylindrocarpon effusum*, *Fusarium equiseti* *Monacrosporium bembicodes*, *Penicillium javanicum*, *Penicillium spiculaisporium* and *Graphium* sp. were reported as the fungi associated with roots of root (wilt) affected coconut palms (Joseph, 1978).

Intercropping with fodder grass like *Pennisetum purpureum* (hybrid napier) and legumes like *Stylosanthus gracilis* and *Centrosema pubescens* enhanced the microbial activity in the root region of coconut with respect to total bacteria, nitrogen-fixers and denitrifiers (Sahasranaman *et al*., 1976). Similarly intercropping in healthy and root (wilt) affected coconut garden with napier grass increased the total bacteria, N_2-fixers and phosphate solubilising bacteria (Potty *et al*., 1977). Further, Potty (1977) reported that the crop mixing with *Centrosema pubescens* and *Stylosanthus gracilis* besides *Pennisetum purpureum* (hybrid napier) increased the fungi, bacteria, actinomycetes and indole producing microorganisms in the rhizosphere of coconut. The mixed cropping also favoured the proliferation of N_2-fixers, of which *Beijerinckia* and *Azotobacter* were the predominant asymbiotic N_2-fixers. The physico-chemical factors in coconut rhizosphere like pH, organic carbon, nitrogen and phosphorus showed positive correlation with the microflora. Cover cropping with fodder grasses or legumes showed the difference in the proliferation of physiologically distinct actinomycetes in the rhizosphere of coconut (Potty, 1977).

Influence of mixed cropping on microflora in coconut:

Cacao, clove, nutmeg and to a certain extent Robusta coffee are grown as mixed crops in coconut gardens

(Nelliat and Shama Bhat, 1979). Planting of cacao in the interspaces of coconut is being practiced in Philippines, Malaysia, India and Papua New Guinea. Nair and Subba Rao (1977a) reported the number, nature, specific biological attributes of bacteria, fungi and actinomycetes in the rhizosphere of coconut under mixed cropping with cacao. The N_2-fixers, phosphate solubilising and indole acetic acid (IAA) producing microorganisms were isolated from the rhizosphere soil. The production of gibberellin-like substances (GLS) were also detected.

The term multistoreyed cropping is used to refer to multispecies crop combination involving both annuals and perennials with an existing stand of perennials (Nelliat, *et al*., 1974; Nair et al., 1975; Nair, 1977).

Asymbiotic Nitrogen fixers:

There are several ways for nitrogen to enter the plant-soil component of the eco-system. Rain and snow usually contain small amounts of bound nitrogen but nitrogen fixation by microorganisms play an important role in the natural ecosystem. *Azotobacter*, *Beijerinckia*, *Derxia* and *Clostridium* are reported as asymbiotic nitrogen fixers. Among these *Beijerinckia* is widely distributed in tropical soils (Jensen, 1954; Becking, 1961; Vancura *et al*., 1965; Anderson, 1966; Dobereiner and Campelo, 1971; Nair and Subba Rao, 1977a; Kothandaraman, 1979; Rawther *et al*., 1979; Bopaiah, 1982).

The occurrence of Azotobacter was also reported in some acid soils (Iswaran, 1960; Rangaswami and Sadasivam, 1964). Kluyver and Becking (1950) and Jensen (1954) reported that *Beijerinckia* fixes 16 to 20 mg of nitrogen per gram of sugar. However, Jensen (1948) observed *Beijerinckia* makes better use of ammoniacal, nitrate nitrogen and many aminoacids. Barooah and Sen (1964) have correlated the production of mucilage by *Beijerinckia* with the intensity of nitrogen assimilation in pure culture *in vitro*. Dobereiner (1961) observed an increased number of *Beijerinckia* in the proximity of sugarcane roots. Martin *et al*. (1965) reported that the mucilage of *Beijerinckia* is a polysaccharide, which decomposes very slowly in the soil and it is highly toxic to several soil microorganisms.

Azotobacter isolated from the rhizosphere of sunhemp fixes about 6 mg of nitrogen per gram of mannitol in Fred and Waksman's medium (Iswaran, 1960). The organism fixes significantly higher nitrogen when grown in association with *Trichoderma* sp. and strains of *Rhizobium*, whereas the *Bacillus subtilis* and *Aspergillus* sp. did not help in increasing nitrogen fixation.

The *Azotobacter* culture produces certain biologically active substances such as indole acetic acid (Vancura and Macura, 1960; Brown and Walker, 1970; Brown, 1972) and gibberellins (Vancura, 1961; Azcon and Barea, 1975).

Asymbiotic N_2-fixers have been reported in certain plantation crops (Ravther *et al*. 1979; Kothandaraman, 1979; Nair and Subba Rao, 1977a; Bopaiah, 1982; Subba Rao, 1983). In coconut rhizosphere, *Beijerinckia* was the commonly recorded asymbiotic N_2-fixer (Nair and Subba Rao, 1977a; and Subba Rao, 1983). Kothandaraman (1979) studied the nitrogen fixation by *Beijerinckia* sp. in culture media and its survival in the rhizosphere of rubber plants.

Associative symbiotic N_2-fixers:

Azospirillum, the nitrogen fixing associative symbiotic bacterium is gaining greater importance in recent years (Barkum and Bohlool, 1980). Many of the tropical grasses, cereals and several weed plants have been reported to harbour this bacterium in the root system (Partriquin and Dobereiner, 1978; Dobereiner and Campelo, 1978; Venkateswaralu and Rao, 1983). The association of this bacteria in plantation crops has not been adequately investigated so far. The occurrence of the organisms, however, has been reported in various plantation crops such as coconut, cashew, arecanut, cocoa, rubber, cardamom and banana (Subba Rao, 1983; Govindan and Purushothaman, 1985).

Phosphate solubilising Microorganisms:

Phosphate occurs in soil in bound forms (organic and inorganic forms) which is not readily available to plants. Several workers have demonstrated the phosphate solubilising property of soil microorganisms (Pikovskaya, 1948; Sen and Paul, 1957; Sethi and Subba Rao, 1968; Khan and Bhatnagar, 1977; Nair and Subba Rao, 1977b; Arora and Gaur, 1979; Banik and Dey, 1981; Kucey, 1983; Thomas *et al.* 1985; Thomas and Shantaram, 1986). Abbott (1923) studied the phosphate transformation by soil fungi and found that *Aspergillus flavus*, *A. fumigatus*, *Penicillium funiculosum*, *P. luteum* and *Cladosporium herbarum* solubilised rock phosphate. The solubilisation of insoluble phosphates by bacteria and fungi and the production of organic acids by them have been studied (Sperber, 1957; Chandrasekharan, 1969; Mehta and Bhide, 1970; Gaur and Sachar, 1980).

Gerretsen (1948) and Sen and Paul (1957) have demonstrated that plants take up more phosphates in the presence of phosphate solubilising microorganisms.

Bopaiah (1985) reported the occurrence of phosphate solubilising microorganisms in the root region of arecanut palms. Species of *Bacillus*, *Pseudomonas*, *Aspergillus niger*, *Aspergillus*, *Penicillium* and Actinomycetes are the organisms associated and of which *Aspergillus niger* (45.8%), *Aspergillus* sp. (39.8%) and *Pseudomonas* sp. showed greater P-solubilising ability *in vitro*.

Nair and Subba Rao (1977b) reported the two efficient PO_4 solubilising microorganisms, *Pseudomonas* sp. and *Aspergillus niger* isolated from the rhizosphere of coconut and cacao and the organism solubilised 49% and 49.7% inorganic phosphate *in vitro*. Further, Thomas *et al.* (1985) reported that in laterite, alluvial and clayey soils the P-solubilising fungi were more than in sandy soils. The studies on the occurrence and the activity of P-solubilising bacteria in the rhizosphere of different coconut growing soils are also attempted (Thomas and Shantaram, 1986).

The *Azotobacter* cultures produce certain biologically active substances. The production of IAA (Indole Acetic Acid) (Vancura and Macura, 1960; Brown and Walker, 1970; Brown, 1972) and gibberellins (Vancura, 1961) have been reported. Azcon and Barea (1975) have reported the synthesis of auxins, gibberellins and cytokinin by *Azotobacter vinelandii* and *A. beijerinckii*.

VA-Mycorrhizae:

One of the most prominent organism associated with plant roots is the mycorrhizae. The fungus benefits the plant by increasing the uptake of phosphorus, zinc and copper (Bowen and Theodorou, 1967; Hayman, 1970, 1975; Powell, 1981; Krishna and Bagyaraj, 1982). Barea and Azcon-Aguilar (1982) reported that *Glomus mosseae* a vesicular-arbuscular mycorrhizae (VAM) fungus synthesises at least two gibberellin like substances and four cytokinin-like substances. The plants are benefitted from VAM fungi both by nutrient uptake and production of growth promoting substances. The infection with VA-mycorrhizae fungi may also enhance the water status or drought tolerance of plants (Allen *et al.*, 1981).

Association of VA-mycorrhizae has been reported as beneficial to *citrus* sp. (Mosse, 1978; Gerdemann, 1968; Shanmugam *et al.*, 1981; Nemec *et al.*, 1981 and Menge *et al.*, 1982). Association of VA-mycorrhizae in coconut has been reported (Lily, 1975, and Ramesh and Rohini Iyer, 1979). Satyanarayana and Venkataramanan (1979) reported the mycorrhizal association in tea and weeds from soils of north-eastern regions of India.

Microbial biomass:

Soil microbial biomass is the living part of the soil excluding plant roots and soil animals which are larger than about $5 \times 10^3\ \mu m^3$ (Jenkinson and Ladd, 1981). The fumigated soil consumes more oxygen (Jenkinson and

Powlson, 1976) and evolves more carbon dioxide (Walksman and Starkey, 1923; Powlson and Jenkinson, 1976). Lynch and Panting (1982) reported that the soil biomass increased during the growth of wheat crop followed by decrease, till it reached a constant level. The biomass was significantly greater where the soil had been direct-drilled than where it had been ploughed probably because the plant roots were more abundant after direct drilling. Similarly Carter and Rennie (1982) studied the changes in soil quality under zero tillage farming system. The concentration gradient of potential microbial biomass, C and N and potential net mineralizable C and N were significantly greater in the surface soil under zero tillage in comparison with conventional tillage. The reverse trend was observed at the lower depth.

Nannipieri *et al*. (1983) studied the production and persistence of biomass and also urease, phosphatase and Casein-hydrolysis activities with 15_{NO_3} when either glucose or rye grass were added as energy source to a clay loam soil. The increase in phosphatase and urease activities coincided with an increase in bacterial biomass. Tesarova and Repova (1984) studied the total biomass of microorganisms in the root-free soil from submontane grass land ranged from 40.8 to 60.7 mg C/100g, as compared to 69.4 to 143.0 mg C/100g for the rhizosphere soil. Several other workers have studied the soil biomass (Lynch and Panting, 1982; Brookes et al. 1985; Gilmour and Gilmour, 1985; Schnurer et al., 1985; Malal and Sauerbeck, 1986). Fumigation accelerates the decomposition of soil organic matter in the initial stage. The flush of decomposition is defined as the difference in the amount of CO_2 evolved (or O_2 consumed or N mineralised) by a fumigated soil when incubated for a given time, as compared to unfumigated soil. Jenkinson (1966) suggested that the flush was due to the decomposition of organisms killed during fumigation and by the survivors or by the addition of an inoculum. The flush can then be directly related to the biomass by the expression:

$$B = F/_{Kc}$$

Where B is soil biomass C, in μg C g^{-1} soil; F is the difference in CO_2-C (μg C g^{-1}) evolved between fumigated and unfumigated soil during incubation and Kc is the constant ratio (0.41).

Several models have been proposed for the turn over of organic matter in soil (Hunt, 1977; Jenkinson and Rayner, 1977) which include sub-models for the turn over of microbial biomass. Earlier methods of soil biomass estimation were reviewed and modified by Clark and Paul (1970) as described by Barber and Lynch (1977). These estimations were mostly based on various combinations of direct and plate count numbers which is converted to biomass by assuming standard size of different numbers of the soil population.

<u>Soil enzymes</u>:

A number of enzymes are produced by soil microorganisms during their growth. The total activity of soil microflora is the sum total of a number of activities of different microorganisms. Number of investigations have shown that the activities of various enzymes in field or green house soils are affected differentially by the nature of the plant cover (Neal, 1973; Pancholy and Rice, 1973; Kiss *et al.*, 1975). Pancholy and Rice (1973) have demonstrated that the activities of dehydrogenase and urease increased under successional stages of revegetation of prairie grass, Oak forest and Oak-pine forest. No correlation was found between soil enzyme activity and amounts of organic matter.

Urease activities of field soils varied, according to season and were influenced by vegetation (Stojanovic, 1959; Khan, 1970; Pancholy and Rice, 1973; Tarafdar and Roy, 1981; Hoult and McGarity, 1986). Urease activity decreased with depth of soil and it could be correlated with soil organic matter (Myers and McGarity, 1968; Musa and Mukhtar, 1969; Moult and McGarity, 1986). Activities increased after addition of inorganic and especially organic fertilizers to soils (Bei-Bienko, 1970; Nikiforenko, 1971; Stefanic, 1971; Balasubramanian et al., 1972; Thomas and Shantaram, 1984).

Many investigators have found that urease activity in soil can be increased by the addition of glucose or other materials that promote microbial activity (Vasilenko, 1962; Balasubramanian et al., 1972; Zantua and Bremner, 1976, 1977).

Tarafdar et al. (1981) studied the distribution of urease, acid and alkaline phosphatase in some jute soil of West Bengal. The activities were positively correlated with organic carbon, fungal and bacterial population, but there was no correlation with pH and soil actinomycetes. Effects of insecticides, herbicides, nematicides and heavy metals on Urease activities have been determined (Burns, 1978). The application of pesticides caused either inhibition or stimulation of Urease activity.

Moore and Russell (1972) studied the factors affecting dehydrogenase activity as an index of soil fertility and based on this they suggested that it could not be used as a general index of soil fertility, rather it is only a quantitative indication. Dehydrogenase activity increased with an increase in microbial population following the amendment of soils with nutrients (Ladd and Paul, 1973) and decreased when the soils are dried (Pancholy and Rice, 1972). Dehydrogenase activity decreased with depth of soil (Musa and Mukhtar, 1969; Ross, 1973).

Many reactions involving soil organic matter transformations may be catalysed by enzymes existing outside the microorganisms and plant root systems. Phosphatase enzyme activity also decreased with soil depth (Skujins and McLaren, 1967; Kiss *et al.*, 1975). Khaziev and Burangulova (1965) reported that the phosphatase activities correlated with the distribution of microorganisms in the soil. Definite positive relationships between phosphatase activity and organic matter content in soil have also been reported (Galstyan and Tatevosyan, 1964; Arutyunyan and Galstyan, 1975). Rhizosphere phosphatase activities tend to be higher than in non-rhizosphere soil (Kiss *et al.*, 1975) because of increased microbial numbers in the rhizosphere (Greaves and Webley, 1964). The phosphatase activity is directly related to the level of organic phosphorus in soil (Gavrilova *et al.* 1974). The presence of plants, and the type of plants grown in a soil have a marked effect on its enzyme activities (Khan, 1970; Neal, 1973; Dudchenko *et al.*, 1973; Kiss *et al.*, 1975;

Dalal, 1982; Dick, 1984).

Carbon mineralisation:

Soil organic matter comprises of residues of plant and animals at all stages of decomposition mediated by soil microorganisms. The diversity of plant materials that enter the soil and the organic constituents of plant are commonly divided into six broad categories (a) cellulose (varying from 15 to 60 per cent of the dry weight); (b) hemicelluloses (10-30 per cent); (c) Lignin (5-30 per cent); (d) water soluble fraction (5-30 per cent); (e) ether and alcohol soluble fraction; (f) proteins. The decomposition of native organic matter (humus) reflects the biological availability of soil carbon, while the release of CO_2 following the addition of relatively single substrates is an estimation of the potential carbon--mineralising capacity of the mifroflora (Alexander,1961). The rate at which CO_2 is released during the mineralisation of humus varies greatly with soil type and the major factors governing the decomposition are the organic matter level of the soil, cultivation, temperature,moisture, pH, depth and aeration (Sinha et al., 1977; McGill and Cole, 1981). A number of factors affect the mineralisation of added organic matter such as chemical composition and environmental condition (temperature, O_2 supply, moisture, pH, available minerals and the C:N ratio of the plant residue) (Bingeman *et al*., 1953; Lamb, 1976).

The magnitude of carbon mineralisation is directly related to the organic carbon content of the soil and thus the release of CO_2 is proportional to the organic matter level (Alexander, 1961).

Cultivation enhances the organic matter destruction. After cultivation of soil for 25 years the organic matter content of 28 soils of Georgia had decreased from 3.29 to 1.43 per cent. In sandy soil (Virgin prest) contained 2.30 per cent organic matter, but the level had fallen to 1.59 per cent after 3 years of cultivation (Giddens, 1957). The higher rate of CO_2 evolution occurs near the surface of the soil profile, where organic matter content is high and at deeper layer, rate of CO_2 production diminishes (Newman and Norman, 1943).

Gaur *et al*. (1971) studied the rate of decomposition and development of soil microflora with the addition of organic materials viz., berseem hay, farm yard manure and wheat straw.

Nitrogen mineralisation:

Studies on nitrogen mineralisation have been attempted by several workers (Estermann *et al*., 1959; Winsor and

Pollard, 1956; Allison and Sterling, 1949; Weeraratna,1979; Cassman and Munns,1980; Ross and Cairns, 1980; Campbell and Biederbeck, 1982). Vlassak (1970) reported the mineralisation capacity of 24 different soil. Relatively rapid mineralisation and nitrification was recorded with soils from cultivated land and pastures. A close relationship could be established between the nitrogen content of soil and the amount of mineral nitrogen formed during incubation. Ishaque and Cornfield (1972) studied the nitrogen mineralisation and nitrification during incubation of tea soil and found that the nitrate accumulation increased considerably with pH. The counts of ammonifiers and nitrifiers were studied in relationship with rates of nitrogen mineralisation and protease activity (Ross and Bridger, 1978). Cassman and Munns (1980) studied *in vitro* variation in rate of nitrogen mineralisation in soil.

Sato (1981) reported the relationship between soil microflora and CO_2 evolution during decomposition of cellulose. The gram -ve bacteria and cellulose decomposing microorganisms grew well in surface soil crumbs with added cellulose powder.

As cellulose is the main source of energy for all form of life in soil, cellulose decomposition in soil has been studied by several workers (Eggins and Pugh,1962;

Gaur et al, 1971) and reviewed by Dickinson (1974) and Williams and Gray (1974).

The studies on the mineralisation of soil nitrogen in four forest krasnozem soils revealed that nitrification under laboratory incubation was considerably higher in soils from silver wattle (*Acacia dealbata*) and Mountain ash (*Eucalyptus regnans*). In Messmate (*Eucalyptus obliqua*) and Monterey pine (*Pinus radiata*) forest soils ammonifiers were predominant. The occurrence of nitrification in Australian forest appeared to be predominantly related to the amount of N present and its rate of turnover rather than inhibitory effects (Adams and Attiwill, 1982).

Soil physical properties:

Soil aggregation is vital for the growth, movement of air, water and transfer of energy. Gantotti and Rangaswami (1971) reported that the growing of finger millet, sorghum, green gram and soybean have increased the soil aggregates. The rhizosphere microflora in association with roots of growing plants play a pivotal role in improving the soil structure. Among the other physical factors affecting plant growth, Nye (1981) studied the changes of pH across the rhizosphere induced by plant roots.

Chemical properties of soil:

Microbiological decomposition of organic matter is an essential step to release the bound nutrients in organic residue into an available form. Several workers have reported that addition of organic matter improves the soil chemical composition (Sinha *et al.*, 1977; McGill and Cole, 1981). Gaur *et al.* (1971) reported that the addition of berseem hay, farm yard manure and wheat straw increased the humic acid content, organic carbon, total nitrogen and available nutrients like N,P and sulfur.

The effect of intercropping in coconut with groundnut on soil fertility showed a higher N,P,K and Ca status (Leela and Bhaskaran, 1978). Varghese *et al.* (1978) reported the beneficial interaction of coconut-cacao crop combination indicating the improvement in organic carbon content and soil fertility.

Mulching increased the downward movement of phosphorus and decreased the amount of phosphorus fixed in the soil (Othieno, 1973). Gaur and Mukerjee (1980) studied the recycling of organic matter through mulch in relation to chemical and microbiological properties of soil and crop yield.

Effect of pesticides on soil microorganisms:

The non-target microorganisms are exposed to herbicides, insecticides, nematicides, fungicides and bactericides which are applied increasingly to soil. Of these pesticides, only fungicides and bactericides are aimed specifically against microorganisms. The herbicides and insecticides, although not aimed at microorganisms, may nonetheless prove detrimental to the non-target organisms by virtue of their chemical nature (Anderson, 1978; Simon-Silvestre and Fourier, 1979).

Houseworth and Tweedy (1973) reported that captan application (at 0.1 and 1.0 kg/ha) on sandy loam soil initially decreased fungal population but later the population increased to normal level. Mahmoud *et al*. (1972) reported decreased population of actinomycetes. The application of Lindane to sandy loam soil initially reduced the bacterial counts but later increased to normal level (Gawaad *et al*. 1973). Ross (1974) showed slight stimulation effect of carbofuran (0.75 to 50 kg/ha) on microorganisms. Tu (1975) reported that the application of BHC at 1.0, 10, and 100 kg ai/ha on loamy sand did not effect the fungi and actinomycetes, whereas on sandy loam soil fungal population decreased at higher rate but there was no qualitative changes. The addition of aldicarb (0.5 and 50 kg ai/ha) on sandy loam at lower level there was no effect but at higher rate increased the number of fungi and actinomycetes (Kuseske, *et al.*, 1974).

Wainwright and Pugh (1975) studied the effect of captan Dichoran and Milcol on the number of microorganisms and frequency of cellulolytic fungi in soil. The results revealed that all the fungicides initially reduced the fungal numbers but then recovered to level of untreated soil after 157 days. The bacterial count increased in the captan treated soils.

Three fungicides, namely, captan, thiram and verdasan at 5 and 15 µg/g of soil caused a shift of fungal to bacterial dominance (Anderson *et al.*, 1981). At 5 µg/g it caused significant decrease (40%) in the biomass but within 8 days the biomass in captan and thiram amended soil had recovered to that of the control.

Several workers have studied production of cellulase enzyme by fungi (Halliwell, 1961; Mandels and Reese, 1957) and bacteria (Kolman and Cowling, 1965). Hussain and Rich (1958) studied the extracellular pectic and cellulolytic enzymes of *Cladosporium cucumerinum*. Manibhushan Rao (1971) studied the production of cellulolytic enzymes in the culture filtrate of *Pyricularia oryzae* *in vitro*. All the cultures registered higher growth and enzyme activity with the insoluble filter paper cellulose than the soluble (carboxy methyl cellulose) form when both were supplied as sole carbon source in the basal medium. Olutiola (1976) reported the presence of cellulase enzyme in the culture filtrate of *Aspergillus flavus*. Sandhu and Kalra (1985) reported the effect of cultural condition on the production of cellulase in *Trichoderma longibrachiatum*.

Growth promoting substances are produced by certain bacteria and fungi isolated from soil (Nair and Subba Rao, 1977a; Prikyl *et al*., 1985). The inoculation of selected strains of *Pseudomonas* sp. enhanced the plant growth (Suslow *et al*., 1979; Suslow and Schroth, 1982).

Interaction of rhizosphere microorganisms:

Production of antibiotics by soil fungi, actinomycetes and bacteria has been well recognised for the past several decades. The antibiotic produced may influence the pattern of saprophytic colonisation (Garret, 1960). Zak (1964) postulated that fungi may furnish protection to root pathogen by (a) utilising root carbohydrates and other nutrients which would otherwise be attractive to the root pathogen; (b) providing physical barrier for the entry of the pathogen; (c) secreting antibiotics which kills or inhibit the pathogen; (d) supporting a protective rhizosphere microflora.

Several soil fungi have been reported to be antagonisti to plant pathogens (Sudha Mali, 1973; Elad, 1982; Pathak *et* 1981; Khetmalas *et al.* 1984; Venkatasubbaiah and Sufeulla 1984; Venkatasubbaiah *et al.*, 1984. The formation of oospores by *Phytophthora* in response to *Trichoderma* is a defensive response to a potential antagonist or competition (Brasier, 1978). Dennis and Webster (1971a) reported the production of non-volatile antibiotic by *Trichoderma*. The antagonism of bacterial and actinomycetes were also reported (Kulkarni *et al.*, 1980; Rothrock and Gottlieb, 198 Podile *et al.*, 1985). The antagonism of isolate of *Bacillu subtilis* to *Phytophthora cactorum* (Vtekhade, 1984) and that of soil actinomycetes against *Phytophthora* and *Pythium* species and a mycorrhizal fungi (Keast and Tonkin, 1983) wer studied.

Plant roots exude a great variety of organic compounds and the substances exuded include aminoacids, sugars, organic acids, vitamins, nucleotides and many other unidentified compounds (Bhuvaneswari and Sulochana, 1955; Rovira and Harris, 1961; Vancura, 1964; Balasubramanian and Rangaswamy, 1974; Jayman and Sivasubramaniam, 1975). The nature and amount of exudates of certain field crops has been characterised by several workers (Andal *et al.*, 1956; Dey *et al.*, 1958; Vancura, 1975). The areca based cropping system influenced the quantitative exudation of aminoacids and there was no qualitative difference in sugars, organic acids and aminoacids (Nagaraja et al., 1986). The rhizosphere and root region of the crops are the active zone which are directly under the influence of root exudates. The organic substances in soil arise from the decomposition of plant and animal residues and from the plant root exudates. The substances includes aminoacids, sugar, organic acids, vitamins, nucleotides and many other organic compounds (Putman and Schmidt, 1959; Lynch et al.,1987; Gupta and Sowden, 1963 , Barber and Martin, 1977).

The presence of phenolics in soil has been reported by a few workers (Kononova, 1961; Chandramohan et al.,1973; Manikandan and Siddappa,1986). Sullia (1973) studied the effect of root exudates and extract of Cassia tora and Crotalaria sp. on some dominant rhizosphere fungi. The root extracts induced high degree of stimulation in the rate of growth of fungi. Chile and Vyas (1984) reported the antifungal activity of the extract of piper beetle cultivars against some fungi. The growth promotion and inhibition by antibiotic producing flourescent Pseudomonas in citrus root was studied by Gardner et al. (1984).

MATERIALS AND METHODS

MATERIALS AND METHODS

The coconut based multistoreyed cropping system and coconut based mixed farming system are the two separate ongoing experiments at the Central Plantation Crops Research Institute (CPCRI), Kasaragod, Kerala. The crops included in the coconut based multistoreyed cropping system are black pepper (*Piper nigrum* L.), cacao (*Theobroma cacao* L.) or cinnamon (*Cinnamomum zeylanicum* Nees), and pineapple (*Ananas comosus* (L.) Merr.) in different systems of planting. The mixed farming in coconut garden involves the cultivation of fodder grass like hybrid napier var. pusa giant (*Penisetum purpureum* Schum.) in the interspaces of coconut palms, to maintain the milch animals on the fodder produced and recycling the cattle dung slurry through the biogas plant into the coconut grass mixed strand. The soils are sandy loam with pH of 5.0 to 5.4 and are low in organic carbon, N and P contents.

EXPERIMENTAL LAYOUT:

Expt. 1: Coconut based multistoreyed cropping system

The coconut based multistoreyed cropping trial was initiated in 1972 in 20-year-old coconut garden with a spacing of 7.5m x 7.5m. The crop combinations included in the experiments were:

1. Coconut-black pepper-cacao (single hedge)-pineapple (Plate 1A and 2A)
2. Coconut-black pepper-cinnamon (single hedge)-pineapple

3. Coconut monocropping system (Plate 1B and 2B).

The black pepper was trailed on coconut trunk to a height of 8 metres. The number of cacao or cinnamon plants were 350/ha on single hedge planting. The pineapple suckers (3500/ha) were planted in the alley not occupied by cacao/cinnamon. The plots were irrigated during the dry months (December-May). The black pepper and pineapple were fertilised at 15g N, 10g P_2O_5 and 10g K_2O/vine/year and 80 kg N, 30 kg P_2O_5 and 60 kg K_2O/ha/year respectively. The cacao (100:40:140 kg NPK/ha/year) and coconut (500:320:1200 kg NPK per ha/year) were fertilised following the normal agronomic practices. The fertiliser was applied in two split application (February-March and August-September).

Expt. 2: Coconut based mixed farming system

This experiment was also initiated in 1972 in the 60 year old coconut garden (Plate 3). Black pepper was trailed on the trunk of the coconut palms. The coconut palms (500:320:1200 kg NPK/ha/year applied in two equal split doses in February-March and August-September) and grass (20 kg N, 8 kg P_2O_5 and 60 kg K_2O/ha applied after every cutting) received the recommended dose of fertilisers. The grasses were cut at 30-40 days intervals to feed the dairy animals. The cowdung was loaded to biogas plant and the

Plate - 1A

Coconut based Multistoreyed Cropping System with pepper + cacao + pineapple.

Plate - 1B

Coconut monocropping system

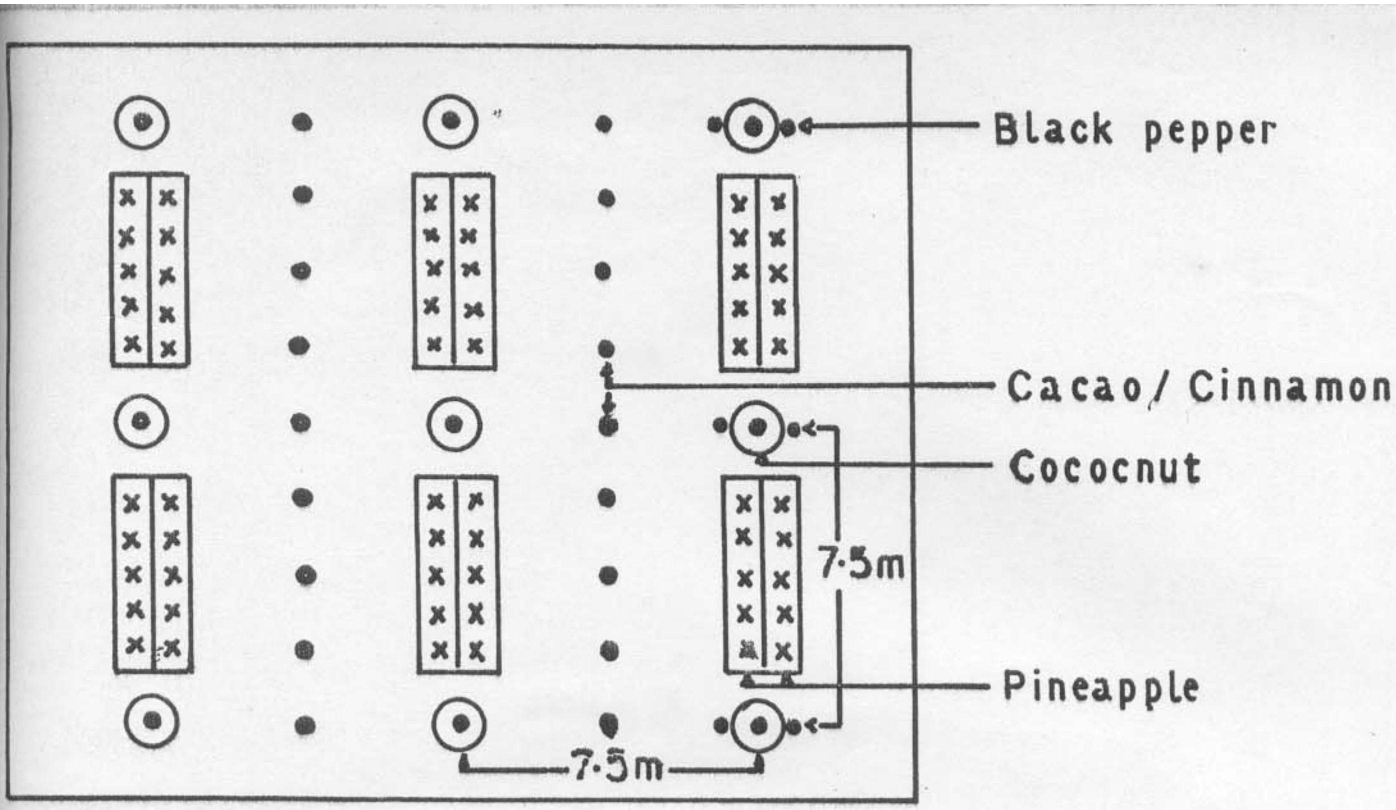

Plate 2 A

cheme of planting of defferent crops in coconut based ultistoreyed cropping system.

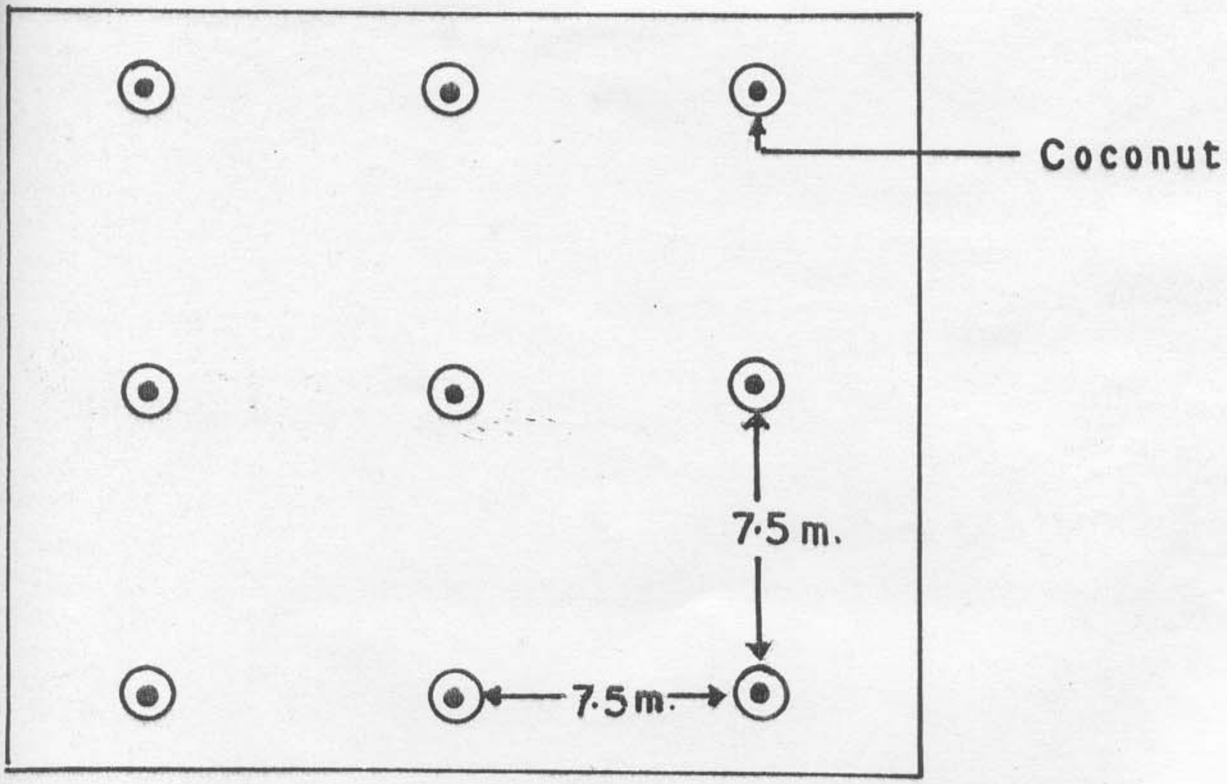

Plate 2B

Scheme of planting of coconut in monocropping(monoculture) system.

Plate - 1

Coconut based mixed farming system with pepper + napier grass

slurry was recycled into the coconut-grass mixed strand. During the dry period (December-May), sprinkler irrigation was provided to ensure the steady growth of grass.

Collection of root region and rhizosphere soils:

The soil samples were collected from the root region and interspaces of various crops in May and October 1983 by core sampling using soil auger from three depths viz., 0-25, 26-50 and 51-100 cm. The root region soils were collected 1 m away from the base of the coconut palm. The samples from cacao and cinnamon were collected from 0.5 m away from the base of the tree. In case of pineapple the sampling was done at 0.25 m away from the base. The root region and interspace soils were collected from the adjoining coconut monocropped plot. In all the cases three samples were collected from each depth. Core samples were collected from four corners of each plant and a composite sample was obtained. Both in May (pre-monsoon) and in October (post-monsoon) samples were collected from the same source to study the seasonal variation in microbial population in the root region of various crops. Weather data were collected during the 1983 and it is presented in the Appendix-II. The samples were stored at 4°C for a period of 6 weeks before analysis.

The rhizosphere samples were collected from the different crops (five samples each) in the month of October 1983 as per the method outlined by Nair and Subba Rao (1977 a). Soil containing roots were cut and transferred to polythene bags. The rhizosphere samples were also collected from the adjoining coconut monocropping plot. The samples were stored at 4°C for 6 weeks before analysis.

Enumeration of root region and rhizosphere soil microorganisms:

The enumeration of root region microflora was done following the soil dilution and plate count method (Timonin, 1940). An estimate of total population of bacteria, fungi and actinomycetes was calculated per gram of root region or rhizosphere soil. The bacterial, fungal and actinomycetes population were determined by using Nutrient Soil Extract Agar (NSEA) (Thornton, 1922); Martin's Rose Bengal Agar (MRBA) (Martin, 1950) and Kuster's Agar (KA) (Allen, 1957) respectively*. The Jensen's nitrogen-free-medium (Jensen, 1942) was used to enumerate the asymbiotic nitrogen fixers. Phosphate solubilizing microorganisms were enumerated by using modified Pikovskaya's medium (Sundara Rao and Sinha, 1963).

To enumerate the rhizosphere microflora, the roots were carefully removed from the block of soil. The roots along with the adhering soil were then cut into one inch size and

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Please see Appendix-III for the composition of different media transferred to flask containing 100 ml of sterile water. Twenty roots were used for each sample. After shaking for 5 min suitable dilutions were prepared aseptically. The dilutions used were: 10^3 for N_2-fixers and fungi, 10^4 for phosphate solubilisers (bacteria and fungi) and 10^5 and 10^6 for bacteria. After pouring the suitable media, the plates were incubated at 30 ± 2°C. The bacteria, fungi, phosphate solubilisers, actinomycetes and N_2-fixers were counted after 48 hrs, 72 hrs and 96 hrs, 1 week and 2 weeks of incubation respectively. Three replications were maintained for each sample. The average numbers of colonies per gram dry weight of soil was calculated from the counts obtained for each group of microorganisms. Isolates of bacteria, fungi and actinomycetes were purified and maintained on Nutrient Agar (NA), Potato Dextrose Agar (PDA) and Kuster's Agar (KA) respectively. The nitrogen fixing bacteria were maintained on Beckings medium and P-solubilisers on modified Pikovskaya's medium*.

Enumeration of Nitrifying bacteria:

Determinations of *Nitrosomonas* and *Nitrobacter* were carried out as per the Most Probable Number (MPN) method (Alexander and Clark, 1965). For *Nitrosomonas*, ammonium-calcium carbonate medium and for *Nitrobacter*, nitrite-calcium carbonate medium were used. Serial dilutions were made using 10g soil in sterile water. One ml aliquots of each dilutions were transferred to test tubes containing 5 ml of sterilised medium. One set of tubes without soil dilution were maintained as control and the tubes were incubated for three weeks at laboratory (30±2°C).

At the end of incubation, three drops of freshly prepared Griess Ilosvay reagent was added to test the presence of Nitrite. The presence of nitrate was tested by adding a pinch of Zinc-copper-manganese mixture (1 g powdered zinc, 0.1 g copper powder and 1.0 g powdered manganese dioxide). The number of <u>Nitrosomonas</u> and <u>Nitrobacter</u> were calculated by referring to the Most Probable Number (MPN) table.

Vesicular-Arbuscular mycorrhizae (VA-mycorrhizae):

VA-mycorrhizae spore count:

The endogone spores were extracted by wet sieving and decanting techniques (Gerdemann and Nicolson, 1963). Fifty grams of soil samples were suspended in 1 litre of water and sieved using 400, 250, 200 and 100 µm pore diameter. The residue retained on each sieve was transferred into beaker containing water. After the heavier particles had settled, the water along with its floating components containing spores were poured through Whatman No.1 filter paper, marked with 15, 2.5 cm squares. The spores were counted using stereomicroscope and expressed as number per 50 g of soil.

VA-mycorrhizae colonisation (Phillips and Hayman, 1970):

The root segments either fresh or fixed in FAA (13 ml formalin, 5 ml glacial acetic acid, 200 ml 50% ethanol) were heated at 90°C for about 1 hr in 10% KOH. Then the roots were rinsed in water and acidified with dilute HCl. The roots were stained by simmering for 5 min in 0.05% Trypan blue in lactophenol. The excess of stain was washed and removed with lactophenol. The root segments were mounted on slide in lactophenol and observed under microscope. In the case of pigmented roots they were heated with 10% KOH at 90°C for 2 hrs. The roots were immersed in alkaline solution of hydrogen peroxide (approximately 10 volume) at 20°C until the roots were completely bleached (10 min.). Then the roots were rinsed thoroughly in water to remove the H_2O_2, acidified with dilute HCl and stained, as described earlier. The percentage infected roots by VA-mycorrhizae was calculated.

Soil enzyme activity assay:

Urease activity

Urease activity of soil was estimated by the method described by Pancholy and Rice (1973) except that the ammonia evolved due to the hydrolysis of urea in the reaction mixture was estimated by the Nesslerisation procedure as described by Jackson (1973). Freshly collected soil samples of 10g were taken in duplicate in 250 ml Erlenmeyer flask. Twenty ml of phosphate buffer (pH 6.7) and 20 ml of 10% urea solution and one ml of toluene were added to flasks. The contents of flasks were shaken for five min and incubated at 30°C for 24 hrs. A control with 20 ml distilled water instead of urea solution was run simultaneously for each sample. At the end of 24 hrs the contents of flasks were filtered and 15 ml of 1N KCl containing 150 ppm $HgCl_2$ was added to the soil in the flasks. After 10 minutes this solution was filtered and the volume of the filterate was made up to 100 ml with distilled water. The amount of ammonia in one ml of filtrate was determined by Nesslerisation. To each, two ml of 10% solution of sodium tartrate was added. After that 0.5 ml of Nesslers reagent was added and the volume was made up to 20 ml. After 20 min, the yellow colour developed was read at 410 nm spectrophotometrically. The results obtained were expressed as microgram of NH_4 liberated per gram of soil (oven dry basis) with reference to a standard curve. A standard curve was obtained with NH_4Cl or $(NH_4)_2SO_4$ over a range of 0 to 100 µg per ml.

Dehydrogenase activity assay:

The dehydrogenase activity of the soil was determined as described by Casida *et al.* (1964).

Ten gram of freshly collected soil was dispensed in each of two sets of test tubes. To the contents of the test tube $CaCO_3$ (0.2g) was added followed by one ml of 1.5% aqueous solution of 2-3, 5 Triphenyl Tetrazolium Chloride (TTC). One set of tubes received one ml of 1.0% glucose solution and eight ml distilled water, whereas the other set received nine ml of distilled water, alone. The quantity of liquid was found to be sufficient to saturate the soil and form a liquid layer above the soil surface. The contents of each tubes were mixed well, plugged tightly with rubber stopper and incubated at 37°C for 24 hrs. The 2-3, 5 triphenyl formazan (TPF) formed was extracted with methanol at the end of incubation period and filtered through Whatman No.42 filter paper. Residue was washed down into a 100 ml volumetric flask with small additions of methanol till the filtrate ran free of pink colour. The combined volume of the filtrate was made upto the mark with methanol. The absorbance of the filtrate was determined at 485 nm in a Spectrophotometer using methanol extract. A control without soil served as blank. The results were expressed in microliters of hydrogen ion evolved per gram of oven dried soil. A standard curve of TTC solution was prepared over a range of 0.0 to 100 µg per ml. To each of these concentrations 150 mg sodium dithionate ($Na_2 S_2O_4 H_2O$) was added to obtain rapid reduction of TTC to TPF. The absorbance of the pink colour of TPF was read at 485 nm.

Soil phosphatase activity:

Soil phosphatase activity at pH 6.5 was determined by measuring the liberated P-nitrophenol after incubation with P-nitrophenyl phosphate disodium (PNPP) (Tabatabai and Bremner, 1969).

Reagents:

1. Modified universal buffer (MUB) Citrate phosphate buffer, pH 6.5 (Skujins et al., 1967).
2. Toluene - Fisher certified reagent.
3. P-nitrophenyl phosphate (PNPP) solution 0.115 M: 1.927g of disodium p-nitrophenyl phosphate tetrahydrate (Sigma 104) was dissolved in MUB and diluted the solution to 50 ml with MUB. The diluted solution was stored in a refrigerator.
4. Calcium chloride 0.5M - 73.5g of $CaCl_2$ was dissolved in water and solution was diluted to 1 litre.
5. Sodium hydroxide 0.5M - 20g of NaOH was dissolved in water and diluted to 1 litre.
6. Standard p-nitrophenol solution : 1.0g of p-nitrophenol was dissolved in water and diluted to 1 litre and stored in refrigerator

Procedure:

To one gram of soil (on oven dry weight basis, 2 mm) in 50 ml Erlenmayer flask, 4 ml of Modified Universal Buffer (MUB) solution, 0.25 ml of toluene and 1.0 ml of 0.115M aqueous solution of disodium p-nitrophenyl phosphate tetrahydrate were added and incubated at 37°C for 1 hr.

After the incubation, 1 ml of 0.5M calcium chloride and 4 ml of 0.5M sodium hydroxide were added and filtered through Whatman No.12 filter paper. The amount of p-nitrophenol released upon incubation was determined spectrophotometrically at 410 nm. Standard curve was obtained by adding known amount of p-nitrophenol to soil instead of p-nitrophenyl phosphate. A suitable control was run by inactivating the soil enzyme by $CaCl_2$ and NaOH before adding the substrate.

Carbon mineralisation:

The rate of carbon mineralisation of the soil samples was analysed by estimating the amount of CO_2 evolved at different intervals by the procedure outlined by Pramer and Schmidt (1964).

Reagents required are:

1. Sodium hydroxide (1N)
2. Barium chloride (3N)
3. Hydrochloric acid(1N)
4. Phenolphthalein indicator-1.0g of phenolphthalein was dissolved in 100 ml 95% ethanol

Fifty grams of air dried soil was taken in 350 ml capacity glucose drip bottles in duplicate. Glucose and Cellulose were added separately to supply 0.5% carbon. Another set without the addition of either glucose or cellulose served as control (native carbon). The contents in the bottles were mixed and the moisture content was adjusted to 60% of maximum water holding capacity (field capacity). A test tube containing 10 ml of 1N NaOH was introduced into each bottle and the bottles were tightly stoppered with rubber corks and sealed. They were incubated at room temperature (30±2°C) and the CO_2 absorbed in the alkali was determined. In the beginning the CO_2 evolved was measured at weekly intervals and as the decomposition progressed, the determinations were carried out at less frequent intervals.

One ml of saturated barium chloride solution was added to alkali containing evolved CO_2 to precipitate carbonate as barium carbonate and the excess of alkali was titrated against 1N HCl using phenolphthalein as indicator. The amount of CO_2 (mg) evolved was estimated and expressed as percentage of organic carbon mineralised by the formula given below:

$$CO_2 = (B-V)\ NE$$

Where B=Volume (ml) of acid to titrate the alkali in the CO_2 collected from controls to the end point

V=Volume (ml) of acid to titrate the alkali in the CO_2 collected from treatment to the end point

N=Normality of the acid and

E=22 or 6 (Equivalent weight, if the data are expressed in terms of CO_2 E=22 or if expressed as Carbon E=6)

Nitrogen mineralisation:

Rate of nitrogen mineralization of soil was determined as described by Stanford and Smith (1972). Twenty g soil was mixed with an equal weight of 20 mesh quartz sand. The soil and sand were moistened with fine spray of distilled water and transferred to 50 ml glass filter tubes containing glass wool pads. Moistening the mixture prevented the segregation during transfer. Initial mineral nitrogen was removed by leaching with 10 ml of 0.01M calcium chloride. The soil-sand mixture was then leached with 25 ml of nitrogen deficient nutrient solution. After applying suction (60 cm Hg) to remove excess solution the filter tubes were stoppered and incubated at 30°C for four weeks. The glass wool pad in the open stem of the tube allowed air circulation. Nitrogen mineralised during this period was recovered by leaching with 100 ml of 0.01M calcium chloride. Again 25 ml of the nutrient solution was added and leaching experiment was continued at weekly intervals. The mineralised nitrogen was analysed by procedure given for ammonical nitrogen, nitrite nitrogen and nitrate nitrogen. The mineralisation was studied upto a period of 12 weeks.

(i) Ammonical Nitrogen: Ammonical nitrogen in the leachate was determined colorimetrically by Nesslerisation method (Jackson, 1973).

To twenty ml of the leachate two ml of 10 per cent sodium tartrate and three ml of Nessler's reagent were added to it and finally volume was made up to 100 ml with water. The absorbance of the colour developed was read at 410 nm in spectrophotometer. The ammonium content of the sample was determined by using a standard curve obtained with ammonium chloride over a range of 0-100 µg/ml.

(ii) Nitrite Nitrogen: Nitrite was determined colorimetrically by following Griess-Illosvay's method modified by Bremner (1965). Nitrite nitrogen was determined in the leachate collected from perfusion tube, using sulfanilamide as diazotizing reagent and N-(1-naphtyl)-ethylene diamine as coupling agent. Absorbance of the pink colour developed was read at 520 nm. A standard curve with $NaNO_2$ was obtained over a range of 0.0-10 µg nitrite-N per ml.

(iii) Nitrate Nitrogen: Nitrate nitrogen was determined colorimetrically by following phenol-2-4-disulphonic acid method (Jackson, 1973). Twenty ml of leachate were transferred to 7.5 cm diameter porcelain dish and evaporated on hot water bath. After completing evaporation three ml of phenol-disulphonic acid reagent was added to the dish and mixed. After 10 min 15 ml of water was added. Then the solution was made alkaline by adding 6 N ammonium hydroxide as indicated by development of yellow colour. The ml of ammonium hydroxide was added to ensure excess of the reagent. Contents of the dish were made up to 100 ml with distilled water and absorbance was read at 420 nm. A standard curve with KNO_3 was prepared over a range of 0-100 µg of NO_3-N.

Microbial biomass determination:

Chloroform fumigation-incubation technique was followed for microbial biomass estimation (Jenkinson and Powlson, 1976). The flush of decomposition of the soil (evolved CO_2-C or accumulated NH_4-N minus that from an unfumigated soil) during a 10 day incubation period, following the fumigation and removal of chloroform vapours, was used as the basis for microbial biomass estimation.

The unsieved moist soil samples (50 g oven dry weight basis) were first adjusted to 60% of available moisture in 100 ml beakers (and incubated at 25°C for 3 days, prior to fumigation. Both pre-incubation and fumigation were carried out in large desiccators lined with moist paper. After fumigation, the soils were transferred to glucose drip bottles of 0.5L. Then 10 ml of 0.5N NaOH in 25 ml large test tubes were introduced to absorb the CO_2.

The alkali (NaOH) containing the evolved CO_2 during the 10 day incubation period was titrated against 1N HCl.

since only a portion of the microbial biomass is mineralised during the incubation period, the size of the microbial biomass C was determined by dividing the flush of CO_2-C by a K_c factor of 0.45 (Jenkinson and Ladd, 1981).

$$B = F/K_c$$

where B is soil biomass C in $\mu g\ C\ g^{-1}$ soil; F is the CO_2 evolved by fumigated soil during incubation less that evolved by unfumigated soil incubated for the same time under the same conditions, also in $\mu g\ C\ g^{-1}$ and K_c is the fraction of the biomass C mineralised to CO_2 during the incubation.

The NH_4-N accumulated was extracted with 2N KCl and determined by Nesslerisation. The biomass N was calculated using a K_n value of 0.40 (Jenkinson and Ladd, 1981).

Cellulose decomposing capacity of the soil:

To find out the cellulose decomposition capacity of the soil Czapek-Dox medium with Cellulose as carbon source was used as described by Sankaran, (1966). Two grams of filter paper strips was added to 50 ml of medium in 100 ml conical flask and sterilised. Then 10 ml of 10% $CaCO_3$ solution was added to each of the flask under aspetic condition. One gram of root region soil of different crops were added to each flask. For control, 1.0g of soil was added and sterilised. All the flasks were incubated at room temperature (30±2°C) for 2 months. After the incubation period, 20% HCl was added to each flask till the residue ($CaCO_3$) was dissolved. The contents were filtered through a funnel fitted with a dry and weighed filter paper and washed with hot water till the washings run free of chloride. Then it was dried in oven (100 ± 3°C) and weighed. The cellulose decomposed was expressed as the percentage of the original amount of cellulose supplied in the medium.

Chemical analysis of soils:

For the chemical analysis, the soil samples were air dried in shade for a week, ground finely, sieved through a two mm sieve and stored.

Organic carbon:

The determination of organic carbon was made on 0.5g samples by following the Walkley and Black Wet Oxidation method (Black, 1965) using potassium dichromate as oxidant in presence of excess of concentrated sulphuric acid. The excess of chromic acid was titrated against standard ferrous ammonium sulphate. Blank determination was carried out without soil, along with the samples.

Total nitrogen:

Total nitrogen in the samples was estimated by following the procedure outlined by Bremner (1965). Soil (0.5g) was digested with concentrated H_2SO_4 containing 4% salicylic acid in the presence of 250 mg of catalyst mixture containing

K_2SO_4, $CuSO_4$ and Selenium powder in the ratio of 100:10:1 for 8 hr. (Microkjeldahl method). It was then diluted with water and distilled after the addition of sufficient quantity of 40% NaOH. The ammonia evolved was absorbed in two per cent boric acid solution and titrated against 0.05N H_2SO_4. The percentage nitrogen in the soil was calculated from the volume of acid consumed.

Moisture content:

The moisture content of the soil samples was determined after bringing to the laboratory, from the loss of weight of ten gram soil when dried at 105°C to a constant weight.

Total phosphorus estimation:

Two gram of the soil was taken in 150 ml conical flask. Ten ml of concentrated nitric acid was added and kept for 10 min. To this, 10 ml of nitric acid and perchloric acid mixture (1:1) were added and digested on a hot plate till the liquid got clarified (white colour). Then it was dissolved in double distilled water and filtered. The final volume was made up to 100 ml.

Total P was estimated following the procedure of Jackson (1973). Five ml aliquot was pipetted out in 25 ml volumetric flask and to which 5 ml of ammonium molybdate reagent and 1 ml of stannous chloride were added. The volume was made up to 25 ml and read at 660 nm. Total P was calculated and expressed as per cent.

Total potassium:

The potassium in the acid digested sample was estimated by flame photometry. A standard curve over a range of 2-20 ppm was obtained.

Available phosphorus:

Available phosphorus was estimated by the Bray's method No.1 (Jackson, 1973). Fifty ml of Brays-1 solution was added to 5.0 g of soil with 2.0 g of activated charcoal and shaken for 5 minutes. It was filtered through Whatman No.1 filter paper and made to 100 ml. To 5 ml of filtrate, 5 ml molybdate reagent was added and diluted with distilled water to 25 ml after the addition of dilute stannous chloride solution. The colour was read after 10 min at 660 nm. Phosphorus content was determined from the standard curve prepared using K_2HPO_4.

Available potassium

Available K^+ was determined as described in Jackson(1973). Ammonium acetate (1N) was added to 5.0g of soil, shaken for 5 min and K^+ was determined by flame photometry.

Soil pH:

Twenty grams of soil was suspended in 50 ml of distilled water (1:2.5) and pH was measured using ECL digital pH meter.

Soil moisture and root biomass in coconut based cropping systems:

The soil samples collected from various depth viz., 0-25, 26-50 and 51-100 cm were dried at 105±2°C. The soil moisture was estimated. From the core sampling, the roots were separated and dried in an oven. After drying the weight of roots were recorded and root biomass was calculated and expressed as g/100g soil.

Rhizoplane microflora:

Collection of root samples:

The root samples were collected from coconut,pepper, cocoa, cinnamon, pineapple and napier grass of coconut based cropping systems. Twenty roots, after clearing the adhering soil (for each crop), were transferred to 250 ml flask containing 100 ml of sterile water and the flasks were shaken in rotary shaker for 15 min. The dilution plating method was followed to enumerate the microflora such as bacteria (10^7), fungi (10^4) and actinomycetes (10^4). The bacteria, fungi and actinomycetes were enumerated using Nutrient Agar, Martin's Rose Bengal Agar and Conn's Asparaginate agar respectively. After pouring the media, the plates were incubated at room temperature (30±2°C). The bacterial, fungal and actinomycetes populations were counted after 48 hrs, 72 hrs and 7 days of incubation respectively. Bacteria and fungi were purified and maintained on Nutrient agar and Potato dextrose agar respectively.

Asymbiotic N_2-fixers:

The root bits (20 numbers each) were plated on Jensen's and Beckings medium to record the occurrence of asymbiotic N_2-fixers. The plates were observed after 6 days of incubation. The colonies were streaked on fresh media to purify the isolates. The percentage of root inhabiting N_2-fixers were calculated for different crops. The isolates were maintained on Beckings medium. The N_2-fixers were identified after studying the cultural, morphological, physiological, and biochemical characters.

In-vitro Nitrogen fixing ability of the isolates:

N_2-fixers were inoculated into 100 ml Beckings medium (in triplicate) in 250 ml flask and incubated at 28±2°C for 14 days. The contents were homogenised and 2 ml of homogenate was used for the nitrogen estimation by Microkjeldahl's method. The amount of nitrogen fixed was calculated and expressed as mg per g of sucrose.

Phosphate solubilisers:

The root bits from different crops (20 each) of 0.5 cm length were plated aseptically on modified Pikovskaya's medium and the plates were incubated at 30°C for 5 days. The colonies producing clear zone were streaked on fresh medium for purification. The isolates of bacteria and fungi were subjected to P-solubilising ability *in vitro* using tricalcium phosphate (Sethi and Subba Rao, 1968). Fifty ml of Pikovskaya medium in 100 ml flask were sterilised. For P-solubilising ability of bacteria, 25 mg and for fungi 50 mg of tricalcium phosphate were used (in triplicate). After inoculation, the flasks were incubated at 30±2°C for one week and three weeks for bacteria and fungi respectively. The pH of the medium was recorded after the incubation period and passed through seitz filter to remove the spores and mycelial fragments, decolourised with activated charcoal and filtered through Whatman No.41 filter paper. The filtrate (0.2 ml) was used for determining the amount of available phosphorus by Olsen's method (Jackson, 1973). The intensity of blue colour was measured at 610 nm using double beam spectrophotometer. The amount of P_2O_5 was calculated using the standard curve obtained for KH_2PO_4.

Occurrence of associative symbiotic N_2-fixers (*Azospirillum* spp.) in the root of different crops:

The occurrence of *Azospirillum* spp. was investigated using the 2,3,5 Triphenyl tetrazolium chloride reduction procedure (Dobereiner and Day, 1976) and enrichment culture method (Day and Dobereiner, 1976). Root pieces (20 for each crop) after washing were cut into 0.5 to 1.0 cm size and were aseptically placed in 5 ml of semi-solid malate medium in 15 ml screw cap test tubes supplemented with 50 mg per litre of yeast extract. Appearance of white pellicle 2-4 mm below the surface after 48 hrs of incubation indicated the presence of *Azospirillum* spp.

Identification of various microorganisms:

A. **Bacteria:** The cultural, morphological and biochemical properties of the bacterial isolates were studied based on the routine tests. Wherever possible references have been made to the various tests performed.

The staining method used to study the morphology of the bacterial isolates are given below:

1. Gram's staining I
2. Spore staining I
3. Capsule staining I * MMT Chap. 2
4. Acid fast staining I
5. Flagella staining I

The various biochemical tests conducted are as follows:

1. Fermentation of sugars (Glucose,sucrose,lactose,galactose,arabinose and mannitol) ** Microbes in action (MA) -a laboratory manual of Microbiology. Chpt. VIII & IX

2. Hydrolysis of starch I
3. Hydrolysis of casein I
4. Liquification of gelatin I
5. Indole production I** MA Chpt. VIII
6. Production of ammonia I
7. Reaction of litmus milk I
8. Hydrogen sulfide production I
9. Catalase test I-- Whittenbury (1964)

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* MMT refers to the Manual of Microbiological Technique, Society of American Bacteriologist, McGraw Hill Book Company Inc., New York, 1957.

** Microbes in Action-A laboratory manual of Microbiology by H.W.Seeley Jr. and P.J.Van Demark, W.H. Freeman and Company,1972.

Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

B. *Fungi*: The fungal isolates were identified by mounting fungal mycelium with spores on glass slide in lactophenol cotton blue and observed under light microscope. The fungal isolates were identified to generic level (Gilman, 1959). Some of the isolates were sent to Commonwealth Mycological Institute (CMI) London and the identification was confirmed.

Among the various isolates, 21 isolates of bacteria and six isolates of fungi, which are commonly occurring on the rhizosphere were used for the various studies.

Growth studies of rhizosphere fungi:

Six fungi viz., *Trichoderma harzianum*, *T. lignorum*, *Fusarium* sp., *Aspergillus niger*, *Rhizopus* sp. and *Cladosporium* sp. from the rhizosphere were used to determine the growth in Czapek-Dox broth medium. Fifty ml of medium in 100 ml flasks were inoculated with fungi. The growth of the fungi (mycelial dry weight) was recorded at 3, 6, 9, 12 and 15 days intervals. At the end of each incubation period the contents of the flasks were filtered and the filter paper containing the mycelial mat was dried at 103.

Growth of rhizosphere fungi in different cellulose compounds in vitro:

Six isolates of fungi viz., *Trichoderma harzianum*, *T. lignorum*, *Fusarium* sp., *Aspergillus niger*, *Rhizopus* sp. and *Cladosporium* sp. were inoculated into the Czapek-Dox broth medium, where in the carbon source sucrose was replaced by the different cellulose compounds (2% level). The initial pH was adjusted to 6.0. The cellulose materials used were:

1. Carboxy methyl cellulose (CMC)-hemicellulose
2. Cellobiose
3. Cellulose powder (Whatman No.1 filter paper powder)
4. Cellulose filter paper (Whatman No.1 filter paper)
5. Pectin
6. Control (Czapek-Dox broth)

Fifty ml Czapek-Dox broth medium without sucrose was distributed in 100 ml Erlenmeyer conical flask. To this flask respective cellulose materials (2 per cent) were added. The flasks were sterilised. The flasks were incubated for six days (three replication for each treatment) and filtered. The mycelial biomass was calculated as dry weight of the mycelium in mg. The pH of the culture filtrate was also determined.

In vitro Production of cellulase enzymes:

The *in vitro* production of cellulolytic enzymes was studied by following the method of Manibhushan Rao (1971).

For studies on cellulase (C_x) activity, the six different fungi were grown on basal medium, (Czapek-Dox broth) (-carbon) + 2% (W/v) Carboxy methyl cellulose (CMC-hemicellulose). Fifty ml aliquots of the media were distributed in 100 ml Erlenmeyer flask. The pH of medium was adjusted to 6.0 with 1N NaOH or HCl before autoclaving. The media were sterilised at 15 P.S.I (lbs/sq.inch) for 15 min. Then it was inoculated with mycelial discs from 7-day-old cultures. Incubation was done at room temperature (30±2°C). The culture broth was decanted to obtain the enzyme source to study the cellulolytic activity (C_x activity) by viscocity reduction method after 6 days of growth. For C_1 activity three replicates were removed at 2 days intervals for 10 days.

(i) <u>Cellulase enzyme assay:</u> The culture filtrate free from mycelium was centrifuged at 12,000 rpm for 30 min at 4°C. The clear supernatants were used as enzyme source.

The reduction in the viscocity of carboxymethyl cellulose (CMC) has been established as an estimate of cellulase (C_x) activity (Kelman and Cowling, 1965). To determine the efflux time of the mixture at pre-fixed interval, suction was applied in the small arm of the viscometer. The per cent decrease in viscocity was calculated. The viscocity changes were recorded in Ostwald-

Cannon-Fenske viscometer. The viscometer was kept in water bath at 35 ± 0.5°C. The efflux time was recorded after incubation for 2, 5, 10, 20, 25 and 30 min respectively. Per cent decrease in viscosity of the substrate was calculated using the following formula:

$$V = \frac{To - T}{To - TH_2O} \times 100$$

Where, V = per cent decrease in viscosity

To = flow time in seconds at zero time

T = flow time of the reaction mixture at time t and

TH_2O = flow time of distilled water

The reaction mixture was mixed in a small flask in proportion 4 ml of CMC, 1 ml of the buffer and 2 ml of the enzyme into the viscometer kept in water bath. The contents were mixed by drawing air gently through the large arm of the viscometer.

(ii) *Cellulase enzyme*: The fungi were screened for cellulase production. Cellulase (C_1) activity was assayed by the colorimetric method (Norkrans, 1950), where the reduction in the absorbance of CMC by the enzymatic activities of the fungi was determined.

Growth of rhizosphere fungi and bacteria at different pH, sodium chloride and sucrose concentration:

(i) *Fungi*: Six fungi were used. The pH of Czapek-Dox broth medium was adjusted to 3, 5, 7 and 9 using NaOH or HCl.

To 100 ml Erlenmeyer conical flask 50 ml of the medium were transferred and sterilised. The flasks were inoculated with the fungal isolates (in duplicate) and incubated at 30°C for 6 days. At the end of the incubation the mycelial dry weight was recorded. The sodium chloride at 0.5, 2.5 and 5.0% and sucrose at 5 and 10% concentration were used respectively in Czapek-Dox medium to study the growth of the fungi.

(ii) *Bacteria:* The growth of 21 isolates of rhizosphere bacteria was studied at different pH (3, 5, 7 and 9) in Nutrient Agar (NA) broth and agar medium. The growth of the isolates at different levels of sodium chloride (0.5, 2.5 and 5%) and sucrose (5.0 and 10%) was studied by incorporating it in to the NA broth and agar medium. The growth was recorded after 48 hrs of incubation at 30 ± 2°C.

Effect of pesticides on the growth of rhizosphere fungi and bacteria:

The effect of certain soil applied fungicides and insecticides was studied *in vitro* on the rhizosphere microflora of coconut. The pesticide common names, trade names, chemical names and the dosages used are given in Table 1. Apart from the recommended dose, lower concentrations were also tested. In all the studies the same concentration was used and the media incorporated with pesticides were incubated at 30 ± 2°C.

Table 1. The pesticide common, trade and chemical names and the dosage used

Pesticide			Dosage	
Common name	Trade name	Chemical name	recommended	lower
Bordeaux mixture	Copper sulphate and lime	Copper sulphate + hydrated lime	1.0%	0.5% 0.1%
Blitox	Blitox	Copper oxychloride	0.1%	0.02%
Captan	Captan	N-(Trichloromethylthio)-4-cyclohexene-1,2-dicarboximide	0.2%	0.04%
BHC	50% wettable powder (soil application, 120 kg/ha)	Benzene-hexachloride	0.1%	0.02%
Carbofuran	Furadan 3%G (3 kg a.i./ha)	2,3-dihydro-2, 3-dimethyl-7-benzofuranyl methylcarbamate	0.2%	-

Effect of pesticides on the growth of rhizosphere fungi *in vitro*:

(i) *Liquid medium:* The basal medium, Czapek-Dox 50 ml was prepared and dispensed in 100 ml flask and sterilized at 15 p.s.i. for 15 min. The pesticides were incorporated aseptically in to flask and the 6 isolates of rhizosphere fungi were inoculated. The fungal growth was recorded on 3 and 6 days of incubation and mycelial weight was recorded after 6 days of incubation. The growth and mycelial biomass production in each treatment were recorded.

(ii) *Growth of rhizosphere fungi in solid medium:* The Czapek-Dox agar medium was used for the studies. After melting and cooling the medium, the pesticides were incorporated and poured into plates. The test organisms (6 fungi) were grown on Czapek-Dox agar for 5 days. Mycelial discs were cut from the edge of the colony with a cork borer (10 mm diameter). The discs were placed upside down at the centre of plates and incubated at 30 ± 2°C. The mycelial growth was recorded on 3rd and 5th day of incubation. The colony diameter was measured in two directions at right angles to each other and the average of two measurements was taken.

Effect of pesticides on the bacterial growth *in vitro*:

(i) *Liquid medium:* Nutrient broth medium was used (50 ml in 100 ml flask). The pesticides were incorporated after sterilising the media. The bacterial isolates (21 numbers) were inoculated and two replications were maintained for each treatment. They were incubated at 30 ± 2°C and the growth was recorded after 48 hrs.

(ii) Solid media: The nutrient agar medium was prepared and sterilised in 50 ml lots. After melting and cooling to 50°C, the pesticides (Table 1) were added and poured into Petri plates. The bacterial isolates (21 numbers) were inoculated in duplicate and the plates were incubated at 30 ± 2°C for 48 hr to record the growth.

Effect of pesticides on the growth of asymbiotic N_2-fixers and P-solubilisers in vitro:

The growth of 12 isolates of asymbiotic N_2-fixers were studied using Beckings medium after incorporating the pesticides. The growth was recorded on 5th and 10th day of incubation. The effects of pesticides on the growth of P-solubilisers (2 bacteria; Bacillus sp. and Pseudomonas sp. and four fungi (Aspergillus niger - isolate 39 and 40) and Penicillium spp. (isolate No.41 and 42) were also studied in both solid and liquid medium (modified Pikovskaya's medium).

Antagonism of fungi and bacteria against Phytophthora spp.:

The fungal isolates (62) and bacteria (26) isolated from the rhizosphere and rhizoplane of various crops of coconut based cropping systems were screened for the antagonistic properties against *Phytophthora arecae* isolates from arecanut (*Areca catechu* L.). Potato Dextrose Agar (PDA) medium was used throughout the studies. The mycelial discs of (10 mm size) *Phytophthora* were inoculated in the centre of the plate and into each of the plates two test organisms were inoculated. The plates were incubated at 28 ± 2°C and the antagonism was recorded after 3 and 5 days of incubation. The fungi and bacteria inhibiting the growth of *Phytophthora arecae* were selected.

Antagonistic studies of the selected fungi and bacteria:

The antagonistic properties of the selected fungi and bacteria were tested against *Phytophthora arecae* and *Phytophthora palmivora*, isolated from 'mahali' or 'koleroga' disease of arecanut and black pod disease of cacao, were used by following the standard agar disc method and antibiotic cup assay method (Johnson *et al*., 1960).

(i) *Agar disc method:* The spore suspension of all the antagonistic fungi and the two *Phytophthora* were prepared separately in sterile water. The lawn of antagonistic fungus was prepared in petriplates using PDA two days before the test. The mycelial cum spore suspension of *Phytophthora* was seeded and allowed to solidify. After solidification agar disc of 10 mm diameter size was cut and removed from the centre of each plate by using cork borer. Then it was replaced with the same size agar disc of antagonistic test organisms from the lawn prepared earlier. The plates were incubated for 3 days and the inhibition zone was measured. Three replicates were maintained for each organism.

(ii) *Antibiotic cup assay method*: The test organisms were grown in Czapek-Dox (100 ml in 250 conical flask). The flasks were inoculated with 1 ml spore suspension of antagonistic fungi (in triplicates) and incubated at 26 ± 2°C for 10 days. In case of bacteria 1 ml of 48 hr old culture suspension was used. The culture broth was homogenised and passed through double layer, cheese cloth. The filtrate was centrifuged at 10,000 rpm for 10 min to remove the spore and mycelium strand. The bacteria inoculated suspensions were passed through seitz filter. The PDA medium was seeded with the mycelium cum spore suspension of *Phytophthora* spp. with antibiotic cup in the centre. Each cup was filled with 0.2 ml of culture filtrate and plates were kept for 8 hr in refrigerator (4°C) to allow the filtrate to diffuse into the medium. Then the plates were taken out and incubated at 26 ± 2°C for 4 days and inhibition zone was measured.

Biochemical characterisation of root exudates of coconut:

Collection of root exudates: The root exudates were collected from intact roots of coconut. Roots were cleared from soil, washed with water to remove the adhering soil and cleaned with distilled water. The cleaned root was dipped sequentially in 80% ethanol for 30 sec and 0.1% $HgCl_2$ for 3 min. The root was then washed with sterile water and again dipped sequentially in 0.003% streptomycin sulphate for 1 min and 0.2% aqueous suspension of copper oxychloride for 1 min. The root was then immediately introduced aseptically into previously sterilized test tubes (150 mm x 22 mm) containing fluted Whatman No.1 filter paper strips (10 cm x 8 cm, 0.70 to 0.75g) and plugged with cotton. The test tube with the root was covered with a polythene bag, fixed at the top of the test tube and burried into soil. After 5 days, the test tube was removed from the soil and root was cut at the top of the test tube. In all 12 set of roots were installed for the collection of root exudates.

The filter paper strips were plated to check for bacterial and fungal contamination before extraction. The filter paper strips showing the presence of bacteria or fungi were discarded. The filter paper strips without any microorganisms were taken for extraction. The cut roots were dried and its weight was recorded.

Extraction, estimation and identification of root exudates: The filter paper strips were cut into small pieces and extracted thrice with 80% ethanol. A filter paper control was also simultaneously extracted with ethanol. The ethanol extract after filtration was concentrated and the residue taken in 2 ml distilled water and fractionated into aminoacid, sugar and organic acid fraction by ion exchange chromatography with Dowex 50H$^+$ and Dowex 1 (formate form) resins. Aminoacid, using glycine as standard (Yemm and Cocking, 1955), total sugar (Dubois et al., 1951) and reducing sugar (Nelson, 1944), using glucose as standard, were estimated.

Aminoacids in the aminoacid fraction were separated by descending paper chromatography (Whatman No.1), using n-butanol : acetic acid : water (80 : 20 : 20 V/v), phenol : water (74 g molten phenol : 24 ml water) and propanol : water (70 : 30) solvent system and the chromatograms were sprayed with 0.3 per cent ninhydrin in acetone for detection. Sugars were separated by descending paper chromatography (Whatman No.1) using n-butanol : ethanol : water (10 : 1 : 2 V/v) and n-propanol : ethanol : water (7 : 1 : 2 V/v) solvent systems and sprayed with silver nitrate (Trevelyan et al., 1950), aniline phthalate reagent (Patridge, 1949) and Urea-HCl reagent (Dedonder, 1952).

Organic acids were separated by descending paper chromatography (Whatman No.1) using n-butanol : formic acid : water (60 : 15 : 25 V/v) solvent system and chromatogram was developed by dipping the paper, after thoroughly drying in the even in 0.04% bromophenol blue in 95% ethanol.

Biochemical characterisation of root region soils of coconut based cropping system:

Collection of soil: Soil samples were collected from the following cropping systems:

1. Coconut based multistoreyed cropping system with coconut-pepper-cocoa-pineapple
2. Coconut based mixed farming system
3. Coconut monocropping system (coconut basin and interspace)

Six samples were collected from each system. The root region soils were collected by core sampling using soil auger from 0-25 cm depth. The sampling was done from four locations of the coconut basin and from this a composite sample was obtained. The soil samples were oven dried at 103 ± 2°C. Soil sample (10 g) was taken and extracted with 80% ethanol. Residue after ethanol evaporation was dissolved in 2 ml of distilled water and fractionated into aminoacids, sugars and organic acids using Dowex 50H$^+$ and Dowex 1 resin columns. These fractions after evaporation were taken in 0.5 ml of double distilled water. The quantitative and qualitative estimations of sugars, aminoacids and organic acids were carried out as per the procedure described earlier for the biochemical characterisation of root exudates of coconut. The total phenols were estimated following procedure of Folin-Ciocalteu reagent (Bray and Thorpe, 1954) using catechol as standard.

Effect of root extract on the growth of Rhizosphere micro-organisms

The roots of coconut, cacao and pineapple were collected from the coconut based cropping systems. Ten grams of roots were ground in 20 ml of water. The filtrate was collected and made up to 30 ml. The extracts were tested against fungi and bacteria for its growth *in vitro*.

A. Effect of root extract on fungal growth:

(i) *In liquid media*: Czapek-Dox broth media was used and the root extract (5 ml each) was incorporated into the medium (45 ml medium in 120 ml flask) after sterilisation. Six isolates of fungi were inoculated in triplicqte and the flasks were incubated for 6 days. After the inoculation, it was filtered and mycelial dry weight was recorded.

(ii) *Solid medium*: The root extract of coconut,pepper and cacao was filtered using seitz filter. Each fungus was seeded in Martin's Rose Bengal agar. Agar disc were cut from centre of the plate using cork borer and removed. The root extract (0.2 ml) was poured into the well and the plates were incubated for 3 days. The control without the root extract was maintained. All the treatments were replicated thrice.

B. Effect of root extract on bacterial growth:

Nutrient agar plates were seeded with the bacteria (12 isolates - 2 plates each). The extracts were poured (0.2 ml each) into the well at the centre of the plate and incubated for 48 hrs to record the growth.

RESULTS

Results

1. Soil Microflora and Micro Biological Activities in Coconut Based Multistoreyed Cropping system and Coconut Monocropping System:

1.1. Soil microflora and beneficial microorganisms in the root region:

The results of the studies on root region microflora of *coconut based multistoreyed cropping system consisting of* pepper, cinnamon and pineapple during pre- and post-monsoon season, are given in Table 2. The data revealed that during pre-monsoon season there is no difference in the bacterial population between multistoreyed cropping and coconut monocropping system. But it was significantly less in the monocropping system during post-monsoon period. The bacterial counts in the interspace soil were less as compared to the root region soils in both multistoreyed and monocropping systems. The fungal counts showed a reverse trend as that of bacteria, indicating the higher population in the pre-monsoon period. The interspaces of multistoreyed crops recorded higher microbial load as compared to the interspace of coconut monocropping. However, the actinomycetes population did not show any definite trend during both the pre-monsoon and post-monsoon periods. The bacterial, fungal and actinomycetes numbers decreased with the increase in depth of soil and the population was least in 51-100 cm depth. The interspace soils in multistoreyed cropping system showed varied trend in the microbial load in different soil depths.

Table 2. Microflora in the root region and interspaces of coconut based multistoreyed cropping (MS) system – cinnamon + pepper + pineapple and coconut monocropping system in pre- and post-monsoon seasons.

Crop/Inter-space	Soil depth (cm)	Microbial counts* (No./g soil)					
		Pre-monsoon (May)			Post-monsoon (Oct)		
		Bacteria $\times 10^5$	Fungi $\times 10^3$	Actinomycetes $\times 10^5$	Bacteria $\times 10^5$	Fungi $\times 10^3$	Actinomycetes $\times 10^5$
Coconut (MS)	0-25	5.34	39.00	5.37	17.68	11.68	3.35
	26-50	4.37	34.00	1.35	18.46	20.67	1.35
	51-100	1.33	16.38	2.68	8.60	6.35	1.00
Cinnamon	0-25	4.57	21.35	6.35	7.33	6.33	3.00
	26-50	4.40	29.67	1.68	6.00	6.00	0.35
	51-100	3.61	23.00	0.68	3.35	5.69	0.68
Pepper	0-25	9.80	29.00	6.38	10.38	9.38	3.68
	26-50	3.84	27.00	4.68	5.00	10.35	2.35
	51-100	-	-	-	-	-	-
Pineapple	0-25	4.71	25.00	4.68	10.98	7.35	4.67
	26-50	4.82	37.00	5.68	5.68	3.68	1.00
	51-100	1.89	5.00	0.00	3.00	0.68	1.00
Coconut-Coconut interspace	0-25	2.13	18.12	2.00	11.68	5.67	0.68
	26-50	3.57	14.69	0.68	4.33	7.67	0.35
	51-100	0.77	13.29	1.00	2.67	8.67	0.35
Coconut-Pineapple interspace	0-25	2.14	17.38	2.35	9.80	10.33	3.00
	26-50	4.10	10.28	1.00	5.33	4.33	0.35
	51-100	2.80	15.68	0.68	3.58	3.67	0.35
Cinnamon Pineapple interspace	0-25	1.07	12.35	0.68	4.67	13.00	1.00
	26-50	4.20	11.00	3.68	6.28	10.00	0.35
	51-100	0.84	9.68	0.00	7.98	0.00	0.00
Pineapple-Pineapple interspace	0-25	1.57	21.00	2.68	2.33	6.33	0.68
	26-50	1.37	9.33	0.68	1.67	1.67	0.38
	51-100	2.86	10.68	1.00	0.00	0.00	0.00
Coconut Monocropping	0-25	5.67	25.33	1.68	6.67	11.67	3.67
	26-50	7.64	29.35	0.68	7.35	15.33	4.00
	51-100	1.40	15.68	0.00	2.47	5.67	1.68
Coconut Monocrop interspace	0-25	1.38	10.35	1.68	1.87	4.67	4.35
	26-50	0.70	4.68	0.35	0.68	5.67	4.35
	51-100	0.35	3.68	0.00	1.00	2.96	2.00

*Average of three replications

- = Not determined

CD (P = 0.05)						
Crop	1.56	4.48	1.30	2.04	3.09	0.79
Depth	0.98	2.99	0.71	1.59	1.70	0.57
Crop x Depth	2.10	6.48	2.26	3.69	5.29	1.71

The enumeration of microflora in the root region and interspaces of coconut-pepper-cacao (single hedge)-pineapple were carried out in May (pre-monsoon) and October (post-monsoon) period. The bacterial and fungal populations were significantly more in coconut, cacao and pineapple in the multistoreyed cropping system as compared to the root region soils of coconut monocropping system in both the season. Whereas the actinomycetes counts was higher in the pre-monsoon period as compared to the post-monsoon in the multistoreyed cropping system. The bacterial numbers were significantly high in the coconut root region soils as that of cacao and pineapple (Table 3).

The populations of beneficial microorganisms in the root region of coconut-pepper-cinnamon-pineapple cropping system and coconut monocropping system are presented in Table 4. The asymbiotic nitrogen fixers were higher in the root region of pineapple (18×10^3) and cinnamon (15.7×10^3) as compared to other crops ($4.3 - 8.8 \times 10^3$). The phosphate solubilizing bacteria were more in the root region of cinnamon and pepper, whereas the P-solubilizing fungi did not show much variation in the root region of various crops. In general, the population of beneficial microorganisms were more in 0-25 cm depth as compared to 26-50 cm depth. Among the nitrifying bacteria,

Table 3. Microflora in the root region and the interspaces of coconut-based multistoreyed cropping system– cacao+pepper+pineapple and coconut monocropping system in the pre- and post-monsoon seasons

Crop	Soil depth (cm.)	Microbial counts* (No./g of soil)					
		Pre-monsoon			Post-monsoon		
		Bacteria, $\times 10^5$	Fungi $\times 10^3$	Actinomycetes $\times 10^5$	Bacteria, $\times 10^5$	Fungi $\times 10^3$	Actinomycetes $\times 10^5$
Coconut MS	0-25	21.05	32.33	3.35	49.89	18.67	1.33
	26-50	10.95	24.44	2.35	30.00	12.35	1.44
	51-100	4.36	18.67	0.69	11.94	9.38	0.55
Cacao	0-25	14.00	42.65	4.38	14.89	21.35	0.77
	26-50	9.56	26.33	3.69	12.77	13.56	0.77
	51-100	2.00	16.67	1.33	3.35	10.35	1.00
Pineapple	0-25	12.38	26.60	4.89	15.55	15.93	1.89
	26-50	6.29	20.47	2.68	8.22	12.39	3.22
	51-100	-	-	-	-	-	-
Coconut-cacao interspace	0-25	5.38	21.22	1.69	8.67	10.68	2.00
	26-50	5.56	17.32	1.35	8.44	6.78	1.10
	51-100	1.33	8.66	0.67	2.00	3.59	1.00
Coconut Monocropping	0-25	6.36	13.67	4.00	7.69	10.75	3.67
	26-50	5.96	14.00	2.69	5.44	10.45	4.00
	51-100	1.66	6.63	0.16	2.04	5.98	1.67

*Average of three replications

CD (P = 0.05)						
Crop	3.18	4.96	0.97	3.58	4.12	0.64
Depth	2.76	3.84	0.73	3.04	3.66	0.50
Crop x Depth	6.10	8.58	1.35	6.73	6.30	1.11

able 4. Beneficial microorganisms in the root region of coconut based multistoreyed cropping system and coconut monocropping system

Cropping system	Soil depth (cm)	N_2-fixers x10^3	P-solubilisers		Nitrifying bacteria		VA-mycorrhizae	
			Bacteria x10^4	Fungi x10^3	Nitrosomonas x10^2	Nitrobacter x10^3	Spore count (No./50g soil)	% root colonised
A. Coconut-cinnamon-pepper-pineapple								
Coconut	0-25	4.3	2.4	1.4	28.5	48.0	163.0	61.4
	26-50	7.0	2.0	0.5	-	-	32.0	31.3
Cinnamon	0-25	13.7	10.7	3.4	21.0	40.0	186.7	70.0
	26-50	4.7	20.7	2.0	-	-	31.8	37.0
Pepper	0-25	5.4	15.3	2.0	-	-	-	-
	26-50	4.6	2.0	0.0	-	-	-	-
Pineapple	0-25	18.0	8.7	1.7	47.0	20.0	173.0	67.3
	26-50	4.4	3.3	2.0	-	-	40.5	28.0
B. Coconut-pepper-cacao-pineapple								
Coconut	0-25	8.8	3.5	2.0	42.5	28.0	169.8	63.3
	26-50	5.2	2.3	1.2	-	-	51.3	27.3
Cacao	0-25	11.1	5.2	1.2	25.5	43.0	151.5	68.7
	26-50	6.5	2.5	0.9	-	-	31.5	28.3
Pineapple	0-25	10.2	8.0	1.7	34.0	36.0	180.5	64.0
	26-50	5.4	2.0	1.0	-	-	38.2	34.0
C. Coconut Monocropping								
	0-25	6.0	2.7	1.5	29.0	40.0	139.5	58.5
	26-50	4.6	1.6	0.7	-	-	29.5	31.2

*Average of three replications

- = Not determined

Microbial count = No./g dry soil

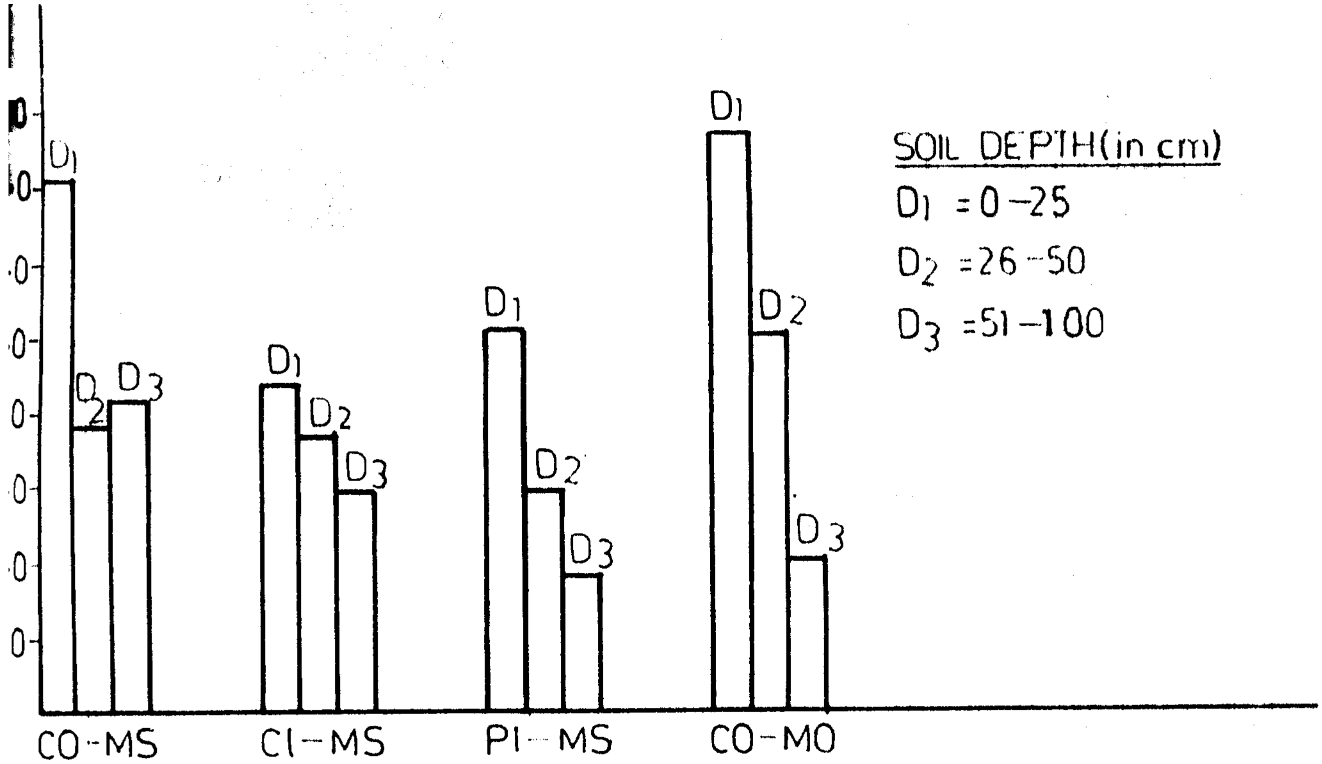
the population of Nitrosomonas sp. was higher in the root region of pineapple of the multistoreyed cropping system. The Nitrobacter sp. did not indicate any variation in counts between the cropping systems.

The data on beneficial microorganisms, such as asymbiotic N_2-fixers, P-solubilisers (bacteria and fungi) and nitrifying bacteria (Nitrosomonas and Nitrobacter) for the coconut-pepper-cacao (single hedge)-pineapple cropping system and coconut monocropping systems are given in Table 4. The load of asymbiotic N_2-fixers and P-solubilisers (bacteria) were higher in the root region soils of multistoreyed cropping system as compared to coconut monocropping system. The counts of Nitrosomonas were higher in the multistoreyed system but the Nitrobacter numbers did not reveal any variation. The endogone spore counts and the VA-mycorrhizal colonisation of the coconut based multistoreyed cropping system and coconut monocropping, indicated that the endogone spores and VA-mycorrhizal colonisation was slightly higher in cinnamon as compared to the coconut and cacao in the multistoreyed cropping system and coconut monocropping (Table 4). A progressive decrease in spore population and VA-mycorrhizal colonisation was observed with the increase in soil depth.

1.2. Soil enzyme activities in the root region:

The soil enzyme (urease, dehydrogenase and phosphatase) activities in the root region and interspace of coconut-pepper-cinnamon-pineapple cropping system and coconut monocropping are presented in Figs. 1 to 3 a and b. The results indicated that urease and dehydrogenase activities showed a marked difference in the surface soil (0-25 cm depth) of the root region of coconut both in multistoreyed and monocropping system as compared to the other crops (Figs.1a and 2a). Urease activity was high in the interspace soils of cinnamon-pineapple (MS), coconut-pineapple (MS) and pineapple-pineapple (MS) as compared to coconut monocropping (Fig. 1b). The dehydrogenase activity was significantly low in the root region as well as in the interspace soils of cinnamon (Figs. 2a and b). The phosphatase activity was significantly highest in the root region soils of coconut in the multistoreyed cropping system (120 µg/g soil/hr) (Figs. 3a and b). In general the urease and phosphatase enzyme activities decreased with increase in soil depths(26-50, 51-100 cm) both in the root region and interspace of various crops.

The enzyme assay for urease, dehydrogenase and phosphatase in the root region of coconut-pepper-cacao-pineapple cropping system and coconut monocropping system revealed that the urease activity was low in cacao and pineapple (Fig. 4). Dehydrogenase activity however did not indicate any difference in the root region soils of various crops. Activities of phosphatase was significantly high in the root region of multistoreyed cropping system. The phosphatase activity



ig.1a. SOIL UREASE ACTIVITY IN THE ROOT REGION OF COCONUT BASED MULTISTOREYED CROPPING (MS) AND COCONUT MONOCROPPING (MO) SYSTEM. (CO-COCONUT, CI-CINNAMON, AND PI-PINEAPPLE)

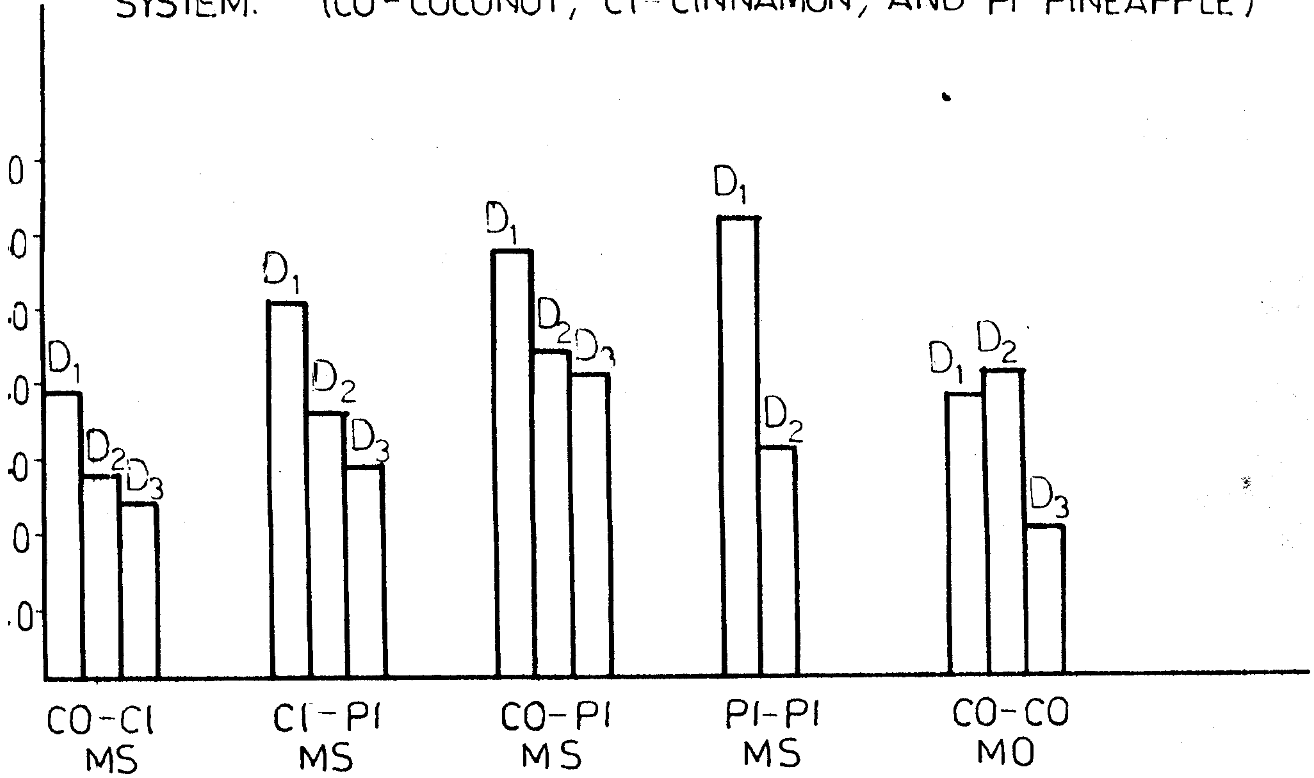


Fig.1b. SOIL UREASE ACTIVITY IN THE INTERSPACE OF COCONUT BASED MULTISTOREYED CROPPING (MS) AND COCONUT MONOCROPPING (MO) SYSTEM.
(CO-COCONUT, CI-CINNAMON AND PI-PINEAPPLE)

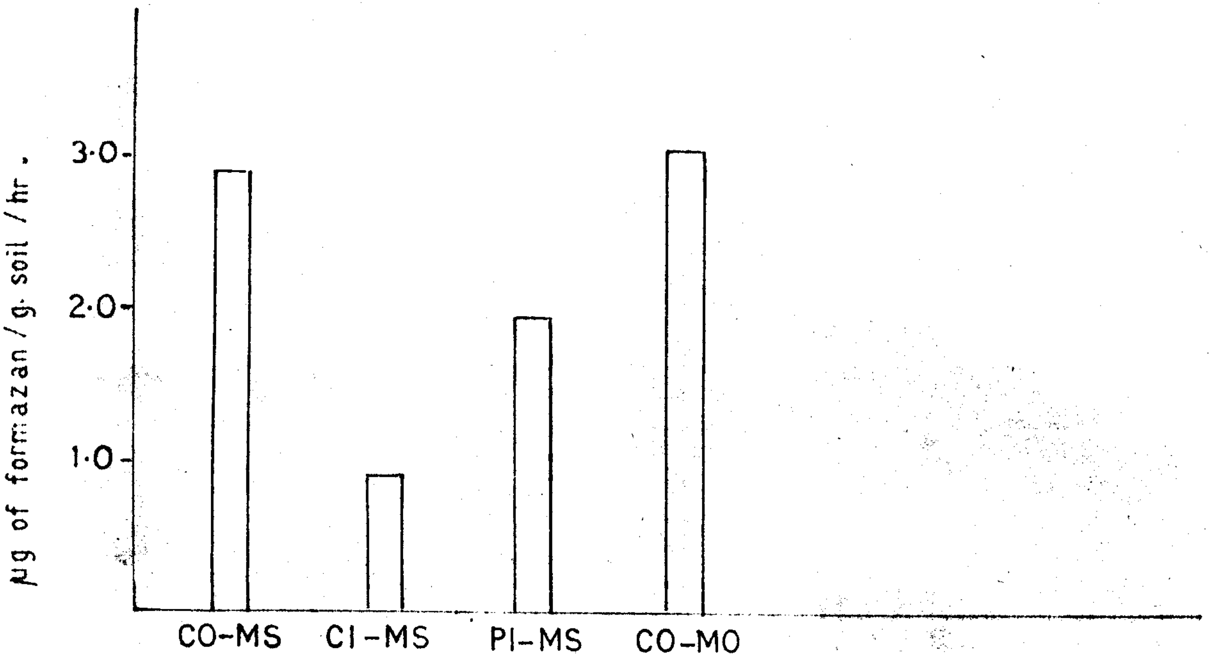


Fig.2a. Dehydrogenase activity of soil in the root region of coconut multistoreyed cropping (MS) and coconut monocropping (MO) system at 0—25 cm. depth.

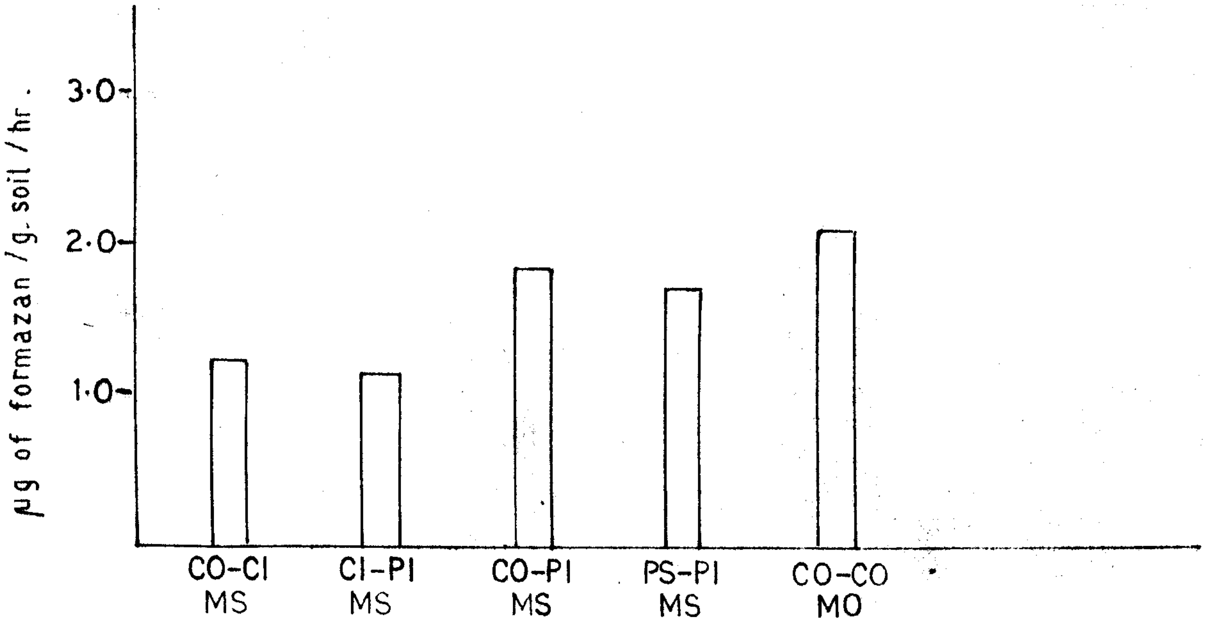


Fig.2b. Dehydrogenase activity of interspace soil of coconut multistoreyed cropping (MS) and coconut monocropping (MO) system at 0—25cm. depth.

(CO = Coconut, CI = Cinnamon, PI = Pineapple .)

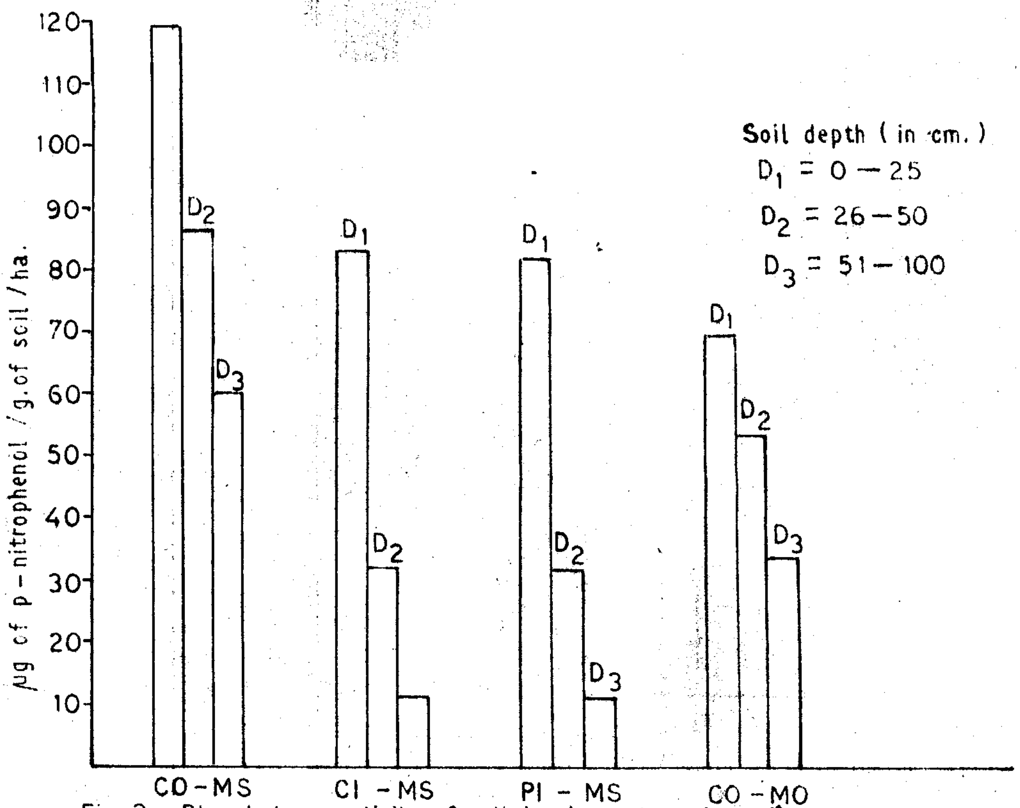


Fig. 3a. Phosphatase activity of soil in the root region of coconut based multi storeyed cropping system (MS) and monocropping systems (MO)

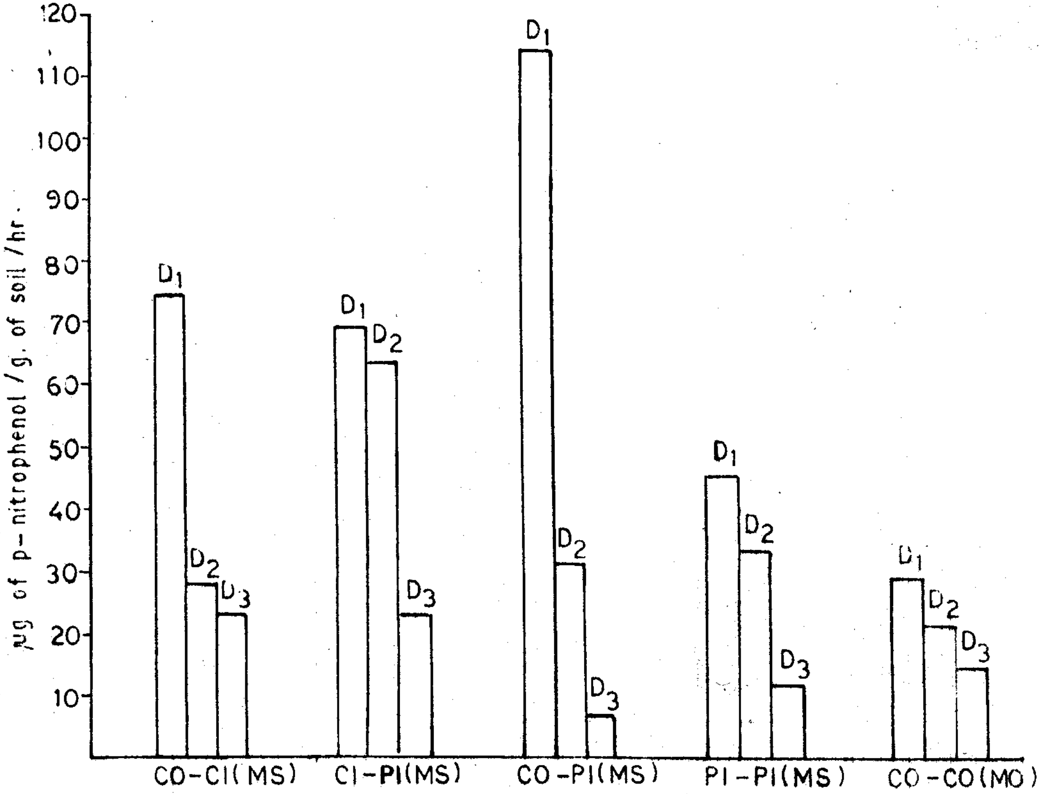


Fig. 3b. Phosphatase activity of interspace soil of coconut based multi-storeyed cropping (MS) and coconut monocropping (MO) system.

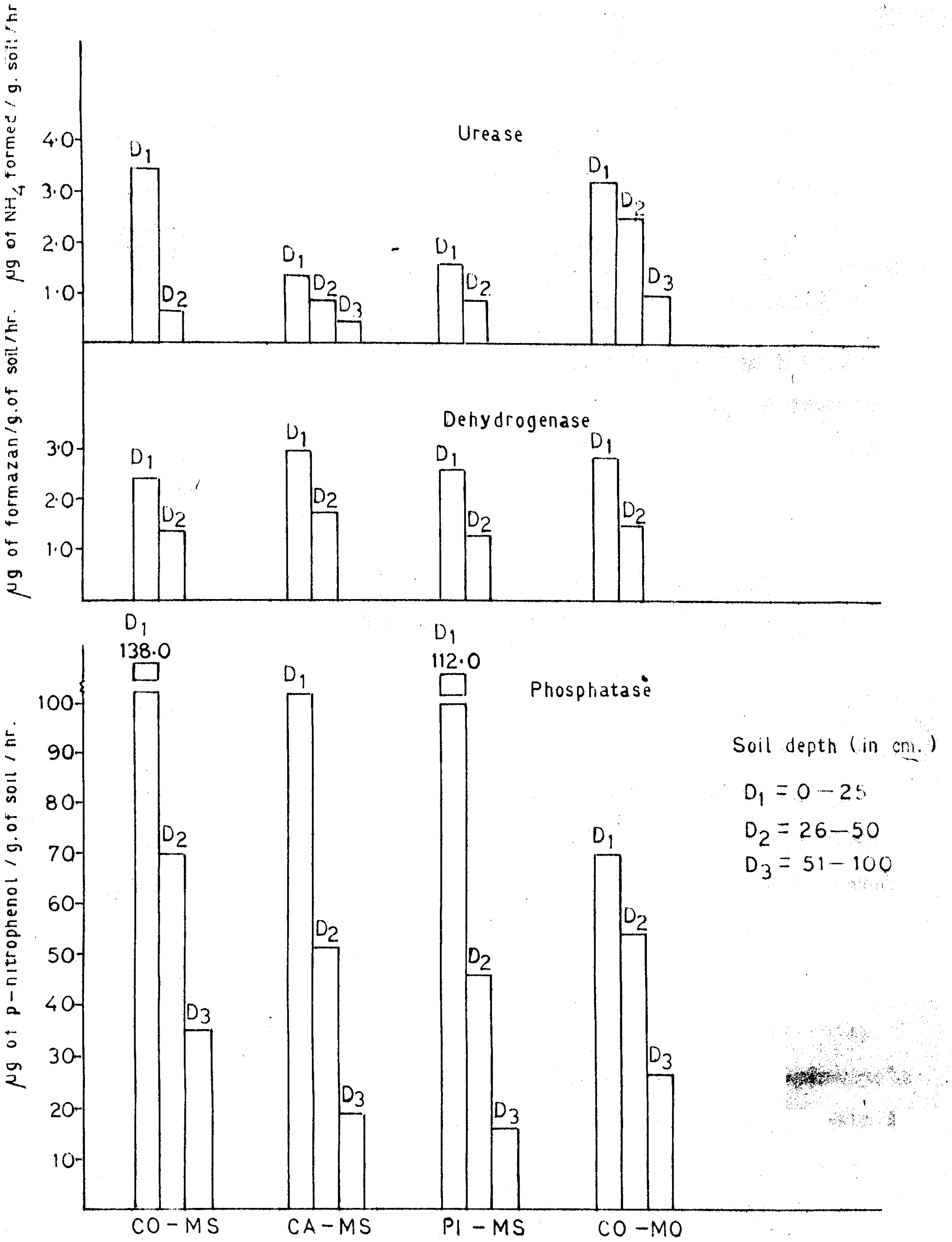


Fig. 4. Soil enzyme activity in the root region of coconut based multi storeyed cropping system (MS)(pepper- cacao - pineapple).and coconut monocropping (M system

(CO = Coconut, CA = Cacao, PI = Pineapple)

(130 µg of p-nitrophenol/g soil/hr) was highest in the root region soils of coconut (MS).

1.3. Soil microflora and enzyme activities in the rhizosphere

Microflora and soil enzyme activities in the rhizosphere of coconut-pepper-cinnamon-pineapple (MS) and coconut monocropping are presented in Table 5. The bacterial counts were high in the rhizosphere of pineapple (16.3 x 10^6) and cinnamon (12.7 x 10^6) as compared to coconut (10.2 to 10.6 x 10^6) in multistoreyed and monocropping system. The population of fungi and actinomycetes did not show much variation between the crops. The rhizosphere of pineapple recorded higher counts of N_2-fixers and P-solubilisers. The urease activity was low in the rhizosphere of coconut in the monocropping system as compared to the crops in multistoreyed system. Dehydrogenase enzyme showed a reverse trend. Activities of phosphatase was low in the rhizosphere of cinnamon and pineapple as compared to coconut both from multistoreyed and monocropping systems.

The rhizosphere microflora and soil enzyme activities were greatly influenced by the coconut-pepper-cacao-pineapple cropping system as compared to coconut monocropping (Table 6). The counts of bacteria, N_2-fixers and P-solubilizers were more in rhizosphere of multistoreyed cropping system. A similar trend in soil enzyme system was observed in this

Table 5. Microflora and soil enzyme activity in the rhizosphere of coconut based multistoreyed cropping and coconut monocropping system*

Crop	Bacteria x10^6	Fungi x10^3	Actinomycetes x10^5	N_2-fixers x10^3	PO_4-solubilizers x10^4 (bacteria)	Urease	Dehydrogenase With out glucose	Dehydrogenase With glucose	Phosphatase
A. Coconut-pepper-cinnamon-pineapple									
Coconut (MS)	10.38	12.50	0.67	4.28	3.68	6.28	2.01	4.3	124.
Cinnamon	12.67	10.73	0.70	6.28	2.70	6.02	1.92	3.8	88.
Pineapple	16.29	11.65	0.70	8.50	5.68	6.18	1.95	4.0	98.
B. Coconut-pepper-cacao-pineapple									
Coconut	17.41	11.54	1.03	4.18	4.48	6.48	2.11	4.68	131
Cacao	24.18	15.52	0.94	5.72	4.80	6.37	2.07	3.72	86
Pineapple	20.43	11.60	0.77	8.27	6.28	6.22	1.97	3.71	94
C. Coconut-monocropping system									
	10.07	10.37	0.37	2.80	2.67	5.41	2.82	4.04	133

*Average of three replications

Urease – µg of NH_4 formed/g soil/hr
Dehydrogenase – µg of formazan/g soil/hr
Phosphatase – µg of P-nitrophenol/g soil/hr

cropping system as that of coconut-pepper-cinnamon-pineapple cropping system. Addition of glucose enhanced the dehydrogenase activity of the rhizosphere soils as compared to the native dehydrogenase activity.

1.4. Carbon and nitrogen mineralisation:

The rate of carbon mineralisation was low in the root region of cinnamon as compared to the root region soils of coconut in both the systems (Table 6). The root region soils of coconut and pineapple recorded a higher rate of carbon mineralisation (42-46 mg/100g soil), and mineralisation was least in the interspace soils of coconut monocropping system (12 mg/100 g soil). The addition of glucose and cellulose separately (0.5%) increased the rate of mineralisation by 3-4 fold and maximum being recorded in the glucose amended soils. Further, the mineralisation rate decreased with increase in soil depth except in interspace soils of coconut monocropping.

The studies on the nitrogen mineralisation revealed lower level of NH_4-N in the surface soil (0-25 cm). But in the interspace soils of coconut monocropping the trend was reverse. The trend for NO_3-N formation in the nitrogen mineralisation was similar to that of native carbon mineralisation.

The mineralisation of carbon and nitrogen in the coconut-pepper-cacao-pineapple cropping and coconut monocropping system revealed that carbon (native) and nitrogen mineralisation

Table 6. Carbon and nitrogen mineralisation in three soil depths of coconut based multistoreyed cropping system (MS) and coconut monocropping system in vitro.

Cropping system & crops	Soil depth (cm)	Carbon mineralisation* mg/100g soil			Nitrogen mineralisation* (mg/100g soil)		
		Native carbon	Glucose (0.5%)	Cellulose (0.5%)	NH_4-N	NO_3-N	Total N
A. Coconut-pepper-cinnamon-pineapple							
Coconut	0-25	42.2	190.8	150.8	0.121	6.68	6.80
	26-50	24.0	168.9	73.2	0.176	4.13	4.31
	51-100	9.2	135.9	52.8	0.180	3.08	3.26
Cinnamon	0-25	20.5	159.0	110.8	0.218	6.35	6.57
	26-50	8.2	160.6	61.2	0.458	4.61	5.07
	51-100	13.2	122.4	48.7	0.208	3.56	3.77
Pineapple	0-25	46.0	147.6	112.8	0.092	6.54	6.63
	26-50	18.0	157.9	70.6	0.206	4.97	5.18
	51-100	13.2	144.8	41.8	0.217	3.58	3.80
B. Coconut-Pepper-cacao-Pineapple							
Coconut	0-25	32.4	196.2	100.0	0.185	3.99	4.18
	26-50	26.4	162.0	77.4	0.425	2.86	3.28
	51-100	20.0	145.0	58.8	0.449	2.86	3.31
Cacao	0-25	33.6	190.8	144.0	0.435	5.25	5.68
	26-50	32.4	165.4	68.0	0.323	5.00	5.32
	51-100	27.4	138.0	48.5	0.377	2.77	3.17
Pineapple	0-25	64.2	162.0	145.8	0.285	6.77	7.05
	26-50	35.4	142.3	81.7	0.282	3.67	3.95
	51-100	-	130.5	-	-	-	-
C. Coconut Monocropping							
Coconut	0-25	29.4	159.2	120.5	0.180	4.63	4.81
	26-50	21.6	122.0	55.8	0.102	4.88	4.98
	51-100	18.3	108.0	38.4	0.084	3.28	3.37
Coconut Monocropping interspace	0-25	12.0	151.4	63.4	0.165	4.43	4.60
	26-50	19.8	136.2	75.8	0.118	3.14	3.26
	51-100	18.0	104.8	61.4	0.078	2.97	3.05

* Average of three replications

CD (P = 0.05)						
	Crop	4.72	10.53	6.78	0.011	0.18
	Depth	3.33	7.28	4.80	0.007	0.12
Crop x	Depth	8.15	18.17	11.76	0.018	0.30

were significantly higher in the root region soils of pineapple, whereas it was least in coconut (monocrop) interspace soils. Addition of cellulose and glucose separately (0.5%) to soil increased the mineralisation rate by 3-4 fold as that of native carbon in all the crops and interspace soils (Table 6).

1.5. Soil chemical properties:

The data on soil chemical properties of coconut-pepper-cinnamon-pineapple cropping system and coconut monocropping system are presented in Table 7. Soil pH was slightly low in the root region of cinnamon. The organic carbon was slightly higher in the root region soil of coconut (0.57%) in multistoreyed cropping system as compared to coconut in monocropping system (0.5%). Levels of both total and available P and K were lower in coconut monocropping as compared to coconut multistoreyed cropping system.

The studies on soil chemical analysis of coconut-pepper-cacao-pineapple cropping (MS) and coconut monocropping indicated that the percentage of organic carbon, total nitrogen, total P and total K was higher in the multistoreyed cropping system as compared to the monocropping system (Table 7). In general the soil pH, organic carbon, total nitrogen, total P, total K, available P and K decreased with increase of soil depth in all the cropping system and the least being recorded in the 51-100 cm depth.

Table 7. Soil chemical properties of coconut based multistoreyed cropping system and coconut monocropping system*

Cropping system and crop	Soil depth (cm)	pH	Organic C (%)	Total nitrogen (%)	Phosphorus		Potash	
					Total (%)	Available (ppm)	Total (%)	Available (ppm)
A. Coconut-pepper-cinnamon-pineapple								
Coconut	0-25	5.80	0.57	0.048	0.280	23.0	0.22	67.9
	26-50	5.15	0.39	0.040	0.242	22.0	0.23	55.0
	51-100	4.75	0.23	0.025	0.040	5.0	0.25	45.0
Cinnamon	0-25	5.10	0.55	0.052	0.245	18.5	0.21	60.0
	26-50	5.10	0.42	0.040	0.240	15.3	0.22	50.0
	51-100	5.01	0.40	0.036	0.205	8.0	0.20	45.0
Pepper	0-25	5.54	0.48	0.045	0.230	21.0	0.23	65.0
	26-50	5.00	0.37	0.035	0.210	17.8	0.20	53.0
	51-100	4.82	ND	ND	ND	ND	ND	ND
Pineapple	0-25	5.25	0.49	0.050	0.260	19.8	0.20	56.0
	26-50	4.95	0.44	0.041	0.230	14.5	0.24	51.0
	51-100	4.98	ND	ND	ND	ND	ND	ND
B. Coconut-pepper-cacao-pineapple								
Coconut	0-25	5.23	0.99	0.092	High	25.8	0.285	121.5
	26-50	4.58	0.50	0.048	0.720	22.0	0.250	89.5
	51-100	4.29	0.47	0.048	0.580	8.5	0.275	79.0
Cacao	0-25	5.36	0.70	0.070	0.800	19.8	0.190	51.5
	26-50	4.70	0.61	0.059	0.550	6.4	0.210	34.5
	51-100	4.40	0.44	0.042	0.070	1.01	0.235	31.0
Pineapple	0-25	5.00	0.61	0.060	0.073	14.4	0.185	31.0
	26-50	4.29	0.55	0.053	0.090	11.2	0.145	33.5
	51-100	ND	ND	ND	ND	ND	ND	ND
C. Coconut Monocrop	0-25	5.15	0.50	0.054	0.075	17.5	0.140	31.0
	26-50	4.39	0.38	0.039	0.035	4.16	0.180	13.5
	51-100	4.29	0.35	0.035	0.043	4.48	0.175	21.0

* Average of three replications

ND = Not determined

1.8 Root biomass, soil microbial biomass and cellulose decomposition:

The data on the root biomass, soil microbial biomass of coconut based multistoreyed cropping system and coconut monocropping system are given in Table 8. The microbial biomass and root biomass was higher in the surface soil (0-25 cm) of the coconut in multistoreyed cropping system as compared to coconut from monocropping system . The root biomass was the highest in 51-100 cm depth in coconut in all the cropping system, whereas in cacao, pineapple and cinnamon the root biomass decreased with increase in soil depth. The microbial biomass was greater in the root region soils of cacao. The root region of coconut in multistoreyed cropping system indicated higher biomass as compared to coconut in the monocropping system. The microbial biomass was less in the root region soils of cinnamon. The cellulose decomposing capacity of root region soil at three depths revealed that the cellulose decomposing ability of the soil decreased with increase in soil depth. There was wide variation in cellulose decomposition between 0-25 and 26-50 cm as compared to 26-50 and 51-100 cm depth (Table 8).

The carbon, nitrogen and phosphorus contents in biomass were calculated for different cropping system from the microbial biomass (µg C/g soil) (Table 9). The carbon nitrogen and phosphorus contents in the biomass were higher in the

Table 8. Root biomass soil microbial biomass and cellulose decomposition of coconut based multistoreyed cropping system and coconut monocropping system*

Crop	Soil depth (cm)	Root biomass (g/100g soil)	Microbial biomass C (μg c/g soil) Period of incubation 0-10 days	10-20 days	Cellulose decomposition (per cent)
A. Coconut-pepper-cinnamon-pineapple					
Coconut	0-25	0.36	152.4	33.9	21.7
	26-50	0.52			10.7
	51-100	0.85			10.5
Cinnamon	0-25	0.30	147.3	33.3	-
	26-50	0.24			
	51-100	0.27			
Pineapple	0-25	0.37	150.0	35.0	-
	26-50	0.33			
	51-100	0.18			
B. Coconut-pepper-cacao-pineapple					
Coconut	0-25	0.51	166.4	37.8	18.5
	26-50	0.55			10.4
	51-100	0.72			6.3
Cacao	0-25	0.28	250.0	49.0	-
	26-50	0.23			
	51-100	0.15			
Pineapple	0-25	0.31	142.0	3.25	-
	26-50	0.25			
	51-100	0.21			
C. Coconut monocropping system					
Coconut	0-25	0.30	130.8	28.0	19.8
	26-50	0.55			12.7
	51-100	0.86			4.9
Coconut Mono-interspace	0-25	0.12			
	26-50	0.21			
	51-100	0.10			

*Average of three replications

- = Not determined

Table 9. Carbon, nitrogen and phosphorus in microbial biomass of coconut based multistoreyed cropping system (MS) and coconut monocropping

	Coconut-pepper-cinnamon-pineapple	Coconut-pepper cacao-pineapple	Coconut monocropping
Microbial biomass from flush (Kg C/ha)	330.0	458.10	287.80
Nitrogen in biomass* (Kg N/ha)	55.0	76.34	47.96
Phosphorus in biomass** (kg/ha)	6.6	9.14	5.76

* Assuming a C/N ratio of 6 for the microbial biomass

**Assuming a C/P ratio of 50 for microbial biomass multistoreyed cropping system as compared to coconut monocropping system. Further, the biomass was higher in coconut-pepper-cacao-pineapple cropping system as compared to coconut-pepper-cinnamon-pineapple cropping system.

2. Soil microflora and micro biological activities in the coconut based mixed farming system and coconut monocropping system:

2.1. Soil microflora and beneficial microorganisms in the root region:

The microflora and microbiological activities in the root region and the interspaces of coconut based mixed farming consisting of coconut-pepper-napier grass (NB-21) and coconut monocropping system in the pre-monsoon and post-monsoon seasons are given in Table 10. The data revealed a significant increase of bacterial and fungal counts in the root region of coconut and napier grass in the mixed farming system as compared to coconut monocropping system in both the season. The population of bacteria and actinomycetes decreased considerably with the increase in depth of soil.

The studies on the occurrence of beneficial microorganisms in the root region of mixed farming system recorded higher number of asymbiotic N_2-fixers and P-solubilisers as compared in the coconut monocropping system (Table 11). The enumeration of nitrifying bacteria by MPN method revealed that the population of *Nitrosomonas* sp. was higher in the root region of

Table 10. Microflora in the root region and the interspaces of coconut based mixed farming (MF) and coconut monocropping system in the pre- and post-monsoon seasons

Crop/interspace	Soil depth (cm)	Microbial counts (No./g dry weight of soil)*					
		Pre-monsoon			Post-monsoon		
		Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^5$	Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^5$
Coconut (MF)	0-25	30.00	53.30	9.50	18.03	45.70	6.68
	26-50	3.25	14.50	2.09	13.23	18.53	3.51
	51-100	3.07	18.55	2.50	3.03	5.25	1.35
Napier grass (MF)	0-25	21.42	26.70	5.23	18.73	23.86	5.20
	26-50	8.18	13.30	2.44	11.87	14.29	2.00
	51-100	1.35	23.30	1.11	3.30	7.38	6.68
Coconut Monocropping	0-25	2.27	25.00	3.67	5.27	15.28	3.28
	26-50	2.73	30.00	4.00	4.68	12.98	2.35
	51-100	1.43	12.00	1.67	2.38	9.23	0.68
Coconut Monocropping interspace	0-25	1.26	6.00	4.33	2.88	5.23	1.69
	26-50	0.67	10.00	4.00	1.35	7.38	1.69
	51-100	0.37	12.33	2.00	0.68	5.05	0.50

* Average of three replications

CD ($P = 0.05$)

Crop	5.15	7.20	1.34	3.54	4.43	1.08
Depth	4.54	6.39	1.16	3.33	3.84	0.95
Crop x Depth	8.95	12.40	2.32	5.58	7.68	1.52

Table 11. Beneficial microorganisms in the root region of coconut based mixed farming and coconut monocropping system*

Crop	Soil depth (cm)	N_2-fixers, $\times 10^3$	Phosphate solubilizers		Nitrifying bacteria		VA-mycorrhizae	
			Bacteria, $\times 10^4$	Fungi $\times 10^3$	Nitrosomonas $\times 10^2$	Nitrobacter $\times 10^3$	Spore count No./50 g soil	Root colonisation (%)
Coconut (MF)	0-25	21.35	18.25	4.3	45.0	20.0	160.8	73.3
	26-50	17.76	7.69	2.6			41.0	37.3
Napier grass (MF)	0-25	38.67	12.69	1.8	36.0	18.0	182.0	78.0
	26-50	19.59	11.37	1.2			26.0	35.3
Coconut monocrop	0-25	10.69	5.67	2.7	20.8	10.6	165.5	60.3
	26-50	5.69	2.10	1.5			45.0	30.0
Coconut monocrop interspace	0-25	3.35	1.69	1.8	-	-	-	-
	26-50	2.69	0.39	-	-	-	-	-

* Average of three replications

\- = Not determined

CD at P=0.95							
Crop	2.92	4.66				12.06	7.94
Depth	2.53	4.04				7.38	6.15
Crop x Depth	5.06	8.07				20.89	13.76

coconut and napier grass of mixed farming system, but the Nitrobacter counts did not show any variation. The endogone spore and VA-mycorrhizal colonisation studies indicated that the root region of napier grass favoured an increased spore count and root colonisation over the coconut both in the mixed farming and monocropping system (Table 11). The spore numbers and root colonisation decreased considerably in 26-50 and 51-100 cm depth.

2.2. Soil enzyme activities in the root region:

The soil enzyme activities in the root region of mixed farming and coconut monocropping (root region and interspace) are shown in Fig.5. The root region of napier grass recorded higher urease and dehydrogenase activity. In general the enzyme activity decreased with the increase of soil depth and it was low in 51-100 cm soil depth. The phosphatase activity was significantly high in the root region soils of coconut in the mixed farming system. Activities of urease, dehydrogenase and phosphatase were significantly lower in the interspace soils of coconut monocropping system.

2.3. Soil microflora and enzyme activities in the rhizosphere:

The rhizosphere microflora and enzyme activity of coconut mixed farming and coconut monocropping system are presented in Table 12. The bacteria, actinomycetes, N_2-fixers and P-solubilisers were more in the mixed farming as compared to monocropping. Urease enzyme assay revealed higher activity in

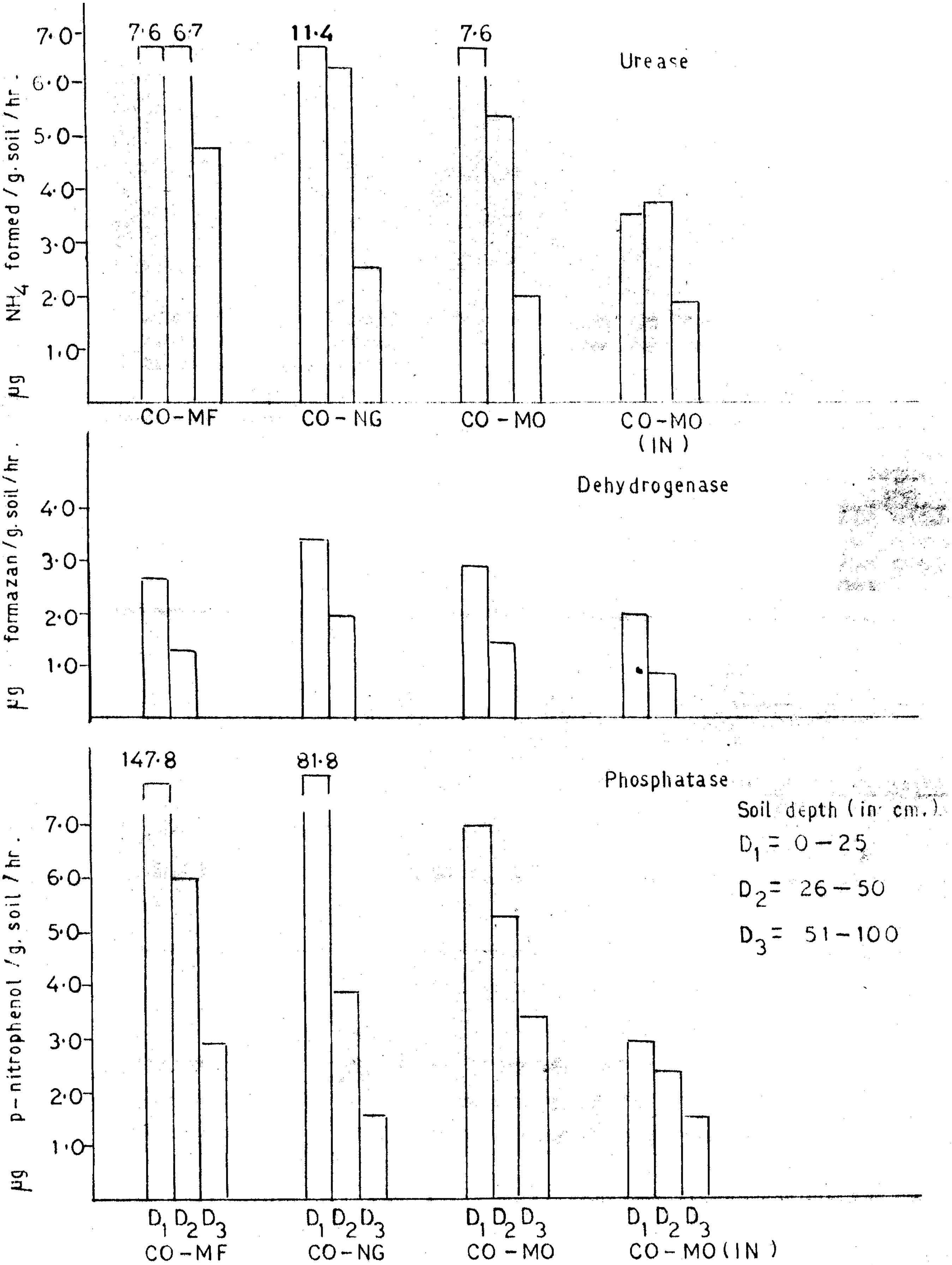


Fig. 5. Soil enzyme activity in the root region of coconut mixed farming (MF) and coconut monocropping (MO) systems
(CO = Coconut, NG = Napier grass, IN = Interspace)

Table 12. Microflora and soil enzyme activity in the rhizosphere of coconut based mixed farming system (MF) and coconut monocropping (MO) system*

Crop	Bacteria X10^6	Fungi X10^3	Actinomycetes X10^5	N_2-fixers X10^3	PO_4-solubilisers X10^4	Urease	Dehydrogenase		Phos pha- tase
							Without glucose	With glucose	
Coconut (MF)	12.25	4.55	2.05	5.30	4.35	9.04	2.61	6.13	161.
Napier grass (MF)	9.28	2.92	0.30	13.50	2.10	8.54	4.71	12.55	136.
Coconut monocrop (Mo)	8.67	4.87	0.38	3.30	2.30	5.41	2.82	6.04	133.

*Average of 5 replications

Urease	μg of NH_4 formed/g of soil/hr
Dehydrogenase	μg of Formazan/g of soil/hr
Phosphatase	μg of P-nitrophenol/g of soil/hr

the rhizosphere of coconut and napier grass in the mixed farming system whereas the dehydrogenase activity was greater only in rhizosphere of napier grass. The coconut rhizosphere (mixed farming) had recorded significantly higher phosphatase enzyme activity.

2.4. Carbon and nitrogen mineralisation:

The results indicated that carbon and nitrogen mineralisation *in vitro* was higher in the root region of coconut in the mixed farming as compared to coconut monocropping system (Table 13). Addition of carbon source in the form of glucose or cellulose (0.5%) considerably increased the rate of carbon mineralisation. The mineralisation rate was more in the glucose amended soil as compared with cellulose. The interspace soils of coconut monocropping recorded low rate of mineralisation both in native soil and with amendment. Similar trend was observed in the nitrogen mineralisation. The formation of NH_4-N was less in the surface soil (0-25cm) of coconut mixed farming system but the NO_3-N was more in the surface soil. Both carbon and nitrogen mineralisation decreased with increase in soil depth.

2.5. Soil chemical analysis:

The soil chemical composition in coconut based mixed farming and coconut monocropping system are given in Table 14. Soil pH did not indicate any difference between the two systems. The organic carbon and total nitrogen contents of

Table 13. Carbon and nitrogen mineralisation *in vitro* of coconut based mixed farming and monocropping system*

Crop	Soil depth (cm)	Carbon mineralisation* (mg/100g soil)			Nitrogen mineralisation* (mg/100g soil)		
		Native carbon	Cellulose (0.5%)	Glucose (0.5%	NH_4-N	NO_3-N	Total-N
Coconut MF	0-25	54.0	144.4	188.8	0.293	6.81	7.10
	26-50	17.4	116.4	169.4	0.550	5.14	5.69
	51-100	16.8	49.4	105.9	1.203	3.23	4.44
Napier grass	0-25	22.2	151.0	168.0	0.145	5.25	5.39
	26-50	14.4	42.0	144.6	0.326	4.54	4.87
	51-100	18.6	36.0	102.0	0.394	3.35	3.74
Coconut Monocrop	0-25	29.4	120.5	159.2	0.180	4.63	4.81
	26-50	21.6	51.0	152.4	0.102	4.88	4.98
	51-100	18.3	38.4	91.0	0.084	3.28	3.36
Coconut Monocrop interspace	0-25	12.0	63.4	151.4	0.165	4.43	4.60
	26-50	19.8	75.8	166.2	0.118	3.14	3.26
	51-100	18.0	61.4	88.0	0.078	2.98	3.05

*Average of three replications

CD (P = 0.05)

Crop	2.95	9.19	9.03	0.03	0.21
Depth	2.56	7.96	7.82	0.02	0.18
Crop x Depth	5.11	15.91	15.64	0.04	0.37

Table 14. Soil chemical composition of coconut based mixed farming and coconut monocropping system*

Crop	Soil depth (cm)	pH	Organic carbon (%)	Total Nitrogen (%)	Phosphorus		Potassium	
					Total P%	Available P (ppm)	Total K%	Available K (ppm)
Coconut (MF)	0-25	5.50	0.71	0.068	0.50	22.0	0.155	88
	26-50	5.10	0.50	0.053	0.36	18.4	0.235	97
	51-100	5.00	0.26	0.029	0.21	16.2	0.235	90
Napier grass MF-NG	0-25	5.35	0.26	0.030	0.30	17.2	0.130	23
	26-50	5.30	0.19	0.021	0.35	18.4	0.235	32
	51-100	5.43	0.18	0.018	0.12	7.9	0.200	24
Coconut Monocrop	0-25	5.55	0.22	0.020	0.42	13.2	0.180	60
	26-50	5.20	0.15	0.015	0.31	12.0	0.190	56
	51-100	4.44	0.10	0.011	0.28	8.4	0.220	40
Coconut Monocrop Interspace	0-25	5.40	0.18	0.018	0.30	9.9	0.175	27
	26-50	4.93	0.09	0.010	0.52	0.8	0.210	13
	51-100	4.80	0.06	0.006	0.40	0.7	0.175	23

* Average of three replications

soil were more in the mixed farming system. The available potash (K_2O) was very low (23 ppm) in the root region soils of napier grass and in the interspace soils of coconut monocropping system (27 ppm).

2.6. Root biomass, soil microbial biomass and cellulose decomposition:

Soil moisture, soil microbial biomass, root biomass and cellulose decomposition of coconut mixed farming and coconut monocropping system are presented in Table 15. The root region of coconut recorded higher microbial biomass (187.8 µg c/g soil) as compared to coconut monocropping (111.2 µg/c/g soil). The root biomass was higher in the root region of coconut (0.41 g/100g soil) in mixed farming system as compared to the coconut monocropping (0.28 g/100g soil). The cellulose decomposing capacity of the soil was higher in the surface soil (0-25 cm) as compared to the other two soil depths (26-50 and 51-100 cm). The root region soils of napier grass exhibited low cellulose decomposition ability as compared to coconut. From the soil microbial biomass, the carbon, nitrogen and phosphorus contents in biomass were calculated (Table 16). The results indicated that carbon, nitrogen and phosphorus contents in coconut based mixed farming system were higher as compared to coconut monocropping system.

Table 15. Soil moisture contents, root biomass, soil microbial biomass and cellulose decomposition in soil profile of coconut mixed farming and coconut monocropping system*

Crop	Soil depth (cm)	Soil moisture content (%)		Root biomass (g/100 g soil)	Soil microbial biomass (µg c/g soil)		Cellulose decomposition (%)
		Rhizosphere soil	Root region soil		0-10 days	10-30 days	
A. Coconut-pepper-Napier grass							
Coconut	0-25		11.70	0.41	187.8	27.8	23.50
	26-50	18.38	13.40	0.55			15.80
	51-100		13.50	0.72			8.20
Napier grass	0-25		7.70	0.20	177.9	41.9	14.35
	26-50	15.00	8.70	0.23			8.67
	51-100		11.75	0.10			10.00
B. Coconut monocrop							
	0-25		6.50	0.28	111.2	19.5	20.67
	26-50	18.00	8.15	0.50			13.00
	51-100		7.70	0.80			6.67

*Average of three replications

– = Not determined

Table 16. Carbon, nitrogen and phosphorus in soil microbial biomass in coconut based mixed farming and coconut monocropping systems.

	Cropping system	
	Coconut mixed farming system	Coconut monocropping system
Microbial biomass from flush (kg/ha)	402.16	244.60
Nitrogen in biomass (kg N/ha)*	67.00	40.80
Phosphorus in biomass** (kg/P/ha)	8.04	4.89

* Assuming a C/N ratio of 6 for the microbial biomass

**Assuming a C/P ratio of 50 for microbial biomass

3. Rhizoplane microflora and the studies on rhizosphere microorganisms:

3.1. Rhizoplane microflora and beneficial microorganisms:

Rhizoplane microflora of the different coconut based cropping system revealed that the bacterial counts in the rhizoplane of cacao was higher as compared to other crops (Table 17). The bacterial numbers were more in the rhizoplane of coconut in different coconut based cropping systems as compared to the rhizoplane coconut monocropping system. The fungal counts were less in the coconut mixed farming system and the maximum population was recorded in the coconut-pepper-cacao-pineapple cropping system. Actinomycetes population did not reveal any variation between the different cropping systems studied.

The percentage occurrence of asymbiotic N_2-fixers, *Azospirillum* and P-solubilisers (bacteria and fungi) in the roots of different coconut based cropping and coconut monocropping systems are presented in Table 18. The asymbiotic N_2-fixers (*Beijerinckia* spp.) were more in the coconut-pepper-cacao-pineapple cropping system. Both TTC reduction and pellicle formation in malate semi-solid medium were observed (*Azospirillum* spp.) in the roots of different crops. The percentage occurrence of *Azospirillum* was higher in the coconut mixed farming system. But the *Azospirillum* occurrence did not reveal any variation between coconut-pepper-cacao-pineapple and coconut monocropping system. The P-solubilising

Table 17. Rhizoplane microflora of the coconut based cropping systems (No./g of dry weight of root)*

Cropping system	Microbial counts		
	Bacteria X10^{6}	Fungi X10^{4}	Actinomycetes X10^{5}
A. Coconut-pepper-cacao-pineapple (MS)			
Coconut	478.62	26.50	10.59
Cacao	604.48	17.52	6.20
Pineapple	520.38	15.60	5.62
B. Coconut-pepper-cinnamon-pineapple (MS)			
Coconut	430.56	13.58	9.38
Cinnamon	488.30	14.35	8.72
Pineapple	481.50	14.78	5.73
C. Coconut-pepper-napier grass (MP)			
Coconut	475.32	11.89	6.73
Napier grass	574.43	16.51	13.05
D. Coconut monocrop (Mo)			
Coconut	397.45	15.20	6.29

* Average of three replications

Table 18. Percentage of root samples showing the occurrence of Asymbiotic N_2-fixers, _Azospirillum_ and P-solubilisers in various crops of coconut based cropping system and coconut monocropping system (percentage)

Crops and cropping system	Asymbiotic N_2-fixers*	_Azospirillum_		P-solubilisers	
		TTC Reduction	Pellicle in Malate medium	Bacteria	Fungi
A. Coconut-pepper-cacao-pineapple					
Coconut	100.0	80.0	75.0	8.3	35.5
Pepper	58.0	56.0	56.0	25.0	33.3
Cacao	72.0	70.0	75.0	25.0	25.0
Pineapple	80.0	64.0	80.0	10.3	20.3
B. Coconut-pepper-napier grass					
Coconut	68.0	92.0	84.0	18.3	9.3
grass	75.0	100.0	100.0	40.0	0.0
C. Coconut monocropping	66.7	80.0	68.0	33.3	5.5

*Asymbiotic N_2-fixers are _Beijerinckia_ spp.

bacteria harboured more on the root surface of napier grass and coconut monocrop. The P-solubilising fungi were more in the coconut-pepper-cacao-pineapple cropping system (20.3-35.5%).

3.2. Fungi and bacteria in the rhizosphere:

The occurrence of different fungi, bacteria and actinomycetes in the rhizosphere of different crops of coconut based cropping and coconut monocropping systems are presented in Table 19. Rhizosphere of different crops showed variation in the occurrence of fungi indicating the influence of crop mixing. *Trichoderma* and *Aspergillus* were predominant in the rhizosphere of different crops. *Penicillium*, *Fusarium*, *Cladosporium*, *Cylindrocarpon*, *Absidia* and *Rhizopus* were also recorded from the rhizosphere. *Trichoderma harzianum*, *Trichoderma* spp. *Aspergillus niger*, *Penicillium* spp., *Rhizopus* spp. and *Fusarium* spp. were observed in the rhizosphere of napier grass. The occurrence of bacteria also revealed variation with respect to different crops and cropping systems. *Pseudomonas* spp. (5 isolates), *Bacillus* spp. (9), *Micrococcus* spp.(2), *Escherichia* spp.(3), *Arthrobacter* sp.(1), and *Klebsiella* sp.(1) were the common genera of bacteria recorded and their relative occurrence in the rhizosphere of different crops are given in Table 19.

The physiological and biochemical tests carried out for the identification of bacterial isolates (21 numbers) revealed,

Table 19. Occurrence of fungi, bacteria and actinomycetes in the rhizosphere of various crops of the coconut based cropping system and coconut monocropping system

	Coconut-pepper-cacao pineapple		Coconut-pepper-napier grass		Coconut monocropping
	Coconut	Cacao	Coconut	Napier grass	
A. *Fungi*					
Trichoderma					
Trichoderma harzianum	+	+	+	+	+
T. lignorum	+	+	+	-	+
T. viride	+	-	+	-	+
Trichoderma sp.	+	-	-	+	+
Aspergillus					
A. niger	+	+	+	+	+
Aspergillus fumigatus	+	+	+	-	+
Aspergillus sp.	+	-	-	-	-
Aspergillus sp.	+	+	-	-	-
Penicillium sp.	+	+	-	-	+
Penicillium sp.	+	+	+	+	+
Penicillium sp.	-	-	-	-	-
Rhizopus sp.	+	+	+	+	-
Absidia sp.	+	-	-	-	-
Fusarium sp.	+	+	+	+	+
Cladosporium sp.	+	+	-	-	+
Cylindrocarpon sp.	+	-	-	-	+
B. *Bacteria**					
Pseudomonas sp. (4)	+	+	-	-	-
Arthrobacter sp. (6)	+	-	+	-	+
Pseudomonas sp. (9)	+	-	+	-	+
Pseudomonas sp. (12)	-	+	-	-	+
Pseudomonas sp. (15)	-	+	-	-	-
Pseudomonas sp. (16)	+	-	-	+	-

Table 19 (Contd..)

	Coconut-pepper-cacao pineapple		Coconut-pepper-napier grass		Coconut monocr pping
	Coconut	Cacao	Coconut	Napier grass	
Escherichia sp. (1)	+	+	+	+	+
Escherichia sp. (2)	-	+	-	-	+
Escherichia sp. (13)	-	-	-	+	-
Bacillus sp. (7)	+				
Bacillus sp. (10)	+	-			
Bacillus sp. (11)	+	-	-	-	-
Bacillus sp. (14)	-	+	-	-	-
Bacillus sp. (17)	-	+	+	-	+
Bacillus sp. (18)	-	-	+	+	+
Bacillus sp. (8)	+	+	+	+	+
Bacillus sp. (20)	+	-	+	+	+
Bacillus sp. (21)	+	-	-	+	-
Micrococcus sp. (5)	+	+	+	-	+
Micrococcus sp. (19)	+	-	+	-	+
Klebsiella sp. (3)	+	-	-	-	-
C. *Actinomycetes*					
Streptomyces sp.	+	+	+	+	+
Nocardia sp.	+	+	+	-	+
Micromonospora sp.	+	-	+	-	+
Actinomyces sp.	+	-	+	-	+

+ Present
- Absent

* Bacterial isolate numbers are given in paranthesis

indole producing and phosphate solubilising ability in one of the *Pseudomonas* isolate (No.5). Another *Pseudomonas* isolate (No.4) produced abundant acid and gas from sugars (glucose and sucrose). Gelatin hydrolysis and P-solubilising ability was observed in one of the *Pseudomonas* isolate (No.15). Two *Escherichia* isolates (No.1 and 2) showed high catalase activity and indole production in Tryptone broth medium. An isolate of *Bacillus* (No.10) showed both starch and gelatin hydrolysis activity. *Bacillus polymixa* (No.18) showed good P-solubilising ability *in vitro*. High catalase activity, getalin liquification and NH_4 formation were observed in another *Bacillus* sp.(No.14). The *Micrococcus* sp. isolate (No.19) showed mild catalase activity and it was positive for both starch hydrolysis and gelatin liquification. The actinomycetes group was represented by *Streptomyces*, *Nocardia*, *Micromonospora* and *Actinomyces* and its occurrence varied with different crops and cropping systems.

3.3. Asymbiotic N_2-fixers and P-solubilisers:

Nitrogen fixing ability of asymbiotic N_2-fixers *in vitro*:

The asymbiotic N_2-fixer, *Beijerinckia* spp. isolates obtained from the rhizoplane of various crops of different coconut based cropping systems were subjected to nitrogen fixing ability *in vitro* using Beckings medium. The isolates obtained from the coconut mixed farming (8.4-12.2 mg/g of sugar) and coconut multistoreyed cropping system (7.0-15.6 mg/g of sugar) have shown greater N_2-fixing ability as compared to the N_2-fixers isolated from the coconut monocropping system (5.6-9.6 mg/g of sugar). The N_2-fixers isolated from the rhizoplane of napier grass (10.0-12.2 mg/g of sugar), cacao (11.3-15.6 mg/g of sugar) and cinnamon (9.3-12.6 mg/g of sugar) also showed higher N_2-fixing ability.

Phosphate solubilising ability in vitro: The P-solubilisers (bacteria and fungi) obtained from the rhizoplane of various crops were further screened by growing them on modified Pikovskaya's medium. All the selected isolates showed good P-solubilising ability (Table 20). The per cent P-solubilising ability in the bacterial isolates, Bacillus sp. and Pseudomonas sp. was 35 and 41.7 respectively. The P-solubilising ability of fungi ranged from 34.1 to 47.7% and maximum P-solubilising ability was recorded with Aspergillus niger (Isolate No.40).

3.4. Physiological studies of rhizosphere fungi and bacteria:

Growth of fungi: The growth of fungi at 3 day intervals up to a period of 15 days in Czapek-Dox Broth medium is depicted in Fig.6. The fungal biomass production (mycelial dry weight) was the maximum in Cladosporium sp. (1230 mg/50 ml) followed by Aspergillus niger (900 mg/50 ml) and Trichoderma lignorum (900 mg/50 ml) after 15 days of growth. The greatest reduction in pH was recorded with Aspergillus niger (pH 2.5) and Cladosporium sp. (pH 2.8).

Table 30. The *in vitro* phosphate solubilising ability and pH of bacteria and fungi isolated from the rhizosphere of coconut based cropping systems*

Isolate No.	Bacteria/ Fungi	P_2O_5 in mg/ 50 ml of medium	$Ca_3(PO_4)_2$ solubilised (%)	pH of the culture filtrate**
P-5	*Bacillus* sp.	4.90a	35.68	4.05
P-6	*Pseudomonas* sp.	5.73a	41.72	3.90
39	*Aspergillus niger* (1)	10.82	44.99	3.50
40	*Aspergillus niger* (2)	11.96	47.74	3.00
41	*Penicillium* sp.	9.65	40.68	3.88
42	*Penicillium* sp.	8.35	34.07	4.28

* Average of three replications

a = 25 mg of tricalcium phosphate incorporated in 50 ml of the medium for bacteria

** = pH of the culture media after sterilization was 6.50

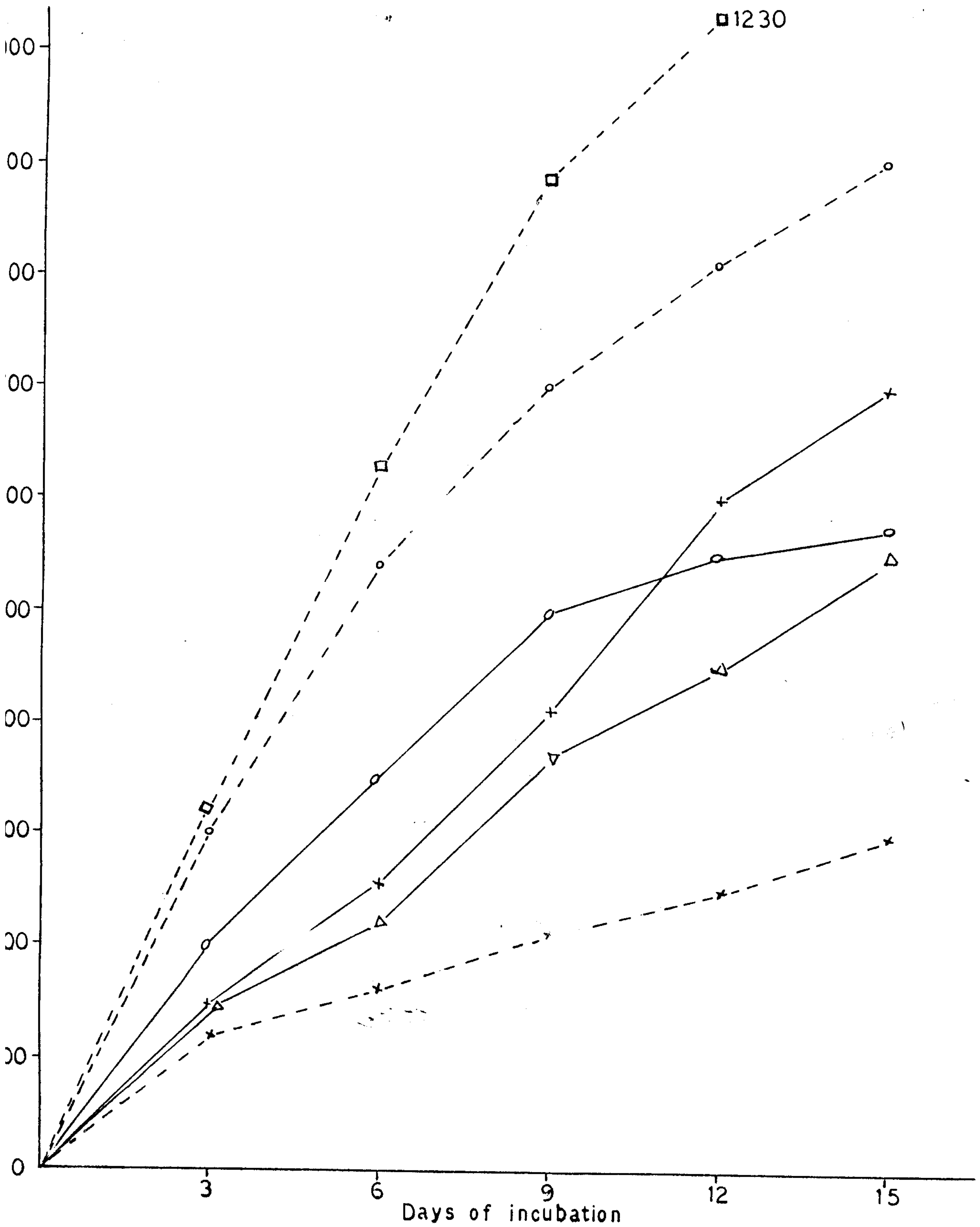


Fig. 6. Growth of fungi at defferent period of incubation on czapex-Dox broth.

o—o *Trichoderma harzianum*

o---o *Aspergillus niger*

x—x *Trichoderma lignorum*

x---x *Rhizopus* sp.

△—△ *Fusarium* sp.

□—□ *Cladospurium* sp.

The growth of fungi (mycelial biomass production) on different cellulose compounds (2%) and pectin (2%) are presented in Fig.7. All the fungi recorded growth in carboxymethyl cellulose (CMC) and cellubiose supplimented medium but the growth in CMC was less as compared to cellubiose. In general the growth of fungi was poor in cellulose powder and cellulose filter paper supplimented medium. Species of *Rhizopus* and *Cladosporium* did not grow when pectin was used as carbon source.

The effect of pH, sodium chloride and sucrose concentration on the growth of rhizosphere fungi are given in Table-21. The biomass production was maximum at pH 5.0. In general the growth of fungi was poor at pH 3.0 and *Cladosporium* sp. did not grow at that pH. At pH 9.0 the growth was observed only in the species of *Fusarium* and *Cladosporium*. All the six fungi showed growth at different sodium chloride concentration (0.5, 2.5 and 5.0%) but the growth varied between the fungi. Similarly the growth of fungi was observed at different level of sucrose (5.0 and 10.0%) except *Cladosporium* spp. which did not grow at 10% concentration.

In vitro production of cellulolytic enzymes: Cellulase enzyme activity (Endoglucanase or C_x) of six rhizosphere fungi towards various substrates are given in Fig.8. *Trichoderma harzianum* showed greater reduction of viscocity in carboxymethyl cellulose (CMC). The maximum viscocity reduction was

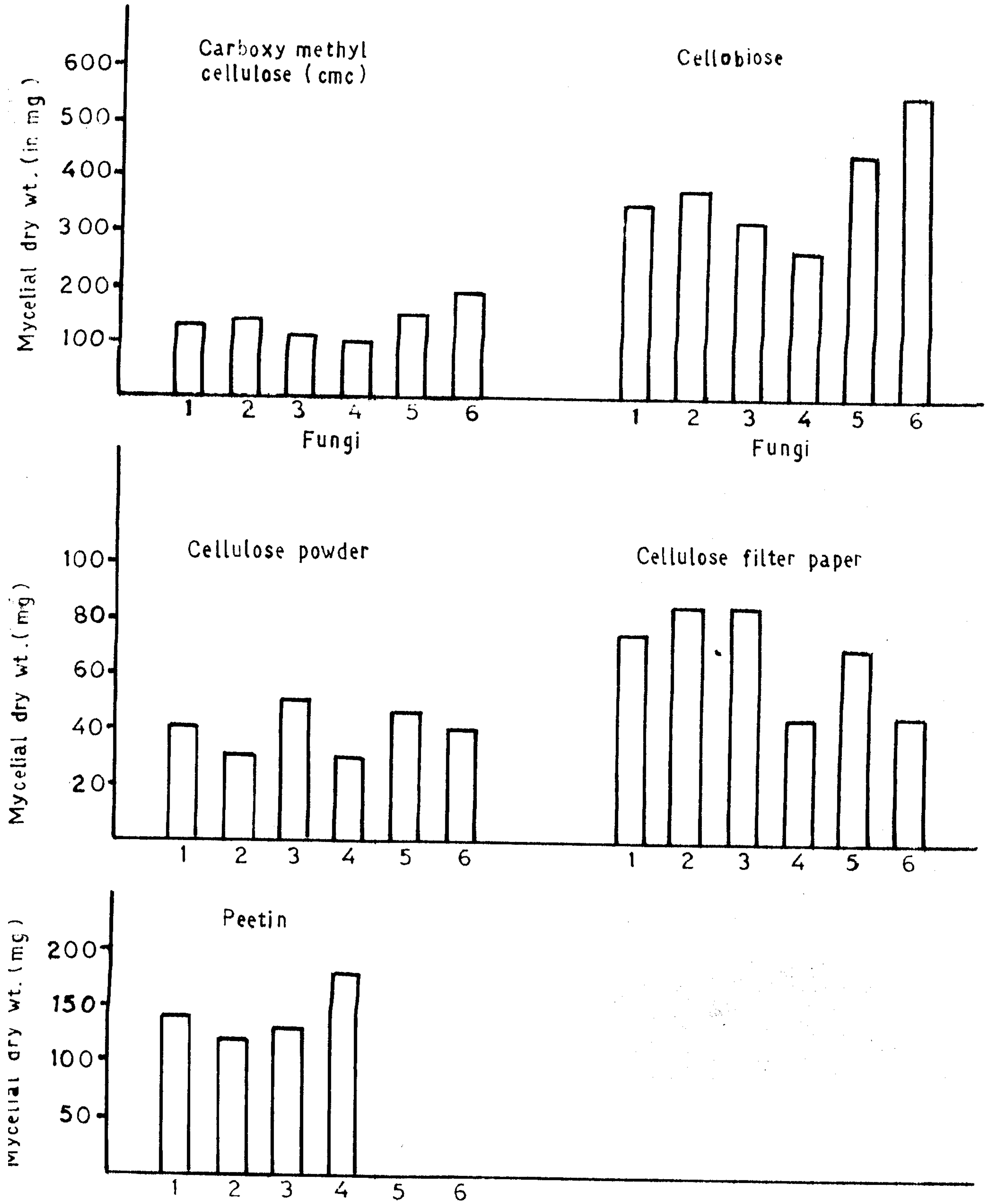


Fig.7. Growth of fungi in defferent cellulose compounds and peetin.

1 *Trichoderma harzianum*

2 *Trichoderma lignorum*

3 *Fusarium* sp.

4 *Aspergillus niger*

5 *Rhizopus* sp,

6 *Cladosporium* sp.

Table 21. Effect of pH, sodium chloride and sucrose concentration on the growth of rhizosphere fungi (mycelial dry wt. in mg)*

Fungi	pH				Sodium chloride (%)			Sucrose (%)	
	3.0	5.0	7.0	9.0	0.5	2.5	5.0	5.0	10.0
Trichoderma harzianum	150	190	410	-	490	470	490	770	480
Trichoderma lignorum	120	210	440	-	440	390	670	570	620
Fusarium sp.	210	340	360	80	570	650	790	740	880
Aspergillus niger	300	630	430	-	600	550	840	820	880
Rhizopus sp.	40	120	-	-	160	650	240	400	220
Cladosporium sp.	0	680	270	130	330	360	570	610	-

*Average of three replications
- No growth

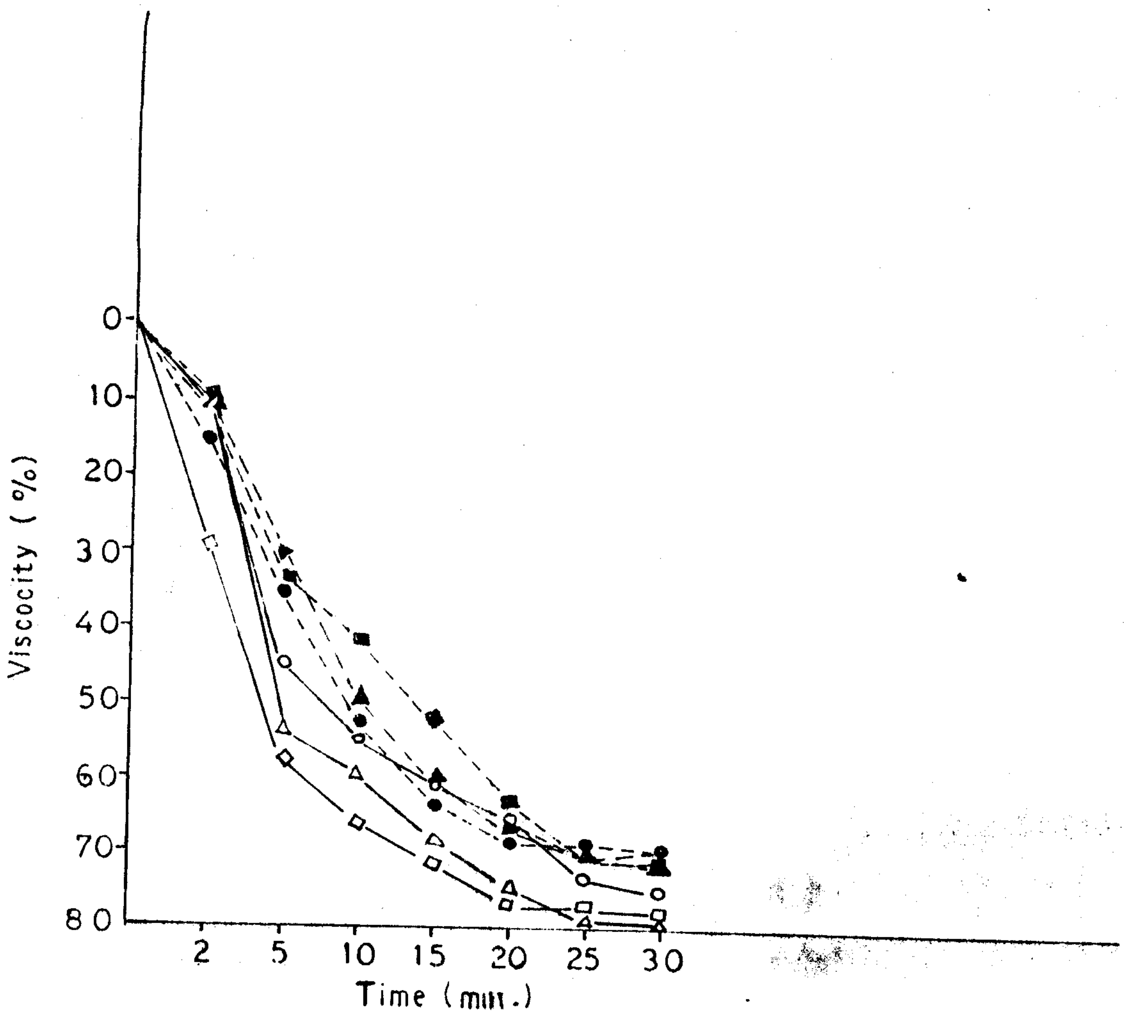


Fig. 8. Cellulase activity (C_x) of the rhizosphere fungi.

□——□ *Trichoderma harzianum* ■- - -■ *Rhizopus* sp.

△——△ *Trichoderma lignorum* ▲- - -▲ *Fusarium* sp.

○——○ *Aspergillus niger* ●- - -● *Cladosporium* sp.

observed upto 5 min (64%).

The results of the enzyme activities of the fungi revealed that both *Trichoderma harzianum* and *Trichoderma lignorum* exhibited the maximum C_1 activity over the other four fungi (Fig.9). Species of *Cladosporium* and *Aspergillus* also showed higher C_1 enzyme activity as compared to the species of *Fusarium* and *Rhizopus*.

The growth of various isolates of bacteria at different pH and sodium chloride concentration are presented in Table 22. Bacterial growth was maximum at pH 7.0. *Escherichia* sp. (3 isolates) and *Bacillus* sp. (1 isolate) showed good growth also at pH 5.0 and pH 9.0 indicating its wide adaptability. *Bacillus* sp. (3 isolates) did not grow at pH 5.0 whereas poor growth was observed in *Bacillus* sp. (isolate No.8 and 10) and *Pseudomonas* sp. (isolate No.15 and 16). The most ideal concentration of sodium chloride for the growth of various isolates of bacteria was 0.5%. *Bacillus* sp.(no.20 and 21) and *Pseudomonas* sp.(isolate no.15 and 16) did not grow at 5% sodium chloride levels. Except *Escherichia* sp. (isolate nos.1, 2, 6 and 13), all the other bacterial isolates failed to grow at 10% sodium chloride concentration.

3.5. Effect of pesticides on the rhizosphere bacteria and fungi:

Bacteria: The results of the *in vitro* studies on the effect of certain common pesticides on the growth of rhizosphere

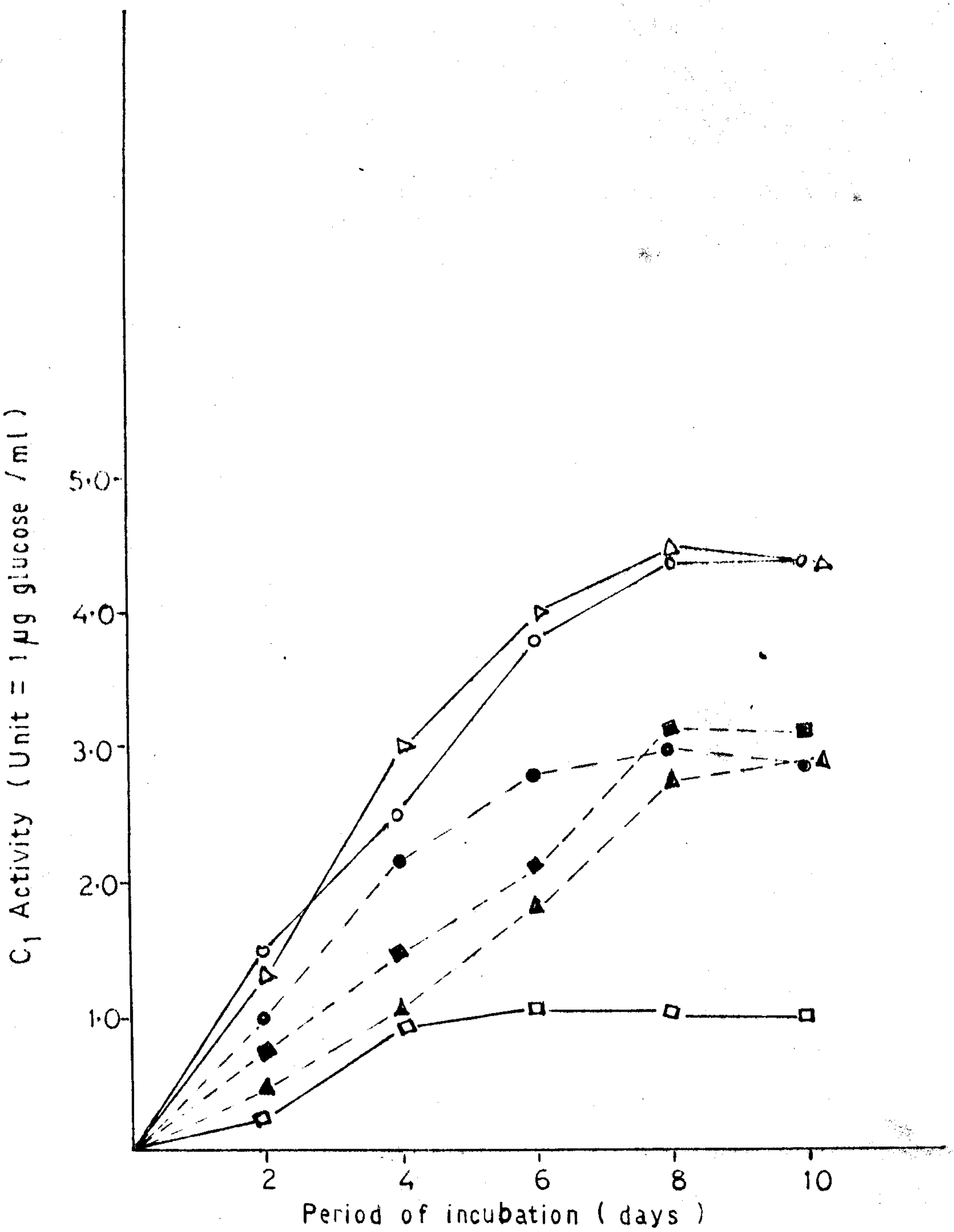


Fig. 9. Cellulase activity (C_1) of the rhizosphere fungi .

○——○ *Trichoderma harzianum* ●- - - -● *Aspergillus niger.*

△——△ *Trichoderma lignorum* ▲- - -▲ *Rhizopus* sp.

□——□ *Fusarium* sp. ■- - - -■ *Cladosporium* sp.

Table 22. Growth of rhizosphere bacteria at different pH level and sodium chloride concentration

Isolate No. and Genera of bacteria	pH			Sodium chloride (per cent)		
	5.0	7.0	9.0	0.5	5.0	10.0
1. *Escherichia* sp.	+++	+++	+++	+++	++	+
2. *Escherichia* sp.	+++	+++	++	+++	+++	+
3. *Klebsiella* sp.	+	++	+(s)	++	+	-
4. *Pseudomonas* sp.	++	++	+	++	+	-
5. *Micrococcus* sp.	+	+++	+	++	+	-
6. *Escherichia* sp.	++	+++	+	+++	++	+
7. *Bacillus* sp.	+(s)	+++	+	+++	+	-
8. *Bacillus* sp.	++	+++	+	+++	+	-
9. *Pseudomonas* sp.	++	+++	++	+++	++	-
10. *Bacillus* sp.	+(s)	++	+	++	+	-
11. *Bacillus* sp.	+++	+++	+++	+++	+	-
12. *Pseudomonas* sp.	+	++	+	++	-	-
13. *Escherichia* sp.	+++	+++	++	+++	++	+
14. *Bacillus* sp.	++	+++	++	+++	+	-
15. *Pseudomonas* sp.	+(s)	++	+	++	-	-
16. *Pseudomonas* sp.	+(s)	+++	+(s)	++	+	-
17. *Bacillus* sp.	-	++	-	+	-	-
18. *Bacillus* sp.	+	+++	++	+++	+	-
19. *Micrococcus* sp.	++	+++	++	+++	++	-
20. *Bacillus* sp.	-	+++	+(s)	++	-	-
21. *Bacillus* sp.	-	++	+(s)	++	-	-

Growth scale +++ = Good ++ = Fair + = Poor
+(s) = Very poor growth
- = No growth

Table 33. Effect of pesticides on the growth of Rhizosphere bacteria *in vitro*

No.	Bacteria	Captan		Blitox		Bordeaux mixture		BHC 50%	
		0.2%	0.04%	0.1%	0.02%	0.3%	0.1%	0.1%	0.02%
1.	*Escherichia* sp.	+++	+++	-	+	-	-	-	-
2.	*Escherichia* sp.	+++	-	+	-	-	-	-	+
3.	*Klebsiella* sp.	+++	++	-	-	-	-	-	-
4.	*Pseudomonas* sp.	++	++	-	-	-	-	-	-
5.	*Micrococcus* sp.	+++	+++	-	+	-	-	-	+
6.	*Escherichia* sp.	+	++	-	-	-	-	-	-
7.	*Bacillus* sp.	++	++	-	+	-	-	-	+
8.	*Bacillus* p/m	++	+++	-	-	-	-	-	-
9.	*Pseudomonas* sp.	++	+++	-	-	-	-	-	-
10.	*Bacillus* sp.	+++	+++	-	-	-	-	-	-
11.	*Bacillus* sp.	++	++	-	-	-	+	-	+
12.	*Pseudomonas* sp.	++	++	-	-	-	+	-	+
13.	*Escherichia* sp.	+++	+++	-	+	-	-	-	-
14.	*Bacillus* sp.	+++	+++	-	-	-	-	-	-
15.	*Pseudomonas* sp.	+++	+++	-	+	-	-	-	-
16.	*Pseudomonas* sp.	++	+++	-	-	-	+	-	+
17.	*Bacillus* sp.(S1)	-	-	-	-	-	-	-	-
18.	*Bacillus* sp.	+++	+++	-	+	-	-	-	-
19.	*Micrococcus*	+++	+++	-	+	-	-	-	+
20.	*Bacillus* sp.	-	-	-	-	-	-	-	-
21.	*Bacillus* sp.	-	-	-	-	-	-	-	-

Growth scale

+++ = Good ++ = Fair + = Poor - = No growth

Table 24. Effect of pesticides on the growth of rhizosphere fungi in liquid and solid media

Pesticides	Fungi	Trichoderma harzianum		Trichoderma lignorum		Fusarium		Aspergillus niger		Rhizopus		Cladosporium		Remarks
		LM	SM	LM	SM	LM	SM	LM	SM	LM	SM	LM	SM	
Bordeaux mixture 1%														
	3rd day	0	0	0	0	0	0	0	0	0	0	0	0	
	6th day	0	0	0	0	0	0	0	0	0	0	0	0	
Bordeaux mixture 0.2%														
	3rd day	0	0	0	0	0	0	0	0	0	0	+	+	*No.4 clear zone was observed (0.5 to 1.0 cm)
	6th day	0	2.5	0	2.5	0	0	0	0	0	0	+	9.0	
Bordeaux mixture 0.1%														
	3rd day	0	0	0	0	0	0	+	+	0	0	+	+	
	6th day	130	+	240	+	0	2.25	650	9.0	0	0	300	25. 0	
BHC 50% wettable 0.1% powder														
	3rd day	0	0	0	0	0	0	0	0	0	0	0	0	
	6th day	0	+	0	+	0	0	0	0	0	0	0	0	
BHC 50% wettable 0.02*powder														
	6th day	+	+	0	+	0	0	+	0	0	0	0	0	
Biltox 0.2%														
	3rd day	++	20.0	+	12.25	0	12.25	+	13.70	0	5.0	+	25.0	
	6th day	320	E	220	E	0	E	700	E	0	0	650	E	
" 0.02%	3rd day	++	16.0	+	12.25	+	12.25	+	2.25	+	16.0	+	30	
Captan 0.2%														
	3rd day	++	4.0	++	4.0	++	4.0	+	2.25	0	0	0	0	
	6th day	570	E	500	E	520	E	690	9.0	80	4.0	300	9.0	
" 0.04%	6th day	++	13.0	++	13.00	++								
Paradon 0.2%	3rd day	++	N	+	N	+	N	+	N	+	N	+	N	
	6th day	670		780		510		730		280		770		

LM = Liquid media (mycelial dry wt. in mg)
0 = No growth

SM = Solid media (Czapek-Dox agar)
(Colony diameter in mm)
E = Growth covered in entire plate
N = Not done

bacteria are presented in Table 23. The fungicide, captan both at 0.2 and 0.04%, did not inhibit the growth of bacteria except three isolates of _Bacillus_ sp. (No.17, 20 and 21). Blitox (0.1%), Bordeaux mixture (0.5%) and BHC (0.1%) inhibited the growth of all the rhizosphere bacteria. Majority of the bacteria failed to grow at lower concentration of Blitox, BM and BHC. However, a few isolates (nos.2, 5, 7, 16 and 19) showed growth, indicating their tolerance to the pesticide at lower concentration.

Effect of pesticides on the rhizosphere fungi: The effect of pesticides on the growth of rhizosphere fungi in liquid and solid media separately are given in Table 24. Bordeaux mixture (BM) at 1% concentration inhibited the growth of all the fungi both in liquid and solid media. _Cladosporium_ spp., _Trichoderma harzianum_ and _T.lignorum_ showed growth on solid media incorporated with BM at 0.5% on 6th day, whereas in the liquid medium no growth of all the fungi was noticed. Blitox (0.1 and 0.02%) and Captan (0.2 and 0.04%) did not inhibit the growth of any of the fungi tested. All the fungi except _Rhizopus_ sp. showed growth on solid and liquid media incorporated with 0.1% BM. The growth of all the fungi were inhibited at 0.1 and 0.02% of BHC except for _Trichoderma harzianum_, _T. lignorum_ and _Aspergillus niger_ at 0.02% concentration. Furadon (0.2%) also did not affect the growth of fungi in liquid media. The effect of furadon was not tried in solid media because the distribution of the granules was not uniform.

3.6. Effect of pesticides on N_2-fixers and P-solubilisers in vitro:

Effect of pesticides on the growth of N_2-fixers: The effect of pesticides on the growth of twelve isolates of *Beijerinckia* spp. are given in Table 25. Tolerance of isolate number 1, 2, 6 and 8 were observed at both the level of all the pesticides tested. Blitox and Bordeaux mixture inhibited the growth of 3, 5, 9, 10, 11 and 12 isolates. Captan and BHC at both the levels did not affect the growth of majority of the isolates except isolate 3,7, 10, 11 and 12.

The effect of pesticides on the growth of P-solubilising bacteria and fungi in both solid and liquid media are given in Table 26. In solid media, the *Pseudomonas* sp. did not grow at lower concentration of Blitox, Captan, BHC and Bordeaux mixture. The *Bacillus* sp. growth was inhibited in the presence of Blitox and Bordeaux mixture. The P-solubilising zone was clear in Blitox but in BHC incorporated Petriplates, only growth was recorded without P-solubilising zone in all the isolates. Similar trend was observed on the growth of P-solubilizers (bacteria and fungi) in liquid media. Blitox and BHC have shown inhibitory to the growth of P-solubilizers (bacteria and fungi) as compared to Captan and Bordeaux mixture treatments.

Table 25. Effect of pesticides on the growth of Asymbiotic N_2-fixers *In vitro**

Name of N_2 fixer Isolate Nos.	Elitox				Bordeaux mixture				Captan				BMC			
	RD		LD		RD		LD		RD		LD		RD		LD	
	5	10	5	10	5	10	5	10	5	10	5	10	5	10	5	10
1.	+++	+++	+++	++	++	+	+++	+++	+	+	++	++	++	+	+++	+++
2.	++	++	+	+	+	++	+	++	+	+	+	+	++	+	++	++
3.	++	+	+	-	±	-	-	-	±	±	+	+	±	-	-	-
4.	-	±	++	+	±	±	+	+	+	++	+	+	++	+	+	±
5.	-	-	+	+	±	-	-	-	+	+	-	+	+	+	+	+
6.	±	+	+	+	±	-	+	++	+	+	+	++	++	++	++	+
7.	-	-	±	-	+	+	+	+	-	-	++	+	±	+	++	++
8.	±	-	±	-	++	++	+	++	+	++	+	++	+++	+++	+++	+
9.	±	-	+	±	±	-	±	±	+	-	+	-	+	+	+	+
10.	±	-	+	±	±	-	±	±	±	+	-	-	±	+	+	+
11.	-	-	±	+	±	±	-	+	+	+	±	-	±	±	±	±
12.	-	-	±	+	±	-	-	±	±	±	±	-	±	±	±	-

* Elitox RD = 0.3%
LD = 0.06%

Bordeaux mixture
RD = 1.0%
LD = 0.02%

Captan RD = 0.2%
LD = 0.04%

BMC 50% WP RD=0.10%
LD=0.02%

Growth scale
- = No growth
± = Poor
++ = Fair
+++ = Good
++++ = Excellent

RD = Recommended dosage
LD = Lower dosage (1/5th the recommended dose)

5 = 5 days 10 = 10 days

Table 26. Effect of pesticides in the growth of P-solubilisers (bacteria and fungi) *in vitro**

Isolate No.	Bacteria/ Fungi	Blitox (0.02%)		Captan (0.04%)		BHC (0.02%)		Bordeaux mixture (0.01%)		Remarks
		SM	LM	SM	LM	SM	LM	SM	LM	
P-5	*Bacillus* sp.	-	+	+++	+++	+++	+++	-	+	
P-6	*Pseudomonas* sp.	-	+	-	+	-	+	-	-	Growth was observed in BHC incorporated plates without P solubilizing zones
39	*Aspergillus niger*	+	++	+	+	++	+	+	++	
40	*A. niger*	+	+	+	+	-	-	+	+	
41	*Penicillium* sp.	+++	++	+++	+++	+	-	+++	++	
42	*Penicillium* sp.	+	+	+	+	+	-	++	+++	

* Based on the growth in three plates/flasks

SM = Solid media LM = liquid media

+++ = Excellent
++ = Good
+ = Poor
- = No growth

4. *Antagonism studies of rhizosphere fungi and bacteria against Phytophthora in vitro:*

After the preliminary screening of sixty two isolates of fungi and twenty six isolates of bacteria against *Phytophthora arecae*, fourteen isolates of fungi and three isolates of bacteria were selected. Antagonistic properties of the selected organisms were tested against *Phytophthora arecae* and *Phytophthora palmivora*. The inhibition on the growth of two species of *Phytophthora* varied with respect to different test organisms (Table 27). In disc method *Fusarium* sp. (No.4), *Penicillium* spp. (No.23 and 30), *Aspergillus* spp. (No.18, 19 and 38), *Trichoderma* sp. (No.22) and all the three bacterial isolates were effective in inhibiting the growth of *Phytophthora arecae*, whereas *Fusarium* sp. (No. 4), *Aspergillus* spp. (No.18, 19, 38 and 41) and *Penicillium* spp. (No. 23 and 30) were effective against *Phytophthora palmivora*. The bacterial isolates were not very effective against *P. palmivora*. The culture filtrates also showed similar inhibitory pattern as in disc method towards *Phytophthora arecae*. The culture filterates of *Trichoderma* sp. (No.12), *Aspergillus* spp. (No.19, 38 and 52), *Penicillium* spp. (No.30 and 34), *Fusarium* sp.(No.37) and all the three bacterial isolates were effective against *P. palmivora*.

Table 27. Antagonism of fungi and bacteria against *Phytophthora arecae* and *Phytophthora palmivora* *in vitro*

Isolate No.	Fungi/Bacteria	Disc method (Inhibition zone in mm)		Culture filtrate method (Inhibition zone in mm)	
		P. arecae	*P. palmivora*	*P. arecae*	*P. palmivora*
A. *Fungi*					
12	*Trichoderma* sp.	-	(E)	20(E)	(E)
18	*Aspergillus* sp.	8	8	14	(E)
19	*Aspergillus niger*	9	8	(E)	12
22	*Trichoderma* sp.	12	5	17	(E)
23	*Penicillium* sp.	18	8	20	6
30	*Penicillium* sp.	8	10	18	10
32	*Aspergillus* sp.	-	3	20	4
34	*Penicillium* sp.	5	6	20	12
37	*Fusarium* sp.	-	2	22(E)	15
38	*Aspergillus* sp.	14	9	10	12
41	*Trichoderma* sp.	3	-	8	6
52	*Aspergillus* sp.	3	9	10	8
14	*Trichoderma* sp.	2	-	-	-
4	*Fusarium* sp.	9	10	8	6
B. *Bacteria*					
B4	*Pseudomonas* sp.	8	5	15	16
B1	*Bacillus* sp.	10	6	16	18
B14	*Bacillus* sp.	6	3	8	10

(E) = Over growth covered the entire plate
\- = no inhibition

5. Biochemical characterisation of root exudates of coconut and root region soils of coconut based cropping systems and effect of root extract on rhizosphere microorganisms

5.1. Biochemical characterisation of root exudates of coconut:

The root exudates from the coconut were collected for the biochemical characterisation. The quantitative estimation of sugars, aminoacids and phenols in the root exudates of coconut are presented in Table 28. The root exudates contained appreciable amount of sugars and phenols. The sugars present in the root exudates are raffinose, lactose, glucose and fructose. Among the aminoacids serine, glycine, glutamic, alanine, phenylalanine, isoleucine/leucine were detected. Succinic acid was the only organic acid detected in the root exudates.

5.2. Biochemical characterisation of root region soils of coconut based cropping system:

The data on quantitative estimation of sugars (total and reducing), aminoacids and phenols of the root region soils are given in Table 29. Significant difference in total sugars were recorded in the cropping systems, as the sugars were more in the root region soils of mixed farming and monocropping system as compared to multistoreyed cropping system and interspace soils of coconut monocropping system. Similar trend was observed in the reducing sugar content of the soils. The aminoacid and phenol contents were

Table 26. The quantity of sugars, amino acids and phenols in the root exudates of coconut*

Sugars	
A. Total sugars	993.67 ± 174.67
B. Reducing sugars	226.17 ± 42.93
Amino acids	10.64 ± 3.05
Phenols	11.60 ± 3.04

* Values are mean of six replications ± SEM in µg/g dry weight of roots

Table 29. Sugars, aminoacids and phenols in the root region soils of coconut based cropping systems

Cropping system	Total sugars	Reducing sugars	Total amino acids	Total phenols
Coconut multi-storeyed cropping	17.59 ± 1.39	3.19 ± 0.28	1.47 ± 0.38	0.24
Coconut mixed farming	27.32 ± 3.27	4.32 ± 0.70	1.18 ± 0.10	0.12
Coconut mono-cropping	29.08 ± 1.09	4.45 ± 0.58	1.24 ± 0.18	0.12
Coconut mono-cropping (interspace)	10.48 ± 1.16	3.16 ± 0.15	-	-
CD at 0.05% level	7.37	[illegible] 1.66	0.90	0.19

Average six values ± SEM

- = Not detected

All values are in µg/g dry weight of soil slightly higher in the soils of multistoreyed cropping system. The results of the qualitative characterisation of the sugars, aminoacids, and organic acids present in the soil extracts are given in Table 30. Raffinose, lactose, maltose, glucose and fructose were identified in all the systems, but sucrose was not detected in the monocropping plot. Aminoacids were same in all the cropping systems. Succinic acid was the only organic acid detected in all the cropping systems.

5.3. *Effect of root extract on the growth of rhizosphere microorganisms:*

A. Bacteria: All the bacterial isolates (12 number) tested for the growth on coconut, pepper and cacao extract incorporated media showed more or less similar growth (Table 31). Neither stimulatory nor inhibitory effects were observed with various isolates of bacteria.

B. Fungi: The growth of fungi (6 number) was not affected much by the addition of root extracts into the solid medium (coconut, pepper and cacao). The addition of the root extract in the liquid medium affected the growth of rhizosphere fungi. The stimulatory effect was observed for *Trichoderma harzianum* by the pepper and cacao root extract (Table 32). The growth of *Aspergillus niger* was enhanced by all the root extract but a reverse trend was noticed for *Cladosporium* wherein the inhibitory effect was evident.

Table 30. Soil biochemical constituents in the root region of coconut based cropping systems

Cropping system in coconut	Sugar	Amino acid	Organic acid
Multistoreyed cropping and mixed farming system	Raffinose, Lactose, Maltose Sucrose Glucose and Fructose	Ser/Glycine Glutamine Alanine Phenylalanine Valine and Isoleucine/ Leucine	Succinic acid

Table 31. Effect of root extract on the growth of Rhizosphere bacteria *in vitro*

Isolate No.	Root extracts			
	Control	Coconut	Pepper	Cocoa
Bacillus sp.(12)	+	+	+	+
Escherichia sp.(5)	+++	+++	++	+++
Bacillus sp.(6)	++	++	++	+++
Bacillus sp.(10)	++	++	++	++
Pseudomonas sp.(4)	++	+++	++	++
Bacillus sp.(11)	+	+	+	+
Micrococcus sp.(9)	++	++	+	+
Bacillus sp.(8)	+	+	+	+
Escherichia sp.(1)	++	++	+++	++
Escherichia sp.(2)	+	+	+	+
Klebsiella sp.(3)	+	+	+	++
Bacillus sp. (8)	++	++	+	++

0 – No growth
\+ – Poor
++ – Fair
+++ – Good

Table 32. Effect of root extract of coconut, pepper and cacao on the growth of Rhizosphere fungi *in vitro*

Fungi	Agar disc method* (Colony diameter in mm)				Root extract incorporated in the medium** (mycelial dry wt. in mg)			
	Control	Coconut	Pepper	Cacao	Control	Coconut	Pepper	Cacao
Trichoderma harzianum	18	21	20	29	280	283	370	420
T. lignorum	20	19	18	22	260	290	370	400
Fusarium sp.	20	18	20	18	380	310	380	340
Aspergillus niger	12	10	11	9	980	710	690	680
Rhizopus sp.	16	15	16	15	200	60	90	40
Cladosporium sp.	24	18	20	19	630	460	430	390

* Average of three replications

**5 ml extract + 45 ml Czapek-Dox broth

DISCUSSION

Discussion

In recent years, there has been considerable interest in intercropping because of imperative need to increase production and productivity from a unit area of land. Multiple cropping, intercropping, mixed cropping and multistoreyed cropping are being practiced in large scale to make use of the available land to the maximum extent and to take two, three or even more crops simultaneously or successively each year (Papendick *et al*., 1976; Nelliat and Shama Bhat, 1979). In coconut the active root zone with maximum concentration and activity are confined laterally within a radius of 2 meter from the base and vertically within 120 cm depths. About 78% of the total area is not being effectively utilised by coconut roots in a pure stand of coconut at normal planting of 7.5m x 7.5m density (Kushwah *et al*., 1973). The intensive cropping systems involving coconut are essentially crop combinations, which envisage the cultivation of other compatible crops in the interspaces between the palms. The crops chosen should create only minimum problems of soil exhaustion, deterioration and pest build-up. The effect of crop combination on the agro-ecological factors such as light, soil microorganisms, climate etc. and the physical aspects of input management affects the productivity of the crops (Trenbath, 1976; Varghese *et al*., 1978).

The coconut based multistoreyed cropping with cacao or cinnamon along with pepper and pineapple hav significantly increased the yield of coconut (Appendix-Ia). Similarly in the mixed farming experiment also an increase in coconut yield was observed (Appendix-Ib). The additional 'synergistic' effect of increase in yield is an excellent example of 'non-monetary' input in crop production. Among the several aspects, the possible role of soil biological activities were attempted in the present investigation. The experiments were planned to study soil microorganisms (bacteria, fungi and actinomycetes), beneficial microorganisms (N_2-fixers, phosphate solubilisers and VA-mycorrhizae) and the soil microbiological activities in the root region and rhizosphere of various crops in multistoreyed, mixed farming and coconut monocropping systems. Activities of the rhizoplane (root surface) microorganisms were also investigated.

Microorganisms in the root region and rhizosphere:

The bacteria, fungi and actinomycetes were observed more in the root region and rhizosphere than in the interspace (non-rhizosphere) soil (Tables 2, 3,10, 12). This is even more pronounced with the occurrence of beneficial microorganisms in the rhizosphere and root region of various crops. The microflora around the living roots are distinctly different from that of non-rhizosphere soil, since the plant creates a unique subterranean habitat for the microorganisms (Starkey, 1929a and 1929b; 1931a; Lochhead, 1940, 1952; Katznelson, 1946, 1965; Clark, 1949, 1969; Rovira, 1956a, 1956b, 1965a). On the other hand, within the rhizosphere soil itself interesting variation in the microbial population were observed. Eventhough total number of bacteria and fungi were more in the coconut rhizosphere, more number of N_2-fixers and P-solubilisers were observed in cacao rhizosphere (Nair and Subba Rao, 1977a). Many explanations can be given for these observation, (a) the rapid stimulation of N_2-fixers and P-solubilizers due to the increased availability of organic matter resulting from periodical shedding of cacao leaves and it augments the organic matter status of soil; (b) the root exudates may be favouring the proliferation of soil microorganisms; (c) better conservation of soil moisture under the multistoreyed cropping system. However, in the mixed farming system, the increased microflora may be due to the addition of cowdung slurry from the biogas plant which is recycled. The microflora enumeration in the month of May (pre-monsoon) and October (post-monsoon) in all the cropping systems in general has indicated increased fungal counts and decreased bacterial populations in pre-monsoon period. The increased fungal counts in May (pre-monsoon) may be due to the sporulation of fungi during the dry spell. In general the bacterial counts increased in the root region of all the crops in the post-monsoon period. The reduction in the number of different microorganisms in July has been attributed to continuous rainfall and other climatic factors (Nair and Subba Rao, 1977a). Similarly, Radha and Rawther (1989) reported the decreased population of fungi in monsoon season.

The intercropping of fodder legumes and napier grass in coconut garden influences the soil microflora (Potty and Jayashankar, 1976; Potty *et al*., 1977). Similarly in the present investigation increased microflora was observed both in the coconut based multistoreyed and mixed farming system as compared to the coconut monocropping system (Tables 2, 3, 10, 12). The influence of intercropping or crop mixing on the rhizosphere microflora have been reported in annual crops like wheat, corn, jute, potato, paddy and moong, and perennial grasses (Rodica *et al*., 1969; Dadchenko, *et al*., 1972; Neal *et al*., 1973; Mukherji and Chattopadhyay, 1979). The coconut and arecanut based high density multispecies cropping systems have shown increased microbial counts in the root region (Bavappa *et al*., 1986). The soil microflora decreased with increase in soil depth, and the least being recorded at 51-100 cm soil depth. The decreased population of rhizosphere microflora with increased soil depth was reported in some crops like wheat, jute,potato paddy and moong (Mishra and Srivastava, 1971; Mukherji and

Chattopadhyay, 1979). The microbial population between 30-90 cm lateral distance decreased with an increase in soil depth in the root region of arecanut palms (Bopaiah and Koti Reddy, 1982). The decreased population of microorganisms at lower depth of soil can be correlated to soil organic carbon, soil pH, total 'N' and total 'P' content of the soil. The organic carbon content of the soil decreased with the increase in soil depth. A considerable decrease in soil pH, organic carbon, total N, total P and available P was recorded at lower depth (Tables 7 and 14). Further, the interspace soils of the multistoreyed cropping system showed varied trend in microflora population between the different crops indicating the root interaction and its influence on the proliferation of microorganisms. The microflora in root region of pineapple at 51-100 cm soil depth was less because of the shallow rooting. However, among the different cropping systems studied, the coconut based cropping system with the combination of pepper, cacao and pineapple favoured an increased microflora as compared to the other crop combinations. The increased microflora and microbiological activities could be due to higher organic carbon content of the soil. The coconut-cacao mixed cropping have shown increased microflora as compared to coconut monocropping system (Nair and Subba Rao, 1977a). The bacteria and fungi were more in the rhizosphere of cacao as compared to other crops in the multistoreyed and coconut monocropping system (Table 5).

The soil microflora were enumerated at three soil depths as the coconut roots vertically go to a depth of 1.2 to 1.5m and to a lateral spread of 2m from the base. The microflora at various soil depths indicated that the microbial counts decreased with increase in soil depth. The higher microbial counts in the root region of various crops may be attributed to favourable environment. The activity of the rhizosphere and root surface microorganisms can affect the nutrient uptake (Bowen and Rovira, 1969; Nair and Subba Rao, 1977a) and wide range of compounds present in the rhizosphere can influence the growth of the plant (Rovira, 1965a, Stevenson,1967). The rooting habit in cacao and cinnamon had relatively less lateral spread (Nelliat and Shama Bhat,1979). Cacao plant had most of its roots with the radius of 80-100 cm and maximum concentration was between 26-60 cm laterally. The fibrous roots of pineapple rarely extended beyond 50 cm laterally and vertically.

Occurrence of beneficial microorganisms:

The occurrence of beneficial microorganisms such as asymbiotic N_2-fixers, P-solubilisers (bacteria and fungi), nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) and endogone mycorrhizae in different cropping system revealed that the population of asymbiotic N_2-fixers were more in the multistoreyed cropping as compared to monocropping system (Table 4). The asymbiotic N_2-fixers were more in the root region of cacao, pineapple and cinnamon.

Beijerinckia has been reported to be widely distributed in tropical acidic soils (Becking, 1959; 1961; Vancura *et al.* 1965; Anderson, 1966; Dobereiner and Campalo, 1971; Subba Rao, 1983). The occurrence and enumeration of asymbiotic N_2-fixers in the rhizosphere of coconut-cacao mixed cropping was studied (Nair and Subba Rao, 1977a). The proliferation of N_2-fixers in coconut based mixed farming experiment with legumes and napier grass has been studied (Potty, 1977). The nitrogen fixing *Azotobacter* and *Beijerinckia* enumerated in arecanut soils, have revealed greater number of *Beijerinckia* in plots with hybrid napier grass (NB-21) (Rawther, *et al.*, 1979). In the present study also, the coconut based mixed farming system encouraged the proliferation of asymbiotic N_2-fixers and *Beijerinckia* was found to be the predominant asymbiotic N_2-fixer. Asymbiotic N_2-fixers were maximum in the root region soils of coconut and napier grass in mixed farming system as compared to multistoreyed and monocropping system. Kothandaraman (1979) reported the occurrence of *Beijerinckia* in the soils of rubber plantation. The role of the asymbiotic N_2-fixers in supplementing the nitrogen in its immediate environment, thereby benefiting the plant can not be ruled out. Several workers have in fact stressed the probable role of *Beijerinckia* in restoring the nitrogen level of many tropical fallow lands (Becking, 1959; Anderson,1966; Dobereiner and Campelo, 1971).

Several microorganisms showing the phosphate solubilising ability have been reported to be associated with the soil (Abbott,1923; Pikovskaya,1948; Sen and Paul,1957; Sethi and Subba Rao, 1968). The occurrence and the activities of P-solubilisers in the rhizosphere of plantation crops have also been reported by a few workers (Nair and Subba Rao,1977b; Bopaiah, 1983; Thomas *et al*., 1985 and Thomas and Shantaram, 1986). Among the several bacteria, fungi and actinomycetes capable of solubilising phosphorus from the rhizosphere of coconut-cacao mixed cropping, the *Pseudomonas* and *Aspergillus niger* solubilised 48.9 and 49.7 per cent respectively of the total tricalcium phosphate *in vitro* (Nair and Subba Rao, 1977b). Thomas and Shantaram (1986) reported that *Pseudomonas* sp., *Micrococcus* sp., *Micrococcus roseus*, *Bacillus subtilis*, *Corynebacterium* sp. and *Alcaligenes* sp. were the phosphate solubilising bacteria encountered in the coconut soil. Similarly in the present study *Pseudomonas* sp. and *Bacillus* sp. solubilised 35.68 and 41.72 per cent of tricalcium phosphate *in vitro* (Table 30). Thomas *et al*.

(1988) also studied the P-solubilising fungi in different coconut soils and the *in vitro* P-solubilising ability was 26 to 74 per cent of tricalcium phosphate. In the present study the P-solubilizing fungi isolated from the root surface of coconut showed 34.07 to 47.74 per cent P-solubilising ability *in vitro* with tricalcium phosphate as substrate.

The occurrence and the activity of P-solubilisers in soils from arecanut plantation has been reported by Rawther *et al.* (1979) and Bopaiah (1983). The P-solubilisers reported in the root region of arecanut soil were *Aspergillus* spp., *Penicillium* spp. and *Pseudomonas* spp. Concurrent with the above observation it was noticed there exists a direct relationship between the population of P-solubilisers and the amount of available phosphorus in soil. The beneficial role of P-solubilizing microorganisms has been reported by Gerretsen (1948), Sen and Paul (1957) and Barber (1968).

The endogone spores and VA-mycorrhizal colonisation was more in all the coconut based cropping system with respect to per cent infected roots and extent of colonisation. The VA-mycorrhizal colonization in coconut and tea has been recorded by the earlier workers (Lily,1975; Ramesh and Rohini Iyer,1979; Satyanarayana and Venkataraman,1979).

However, Nair and Subba Rao (1974) did not report the mycorrhizae in coconut and cacao of coconut-cacao mixed cropping system. The mycorrhizal association is known to play an important role in phosphate, zinc and copper nutrition in plants (Hayman, 1975; Mosse, 1978 and Krishna and Bagyaraj,1982).

Soil Enzyme Activities:

The soil enzyme activities such as urease, dehydrogenase and phosphatase were studied for the different cropping system both for the root region and rhizosphere of crop plants. Urease activities varied with soil depths in Italian rye grass and native pastures (Hoult and McGarity, 1986). The type of vegetation supported by a soil directly influences the urease activity (Khan,1970; Pancholy and Rice,1973). In the present investigation, in general,the soil enzyme activities (urease and phosphatase) decreased with increase in soil depth (Fig.1, 3, 5). The urease activity was low in the root region soils of cacao and pineapple. The dehydrogenase activity did not reveal any variation between the rhizosphere and root region soils of different crops. The biochemical activities in soils have revealed that the dehydrogenase activities appeared to change less with soil depth (Musa and Mukhtar,1969; Ross,1973). Similarly in the present investigation also the dehydrogenase enzyme activity decreased with increase in soil depth. The urease and alkaline phosphatase activities were positively correlated with organic carbon, fungal and bacterial population in jute soils (Tarafdar et al.,1981). The proportionate decrease in soil enzyme activities at 26-50 cm and 51-100 cm soil depth may be due to soil pH and low organic carbon and total nitrogen content of the soil. Dalal (1982) reported a significant close relationship between phosphatase activity and organic and inorganic P in the soil. The phosphatase and dehydrogenase enzyme activities and soil moisture, organic carbon and nitrogen content in the no--tillage soil were significantly higher than those of conventional tillage (Doran,1980a). The microbial counts and relative abundance of various microbial types suggests that the biochemical environment of no-tillage soil is less oxidative than that under conventional tillage. Moore and Russel (1972) studied the factors affecting dehydrogenase activity as an index of soil fertility and suggested that it cannot be used as general index of soil fertility.

The rate of carbon mineralization was more in surface soil and it decreased with the depth of the soil. This decrease in activity parallelled the drop in the organic carbon level, so that the proportion of the total carbon oxidised in a given time interval remained relatively constant (Newman and Norman, 1942). The C : N ratio of 18 : 1

or 10:1 is reasonable for the predominant aerobic flora. Cultivation enhances organic matter destruction (Gidden, 1957). In the present study, the decrease in soil organic matter content was recorded in coconut monocropping system as compared to coconut multistoreyed cropping system and this decrease may be due to the surface ploughing in the monocropping system. The rate of decomposition and development of soil microflora (bacteria, fungi, actinomycetes and *Azotobacter*) increased with the addition of organic matter (Gaur, *et al*. 1971; Debnath and Hajra, 1972). The *in vitro* studies conducted also revealed that the addition of carbon source (cellulose or glucose) enhanced the rate of carbon mineralisation (Table 6 and 13).

In the nitrogen mineralisation studies, the accumulation of NH_4-N was observed in the root region soils of cacao and pineapple. Vlassak (1970), and Adams and Attiwill (1982) reported the establishment of close relationship between the total nitrogen content of the soil and the amount of mineral nitrogen formed during the incubation. The nitrate accumulation has been reported to increase considerably with pH in tea plantation soils. The nitrification of soil; under wide range of pH, organic carbon and total nitrogen and the amount of NO_3-N, was in the range of 0-123 µg/g of soil (Ishaque and Cornfield, 1972). The studies on the variation in the rate of nitrogen mineralisation in soil indicated that forty two per cent of the total estimated N-mineralised was derived from surface soil (0-18 cm), whereas 58 per cent was contributed from the 18-108 cm depth (Cassman and Munns,1980). In the present investigation, the nitrogen mineralisation was more in the mixed farming and the mineralisation decreased with increase in soil depth. The NO_2-N was not detectable during the mineralisation studies.

Soil microbial biomass:

Microbial biomass of the soil is the living part of the soil, excluding plant roots and soil animals. The fumigated soil consumes more oxygen (Jenkinson and Powlson 1976) and evolves more carbondioxide (Waksman and Starkey, 1923; Powlson and Jenkinson,1976). The higher flush of biomass C was recorded in both multistoreyed cropping system and mixed farming system as compared to coconut monocropping system. The higher microbial load might have been reflected in the microbial biomass in the multistoreyed and mixed farming system. The total biomass in root-zone soil from Submontane grassland variant management ranged from 40.8 to 60.70 mg C/100g as compared to 69.4 to 143.0 mg C/100g for the rhizosphere soil (Tesarova and Repora,1984). The soil biomass increased during the growth of wheat crop and then decreased to an approximately constant level

(Lynch and Panting, 1982). The biomass was significantly greater where the soil had been directly drilled than where it has been ploughed probably because the plant roots were more abundant after direct-drilling (Carter and Rennie, 1982).

The soil microbiological activities have revealed higher activities in the multistoreyed and mixed farming system as compared to monocropping system. In multistoreyed cropping system, the soil is under zero tillage,whereas in monocropping the soil is surface ploughed every year. Doran (1980b) reported that maximum aerobic microbial activity in the surface of zero-tillage soil as compared to conventional tillage soil. The biochemical environment of the zero-tillage soil is less oxidative than that under the conventional tillage.

In the present study an increase in soil fertility parameters such as organic carbon and total nitrogen was recorded in coconut multistoreyed cropping system and coconut mixed farming system as compared to their respective monocropping system. Soil fertility depends to a large extent on the microbial profile of the soil. When intercropping or mixed farming are introduced in a coconut garden, congenial conditions are developed for the rapid proliferation of microorganisms in soil. The continuous addition of plant residue by the component crops and the organic recycling facilitated by the multistoreyed or mixed farming exert a favourable influence on the microbial population in the rhizosphere and root region of the plants. The addition of energy sources has increased the biomass and urease and phosphatase enzyme activities (Ross et al.1982; Nannipieri et al., 1983).

The amount of organic matter added to the soil through shed leaves and prunings of cacao under coconut with single and double hedge planting of cacao was 818 and 1985 kg/ha/ year respectively (Varghese et al.,1978). The organic carbon content of soil under mixed cropping of coconut with cacao was found to be higher than that under pure stand of coconut. Further the available phosphorus content in the coconut rhizosphere where cacao was grown (double hedge) was 65 ppm as compared to 41 ppm in single hedge plant, 30 ppm in monocropping and 10 ppm in non-rhizosphere soil (Nair and Subba Rao, 1977a). Similarly, in the present investigation, higher concentrations of soil nutrients were recorded in the multistoreyed and mixed farming systems. The organic carbon content of the soil and the microclimatic conditions might have favoured the proliferation of microorganisms. The multistoreyed cropping system is known to change the microlcimate such as air temperature, evaporation, relative humidity and soil temperature as compared to monocropping system in coconut (Varghese et al., 1978; Nelliat et al., 1979). The crop combinations in the interspace of coconut is bound to change the microclimate in the mixed farming system also.

The greater root volume of the crops per unit volume of soil adds the more organic matter by way of dead roots. Considerable evidences are available to show that there can be formation of soil organic matter during the active root growth (Sauerbeck and Johnen, 1977; Martin, 1977). The addition of roots by napier grass in the mixed farming and by cacao/cinnamon, pepper and pineapple in multistoreyed cropping systems can increase the soil organic matter as compared to the coconut monocropping system. In cacao and cinnamon the lateral spread of the root system is comparatively less. The root volume per unit area is more in the multistoreyed and mixed farming system and hence the overall biological activities were more as compared to the monocropping.

The quantitative microflora in rhizosphere varied with the crops and the microbial load was higher in the rhizosphere as compared to the respective root region soils. This is because the rhizosphere is functioning as the active zone for the proliferation and inhabiting of microorganisms due to the availability of substrates. Similarly higher enzyme activities was also observed in the rhizosphere soils in different cropping systems.

The microorganisms are responsible for ammonification, nitrification, denitrification, carbohydrate utilisation, cellulose decomposition and nitrogen fixation. The abundance of nitrogen fixing and phosphate solubilising bacteria in the rhizosphere of crop plants assumes natural significance from the agronomic point of view. The alteration of rhizosphere microflora is possible by (1) soil amendment and (2) artificial inoculation of seed or soil (bacterisation). The inoculation with *Azotobacter*, *Beijerinckia*, P-solubilising microorganisms and VA-mycorrhizae may help in the establishment in the immediate vicinity of roots of crop plants.

The present study established that the microflora and the microbiological activity were greater in rhizosphere and root regions of coconut, cacao and pineapple in the multistoreyed and coconut based mixed farming system as compared to the monocropping. The build up in soil organic carbon and total nitrogen influences the soil fertility.

Rhizoplane microflora:

Studies on the rhizoplane microflora have indicated that the root surface of various crops inhabited higher microbial load than the rhizosphere. This can be attributed to the availability of substrates from the roots in the form of exudates, which will serve as energy source for the proliferation of microorganisms. The sloughed off roots and dead roots also serve as substrate for the growth of microorganisms in the root region of crops. Several workers have reported the proliferation of microorganisms in the rhizoplanes of different crops (Abdel-Nasser and Makawi, 1977; Odunfa and Oso, 1979).

The grassland species influence the abundance of microbes on each other roots (Christie *et al*., 1974). The occurrence of beneficial microorganisms such as asymbiotic N_2-fixers (*Beijerinckia* sp.), associative symbiotic N_2-fixers (*Azospirillum* sp.) and phosphate solubilising microorganisms (bacteria and fungi) have revealed their relative abundance on the root surface of different crops. The asymbiotic N_2-fixers and P-solubilisers were more in coconut-pepper-cacao-pineapple (multistoreyed) cropping system. The *Azospirillum* were more in the mixed farming system which may be due to the presence of napier grass in the cropping system. The occurrence of *Azospirillum* on the roots of coconut and other plantation crops has been reported by Subba Rao (1983) and Govindan and Purushothaman (1985). The possible role of these organisms on the plant growth and soil fertility has to be ascertained.

Trichoderma, *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Cylindrocarpon*, *Absidia* and *Rhizopus* were found to be associated with the rhizosphere of the various crops in different cropping systems studied (Table 19). Of these, *Trichoderma* spp. and *Aspergillus* spp. were found to be predominant. However, in the rhizosphere of napier grass, *Rhizopus* spp. was frequently associated. These fungi may be commonly occurring in the rhizoplane and root region soils of different crops. The occurrence of *Cylindrocarpon effusum*, *Fusarium equiseti*, *Monacrosporium bembicodes*, *Penicillium javanicum*, *P. spiculisporium* and *Graphium* sp. were reported to be associated with root (wilt) affected coconut palms (Joseph, 1978).

Bacteria associated on the rhizosphere of various crops in different cropping systems were *Pseudomonas* spp. (4 isolates), *Bacillus* spp. (9 isolates), *Micrococcus* spp. (2 isolates), *Escherichia* spp. (3 isolates), *Arthrobacter* spp. (1 isolate), *Achromobacter* spp. (1 isolate) and *Klebsiella* spp. (1 isolate). Some isolates of *Pseudomonas* spp. and *Escherichia* spp. produced indole *in vitro* and several isolates of bacteria possessed good phosphate solubilising ability. *Pseudomonas putida* and *Pseudomonas fluorescence* isolated from Maize and Bean produced indole-3-acetic acid and other auxins when grown in suspension cultures (Prikryl *et al*., 1985). Similarly Nair and Subba Rao (1977a) reported the indole producing *Escherichia* spp. from the coconut-cacao mixed cropping system. *Streptomyces* spp. was the only actinomycetes observed on the rhizoplane of crops. The antagonistic properties are well-known in the actinomycetes groups. The occurrence of various fungi and bacteria on the rhizosphere of various crops showed variation in the different cropping systems studied. This indicates that the plant species are favouring the selective inhabiting of microorganisms on the rhizosphere.

Cellulase enzyme:

Cellulase is an induced enzyme in most microorganisms as it is synthesised only in the presence of specific substrates, cellulose or cellulose derivatives. The cellulase enzyme activity (C_x) in *Trichoderma harzianum* was greater when grown on CMC as carbon source. Cellulolytic activity in *Pyricularia oryzae* was higher when the organism was grown on insoluble filter paper as compared to soluble carboxymethyl cellulose as carbon source (Manibhushan Rao, 1971). The viscosity reduction of carboxymethyl cellulose (CMC) has been established as an estimate of cellulase (C_x) activity (Kelman and Cowling, 1965). Oluliola (1976) studied the cellulase enzymes in culture filtrate of *Aspergillus flavus* with soluble (CMC and Cellobiose) and insoluble (Cellulose powder) cellulose as carbon source. The effect of cultural condition on the production of cellulase in *Trichoderma longibrachiatum* was studied (Sandhu and Kalra, 1985).

Cellobiose was comparatively better utilised by certain isolates of *Pyricularia oryzae* (Manibhushan Rao, 1971). Hussain and Rich (1958) studied the extracellular pectic and cellulolytic enzymes of *Cladosporium cucumerinum*. Similarly in the present investigation the *Cladosporium* has showed a good cellulase enzyme activity *in vitro*. *Trichoderma viride* and *Aspergillus niger* grown on cellulose medium produced a stable cellulose enzyme complex including C_1.

Effect of pesticides on Rhizosphere fungi and bacteria:

The application of herbicides, insecticides, nematicides, fungicides and bactericides may affect the non-target micro-organisms. Studies on the effect of pesticides on bacteria revealed that the growth of all the isolates of bacteria was inhibited by Bordeaux mixture, (BM) Blitox and Benzene hexachloride (BHC), whereas Captan did not inhibit the growth of any of the bacterial isolates tested. Among the pesticides tested *in vitro* Bordeaux mixture and BHC were found to have more deleterious effect on fungi. Many workers have reported the effect of soil application of Captan, BHC and Carbofuran on microorganisms (Houseworth and Tweedy, 1973; Mahmoud *et al*., 1972; Ross, 1974; TU, 1975; Wainwright and Pugh, 1975).

The studies on mycelial biomass production indicated variation in the growth of different root surface fungi. *Cladosporium* sp. *Trichoderma lignorum* and *Aspergillus niger* were the fast growing as compared to *Trichoderma harzianum*, *Fusarium* sp. and *Rhizopus* sp. The growth of fungi was also studied with different cellulose, and pectin as sole source of carbon in the medium. The growth of all the fungi tested was good with CMC and cellubiose as carbon source, which may be due to selective preference for the substrate.

The growth of rhizosphere fungi at different pH, sodium chloride and sucrose concentration showed a different trend with respect to different fungi. A wide adaptability to pH was recorded in the species of *Fusarium* and *Cladosporium*.

N_2-fixers:

The mixed cropping favoured the proliferation of nitrogen fixing microorganisms among which *Beijerinckia* and

Azotobacter were the dominant N_2-fixers (Potty, 1977). In the present investigation, *Beijerinckia* spp. was the predominant asymbiotic N_2-fixers isolated from the root surface of coconut and other crops. The *Beijerinckia* isolates obtained from the coconut based multistoreyed cropping and coconut mixed farming system recorded better nitrogen fixing capacity (8.4 - 15.6 mg/g of sugar) as compared to the isolates obtained from coconut monocropping system (5.6 - 10.1 mg/g of sugar). The *in vitro* nitrogen fixing capacity ranged from 10.6 to 19.0 and 14.6 to 16.8 mg per 100 ml for *Beijerinckia* spp. and *Azotobacter* spp. respectively in the coconut mixing farming experiment (Potty, 1977).

The effect of pesticides on 12 isolates of *Beijerinckia* spp. revealed different response on the growth. Some of the isolates (1, 2, 6 and 8) have shown tolerance to the pesticides *in vitro* (Table 25). However, Blitox and Bordeaux mixture inhibited the growth of all the isolates as compared to other pesticides tested.

P solubilisers

The phosphate solubilising actinomycetes, bacteria and fungi isolated from coconut-cacao mixed cropping system mineralised phosphorus in the range of 20-40 µg/ml, 42-89 µg/ml and 34-81 µg/ml respectively (Nair and Subba Rao, 1977b). In the present investigation, the P-solubilising bacteria and fungi showed good P-solubilising ability *in vitro* with tricalcium phosphate as substrate. Thomas and Shantaram (1986) tested the various bacteria from coconut plantation soils and found that *in vitro* P-solubilising ability was in the range of 19.5 to 54.0 per cent. The P-solubilising fungi occurring in coconut soil solubilised 26-74 per cent of the tricalcium phosphate and among them eight fungi showed high competitive saprophytic ability (Thomas *et al.*, 1985). The P-solubilisers were obtained from the soil of arecanut plantation.

Thus, this study indicates that there is efficient P-solubilising microorganisms inhabiting in the rhizosphere and rhizoplane of coconut soils. Hence the rock phosphate application will be advantageous for the coconut plantation.

The effect of pesticides on P-solubilisers *in vitro* revealed different response both in solid and liquid media. Blitox, BHC and Bordeaux mixture have shown greater inhibitory effect even at lower concentration as compared to captan (Table 26). This calls for the detailed investigations on the effect of pesticide application to soil on the survival and the activity of the phosphate solubilising microorganisms.

Another aspect of plant microbe interrelationships currently attracting the attention is the formation of a fungal mantle around the root system of the plant after invading into the interior of the root cortex. The fungi are often instrumental in absorbing the non-available nutrients from the soil and passing them on to the plant roots (Bowen and Theodorou, 1967; Tinsley and Darbyshire, 1984). Apart from that, the fungi also confer protection to the plants from invading pathogens by virtue of their antibiotic producing abilities (Garrett, 1960; Zak, 1964).

Four different fungi, *Fusarium* sp. (Isolate No.5), *Sepedonium* sp., *Penicillium* sp. (Isolate No.1) and *Trichoderma lignorum* were found closely associated with coconut and cacao root surfaces (Nair and Subba Rao, 1978). The predominant occurrence of *Fusarium* and *Cylindrocarpon* on roots of many plant without any apparent injury suggests that such fungi may be the normal root surface inhabitants. *Trichoderma lignorum* must also be mentioned here because of its ubiquitous occurrence not only in the rhizosphere but also in the rhizoplane and root region soils of coconut.

The interaction among the rhizosphere microflora often assumes considerable significance in the context of root infections and their role in biological control. Production of antibiotics by soil fungi, actinomycetes and bacteria has been recognised for the past several decades.

Zak (1964) postulated that fungi may furnish protection from pathogens by (a) utilizing root carbohydrates and other nutrients which would attract the root pathogen; (b) providing a physical barrier as in the case of mycorrhizae for the entry of pathogen; (c) secreting antibiotics which will kill or inhibit the pathogen; (d) supporting a protective rhizosphere population of other microorganisms; and (e) stimulating the cells of the root during symbiosis to elaborate certain chemicals which will specifically inhibit the pathogens.

Investigation into the natural microbial antagonisms existing in the coconut rhizosphere revealed that several species of *Trichoderma* have shown antagonism against *Phytophthora* *in vitro*. The behaviour of *T. lignorum* is understandable in view of its ability to produce a powerful antibiotic "viridin" well known for its inhibition of several genera of bacteria and fungi. It is highly probable that a good deal of protective influence was bestowed by this species on coconut palm, as not only their rhizosphere but also the rhizoplane was found to be abundant with this organism.

Antagonistic microorganisms:

The rhizosphere is composed mainly of non-pathogenic microorganisms but the very density and the increased microbial interactions-competitive, antagonistic and beneficial can be especially important for the soil borne pathogens, because it must enter through rhizosphere in order to initiate infection. Several isolates of fungi, bacteria and actinomycetes were tested for their antagonistic properties against *Phytophthora arecae* and *P. palmivora*. Though the different species of fungi varied in their antagonism to these two pathogenic fungi, the bacteria showed similar inhibitory effect on both the species of *Phytophthora*. The culture filtrate of bacteria was more inhibitory to *Phytophthora* than these organisms when tested on solid media.

The antagonistic nature of rhizosphere and rhizoplane microflora of coffee against *Rhizoctonia solani* was reported by Venkatasubbaiah *et al.* (1984). The *Trichoderma harzianum*, *Aspergillus niger*, *Penicillium* sp. and *Bacillus subtilis* were found to be antagonistic to *R. solani*, similarly the rhizosphere and rhizoplane of coconut and other crops inhabited several species of *Trichoderma*, *Aspergillus*, *Penicillium*, *Bacillus* and *Pseudomonas* and these organisms have shown great deal of antagonism against two species of *Phytophthora*. The antagonistic microflora such as *Trichoderma viride*, *Chaetomium trilaterale*, *Streptomyces* spp. and *Pseudomonas* spp. in the rhizosphere of potato was found to occur in higher frequency as compared to control soil (Sudha Mall, 1973). The antifungal activity of Western Australian soil actinomycetes against *Phytophthora* and *Pythium* species and mycorrhizal fungi was reported (Keast and Tonkin, 1983). The antagonism of a *Bacillus subtilis* isolate was reported against *Phytophthora cactorum* (Utkhede, 1984) and another isolate of *Bacillus subtilis* against vascular wilt pathogens (Podile *et al.*, 1985). In the present study two isolates of *Bacillus* spp. and an isolate of *Pseudomonas* sp. were found to be antagonistic against two species of *Phytophthora*.

Many isolates of *Trichoderma* produce both non-volatile and volatile antibiotics active against a wide range of fungi and the ability to produce antibiotics varied among the isolates of the same species groups as well as between the isolates of different species groups (Dennis and Webster, 1971a, 1971b). The formation of oospores by *Phytophthora* in response to *Trichoderma* spp. is a defensive response to a potential antagonistic or competition and this form of microbial interaction may be common (Brasier, 1978). The identification of several potential antagonistic fungi, bacteria and actinomycetes paves way for more detailed investigation of their use in the biological control of *Phytophthora* diseases of plantation crops.

Root exudates and soil biochemical characterisation:

The biochemical characterisation of root exudates of coconut revealed that the root exudates contained appreciable quantity of sugars, aminoacids and phenols. The qualitative study showed the presence of various compounds of sugars, aminoacids and organic acids. The report on the biochemical characterisation of root exudates of coconut were not available so far. Ramadasan *et al.* (1987) described the method for the collection of root exudates of coconut. The arecanut based multispecies cropping system has been reported to influence the quantitative exudation of aminoacids in areca root exudates, while it did not effect the exudation of sugars (Nagaraja *et al.*, 1986). The occurrence of malic acid in the root exudates of tea has shown to solubilise appreciable quantity of phosphorus, iron, aluminium by chelating these elements (Jayman and Shivasubramaniam, 1975). The organic acids in the root exudates of coconut may also have similar role which needs to be investigated. Root exudate of sunhemp (Balasubramanian and Rangaswamy, 1974) has been characterised with respect to sugars and aminoacids. Sugars, aminoacids,phenols and organic acids in the root exudates may directly or indirectly influence the soil microorganisms in the rhizoplane, rhizosphere and root region. In the mixed cropping system variation in the root exudates may alter the microflora. Therefore, this may be one of the reasons for the changes in soil microflora under mixed farming or multistoreyed cropping system. The nature and amount of substances exuded by the roots of plant dependent on the species of the plant, age and environmental conditions (Dey et al., 1968). The root exudates may play a role in neutralising the soil pH and altering the microclimate of the rhizosphere through the liberation of water and carbon dioxide (Subba Rao, 1977).

Soil biochemical analysis have indicated the variation between the cropping systems studied. Carbohydrates are energy source for living organisms and free sugars were extracted from soil (Gupta and Sowden, 1963). Glucose, arabinose, fructose, xylose, galactose and ribose are the sugars identified from the soil, of which glucose constituted two-thirds of the total sugar. The polysaccharides of microbial origin are present in soil to the extent of 0.1 per cent. These contain galactose, glucose, mannose and arabinose (Lynch et al., 1957).

The presence of certain compounds in soil may directly or indirectly influence the microflora in the root region. The present study revealed the quantitative variation in sugars, aminoacids and phenols in the root region soil of different cropping system (Table 29) . However, there was not much variation in the qualitative composition of sugars, aminoacids and organic acids, except that sucrose was detected in the root region soils of coconut monocropping system. The organic substances in soil arise from the decomposition of animal and plant residues by microorganisms or from the plant root exudates. The phenol content was low in the root region of coconut based cropping system, whereas the phenol content was high in arecanut soil (Manikandan and Siddappa, 1986). This difference in phenols may be due to the crop species and soil type.

The root extract of pepper slightly inhibited the growth of certain species of bacteria, whereas, the root extract of coconut and cacao did not show any inhibitory or stimulatory effects. The root extract of coconut, pepper and cacao indicated a stimulatory effect on the growth of Aspergillus niger and Trichoderma harzianum. The root extract of pepper and cacao caused a reduction on the growth of Rhizopus sp. and Cladosporium sp. Chile and Vyas (1984) reported antifungal activity of plant extract of piper beetle against certain isolates of Botryodiplodia theobromae. This activity has been attributed to the presence of phenolics and essential oils. However, Sullia (1973) reported that the root extract of Cassia tora and Crotalaria spp. induced a high degree of stimulation in the growth of *Trichoderma lignorum*, *Aspergillus terreus*, *Aspergillus niger*, *Penicillium humicola*, *Cladosporium herbarum* and *Curvularia lunata*. Similarly in the present investigation the root extracts tested showed stimulatory effect on the growth of *Trichoderma harzianum* and *Aspergillus niger* and inhibitory effect on *Rhizopus* sp. and *Cladosporium* sp. Rovira (1965) has concluded after comparing the bacterial and fungal population of the rhizoplane, that bacteria seem to depend more on root exudates and fungi on moribund root cells.

SUMMARY AND CONCLUSIONS

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Coconut (*Cocos nucifera* L.) is an important plantation crop grown in India. The area under coconut is about 1.2 million hectares and about 98% of the holdings are below 2 hectare. In a pure stand of coconut with the normal spacing of 7.5m x 7.5m, about 75% of the land area is not effectively utilised. Therefore based on the pattern of utilisation of soil and solar energy, the coconut palm is amenable to intensive cropping. Several crops can be grown in the interspaces of coconut palms to increase the production per unit area. The multistoreyed cropping and mixed farming systems have shown to increase the yield of coconut as compared to pre-experimental yield or coconut monocropping system. Therefore the possible components of non-monetary inputs are worth examining.

The nature and the activity of microflora and fauna depend upon the crops grown and the management practices. The nature of microorganisms associated with perennials like tree crops are almost constant but the introduction of other annuals or perennials could change the equilibrium and this in turn could be either beneficial or detrimental for the crop community. In the present investigation, the long term effects of coconut based multistoreyed cropping system consisting of pepper-cinnamon-pineapple or pepper-cacao-pineapple and coconut mixed farming system consisting of pepper-napier grass were studied separately in comparison with coconut monocropping on the soil microbiological and fertility parameters. In addition to this, the rhizoplane microflora and the occurrence and the activities of asymbiotic N_2-fixers and P-solubilisers on the root surface of various crops were studied. Growth and certain physiological activities of rhizosphere fungi and bacteria formed a part of the investigation. The antagonism of rhizosphere and root region fungi and bacteria against *Phytophthora* and the biochemical characterisation of root exudates of coconut and soils of different cropping systems were attempted.

Soil samples were collected from the root region of various crops of coconut based cropping system from three depths viz., 0-25, 26-50 and 51-100 cm for studying the microbiological and fertility parameters. Rhizosphere samples were collected from different crops. The results of various studies are summarised as below:

(1) The root region microflora (bacteria, fungi, and actinomycetes) of coconut based multistoreyed cropping system (pepper-cacao-pineapple) recorded higher microbial populations as compared to the coconut monocropping system both in pre-monsoon and post-monsoon periods. The interspaces of multistoreyed crops recorded higher microorganisms

as compared to the interspaces of coconut monocrop. In general, the bacterial, fungal and actinomycetes counts decreased with the increase in soil depth and it was least in 51-100 cm depth region.

(2) The beneficial microorganisms such as asymbiotic N_2-fixers, phosphate solubilisers (bacteria and fungi) were higher in the root region soils of multistoreyed cropping system as compared to coconut monocrop. The Most Probable Number (MPN) method to enumerate the nitrifying bacteria revealed slight variation in the counts of _Nitrosomonas_ but the _Nitrobacter_ count did not indicate any variation. A progressive decrease in endogone spores and VA-mycorrhizae colonisation (per cent) was recorded with the increase in soil depth.

(3) Soil enzyme activities such as urease, dehydrogenase and phosphatase showed marked differences in the root region of surface soils (0-25 cm depth) of coconut plantation: in both multistoreyed and monocropping systems as compared to the root region of other crops. The urease and phosphatase enzyme activities decreased with increase in soil depth both in the root region and interspace soils.

(4) The rhizosphere of pineapple, cinnamon and cocoa recorded higher bacterial counts as compared to coconut in both multistoreyed and monocropping systems. The counts of N_2-fixers and P-solubilisers were more in the rhizosphere of multistoreyed cropping system. Urease activity was low in the rhizosphere of coconut monocrop. The phosphatase activity was low in the rhizosphere of cocoa, cinnamon and pineapple. The addition of glucose increased the dehydrogenase activity of the soil.

(5) Carbon mineralisation (native) was high in the root region of coconut and pineapple. The addition of glucose and cellulose (0.5%) increased the mineralisation by 3-4 fold and maximum was recorded in the glucose amended soils. The mineralisation rate decreased with soil depth.

(6) Nitrogen mineralisation revealed lower level of NH_4-N in the surface soils of the multistoreyed cropping system. The trend in NO_3-N in the nitrogen mineralisation decreased with soil depth.

(7) Soil chemical properties indicated that the organic carbon (%), total nitrogen, total P and total K were high in the root region soils of coconut multistoreyed cropping system as compared to coconut monocrop and all these parameters decreased with increase in soil depth.

(8) The root biomass was high in the surface soil (0-25 cm) of the coconut multistoreyed cropping system as compared to coconut monocrop and the cellulose decomposing capacity was also high in the surface soil.

(9) The soil microbial biomass was greater in the root region soils of coconut and cacao of the multistoreyed cropping system as compared to coconut monocrop. Carbon, nitrogen and phosphorus in biomass were higher in the multistoreyed cropping system.

(10) Soil microflora (bacteria, fungi and actinomycetes), beneficial microorganisms, (N_2-fixers, P-solubilisers), soil enzyme activities (urease, dehydrogenase and phosphatase), carbon and nitrogen mineralisation and soil microbial biomass were greatly influenced in the mixed farming system as compared to coconut monocropping system.

(11) Rhizoplane microflora studies revealed that the bacterial counts were higher in the rhizoplane of cacao as compared to other crops. The fungal counts were more in coconut-pepper-cacao-pineapple cropping system and it was least in the rhizoplane of coconut in the mixed farming system.

(12) The asymbiotic N_2-fixers (*Beijerinckia* sp.) were more in the rhizoplane of coconut-cacao-pineapple cropping

system (MS). The percentage occurrence of *Azospirillum* was higher in the coconut mixed farming system.

(13) The occurrence of different fungi, bacteria and actinomycetes in the rhizosphere of different crops of coconut based multistoreyed, mixed farming and coconut monocropping system were studied. Among the fungi, *Trichoderma* and *Aspergillus* were predominant in the rhizosphere of different crops. The different physiological groups of bacteria were recorded in the rhizosphere of different crops. The bacteria which are capable of phosphate solubilizing, starch hydrolysing, gelatin liquifying and indole producing were recorded in the rhizosphere of the crops.

(14) The nitrogen fixing ability of asymbiotic N_2-fixers *in vitro* revealed that the N_2-fixers obtained from the coconut mixed farming and multistoreyed cropping system have shown better N_2-fixing ability as compared to coconut monocrop. The N_2-fixing ability ranged from 5.6 to 15.6 mg/g of sugar.

(15) The screening of various isolates of fungi, bacteria and actinomycetes were done for P-solubilizing ability *in vitro*. *Pseudomonas* sp. (41.7%) and *Aspergillus niger* (47.7%) showed higher P-solubilising ability.

(16) Six fungi viz. *Trichoderma harzianum*, *T. lignorum*, *Fusarium* sp., *Aspergillus niger*, *Rhizopus* sp. and *Cladosporium* sp. isolated from the rhizosphere were selected for various physiological studies, such as growth (in different cellulose, pH effect, sodium chloride and sucrose concentration) and cellulase enzyme production (C_x and C_1 activity). The effect of certain pesticides on the growth of these fungi was also studied. The result indicated that:

(i) The maximum growth in Czapek-Dox broth was recorded with *Cladosporium* sp. (1230 mg) followed by *Aspergillus niger* (900 mg) and *Trichoderma lignorum* (900 mg)

(ii) All the fungi recorded growth in carboxymethyl cellulose (CMC) and cellobiose supplimented medium.

(iii) The growth of fungi was maximum at pH 5.0 and 7.0. In general, growth was affected at pH 3.0 and 9.0. All the fungi showed growth at different sodium chloride concentrations (0.5, 2.5 and 5.0%). The growth was recorded at two levels of sucrose (5.0 and 10.0%) except for *Cladosporium* at 10% sucrose.

(iv) *Trichoderma harzianum* showed greater reduction of viscosity in carboxymeth-yl cellulose (CMC). Both *Trichoderma harzianum* and *T. lignorum* exhibited maximum C_x and C_1 activity.

(v) Among the pesticides, Blitox (0.1 and 0.02%) and Captan (0.2 and 0.05%) did not inhibit the growth of the fungi. Furadon (0.2%) in liquid media did not affect the growth of fungi. Bordeaux mixture (1.0 and 0.5%) and BHC (0.1 and 0.02%) affected the growth of fungi.

(17) Physiological and biochemical activities of rhizosphere (21 isolates) bacteria were investigated. The study indicated that:

(i) pH 7.0 was optimum for the growth of bacteria. Sodium chloride concentration of 0.5% was most optimum for the growth of various isolates of bacteria.

(ii) The effect of pesticides (Bordeaux mixture, Blitox, Captan, BHC and Furadon) on the growth of rhizosphere bacteria was studied _in vitro_. Bordeaux mixture, Blitox and BHC inhibited growth even at lower concentration levels but Captan did not inhibit the growth.

(18) The effect of pesticides on the growth of N_2-fixers and P-solubilizers was studied _in vitro_. Growth was affected in the majority of the isolates of N_2-fixing bacteria but bacterial isolate number 1, 2, 6 and 8 showed some amount of tolerance to the pesticides tested.

(19) The antagonism studies on *Phytophthora arecae* and *Phytophthora palmivora in vitro* resulted in some potential antagonistic fungi (*Aspergillus* spp., *Trichoderma* spp., *Penicillium* sp. and *Fusarium* sp.) and bacteria (*Bacillus* spp. and *Pseudomonas* sp.)

(20) The qualitative and quantitative biochemical characterisation of root exudate of coconut was studied with respect to sugars, aminoacids and phenols. The biochemical characterisation of soils (sugar, aminoacids and phenols) of different coconut based cropping systems indicated only quantitative variations.

(21) The root extract of coconut, pepper and cacao revealed neither stimulatory nor inhibitory effects on bacteria *in vitro*. The root extract addition enhanced the growth of *Trichoderma harzianum* and *Aspergillus niger*.

The present investigations have clearly shown that the soil microflora and microbiological activities were greater in the rhizosphere and root regions of coconut based multistoreyed cropping systems and coconut mixed farming system as compared to coconut monocropping system. The soil fertility parameters (organic carbon, NPK) were also improved under the multistoreyed and mixed farming systems. Therefore the higher microbiological activities and soil fertility parameters under multistoreyed or mixed farming systems might have served as the non-monetary input (synergistic effect) in increasing the yield of coconut palms. The rhizosphere and rhizoplane microflora studies have revealed the difference in the occurrence of various microorganisms (bacteria and fungi) and their activities indicating the influence of crop-mixing. Some isolates of rhizosphere bacteria and fungi showed antagonism to *Phytophthora arecae* and *P. palmivora* *in vitro* but their potential in the biological control needs to be investigated. The biochemical characterisation of root region soils of coconut based cropping system indicated only quantitative variation in sugars, aminoacids and phenols but there was no qualitative variation.

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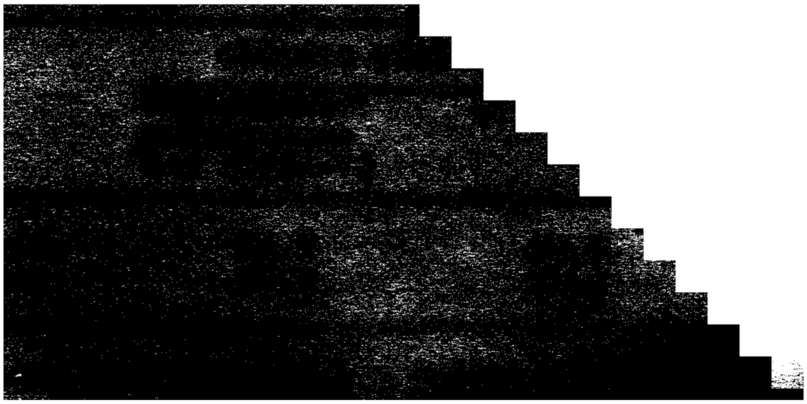
* Original not seen

APPENDICES



APPENDICES

APPENDIX Ia

Yield performance of coconut palms of the coconut based Multi-Storeyed (MS) cropping system and monocropping system*

Cropping system	During pre-experimental period (1969 - 72)	Coconut yield (nuts/palm/year)			
		During 1974-83		During 1981-84	
		Yield	Percentage increase	Yield	Percentage increase
A. Multi-Storeyed Cropping					
i) C+P+Ci(SH)+Pi	40.1	76	188	81.3	103
ii) C+P+Co(SH)+Pi	45.3	99	120	101.2	123
B. Coconut Monocropping	42.0	-	-	62.5	77

C=Coconut P=Pepper Co=Cacao Ci=Cinnamon Pi=Pineapple
SH=Single Hedge

*Source: Annual Reports, CPCRI,
Kasaragod.

Appendix-Ib

Yield of coconut palms of coconut based mixed farming and coconut monocropping system*

Cropping system	Coconut yield (nuts/palm/year)		
	Pre-experimental period 1969—72	1974-83	Increase (%)
Mixed Farming	64.7	69.5	7.4
Monocropping	60.8	51.2	- 15.8

Mixed Farming (MF) = Coconut + Pepper + Napier grass

* Source: Annual Reports, CPCRI,
Kasaragod.

APPENDIX-II

Weather data - Rainfall, temperature and relative humidity during 1983 at CPCRI, Kasaragod.

Month	Rainfall (mm)	Temperature (°C)		Relative humidity (%)
		Maximum	Minimum	
January	0.0	31.3	19.9	56
February	0.0	32.0	20.8	58
March	0.0	32.9	23.5	60
April	0.0	33.1	25.3	63
May	5.0	33.4	24.8	63
June	389.7	30.4	22.8	91
July	988.0	29.3	24.1	87
August	1000.0	28.3	23.4	85
September	672.4	29.5	23.8	80
October	109.6	31.0	23.5	75
November	65.4	31.6	21.9	65
December	46.6	32.7	20.5	50

APPENDIX-III

Composition of different media and reagents

I. Media for enumeration of soil microorganisms

A. Bacteria

1. Nutrient Agar (NA)

Beef extract	3.0 g
Peptone	5.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	6.8-7.0

2. Nutrient Soil Extract Agar (NSEA)

Beef extract	3.0 g
Peptone	5.0 g
Glucose	1.0 g
K_2HPO_4	0.5 g
Soil extract(Stock)*	100 ml
Agar	15.0 g
Tap water	900 ml
pH	6.8

* 1000g of sieved garden soil is mixed with 1000 ml of tap water and steamed in the autoclave for 30 min. A small amount of $CaCO_3$ is added and filtered through a double filter paper.

B. Fungi

1. Martin's Rose Bengal Agar (MRBA) (Martin, 1950)

Glucose	10.0 g	
Peptone	5.0 g	
KH_2PO_4	1.0 g	
Rose Bengal	0.033g	(1 part in 30,000 part of medium)
$MgSO_4 7H_2O$	0.5 g	
Streptomycin	30.0 mg	(Add aseptically before pouring the media into petri plates (45-50°C)
Agar	15.0 g	
Distilled water	1000 ml	

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2. Czapek-Dox Agar (Thom and Raper, 1945)

$NaNO_3$	3.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4.7H_2O$	0.01g
Sucrose	30.0 g
Agar	15.0 g
Distilled water	1000 ml

3. Potato Dextrose Agar (PDA)

Potato, Peeled and Diced	200 g
D-glucose	20 g
Agar	15 g
Distilled water	1000 ml

Boil 200g of peeled diced potato for 1 hr in a litre of water. Filter and make up the volume to one litre.

C. Actinomycetes

Starch-Casein medium (Kusters and Williams, 1964)

Starch	10.0 g
Casein hydrolysate	0.3 g
KNO_3	2.0 g
NaCl	2.0 g
$K_2H\ PO_4$	2.0 g
$MgSO_4.7H_2O$	0.05g
$CaCO_3$	0.02g
$FeSO_4.2H_2O$	0.01g
Agar	18.0 g
Distilled water	1000 ml

D. Media for nitrogen fixing organisms

1. Beckings medium – *Beijerinckia* (Becking, 1959)

Sucrose	20.0 g	Na_2MoO_4	0.005 g
KH_2PO_4	0.8 g	Agar	15.0 g
K_2HPO_4	0.2 g	Distilled water	1000 ml
$MgSO_4 \cdot 7H_2O$	0.5 g	pH	6.5
$FeCl_3$	0.1 g		

2. Jensen's modified N_2-free medium (Asymbiotic N_2-fixers)

KH_2PO_4	0.8 g	Sodium Molybdate	0.005 g
K_2HPO_4	0.2 g	Sucrose	20.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g	Agar	15.0 g
$FeCl_3$	0.1 g	Distilled water	1000 ml

E. Azospirillum medium. N-free semi-solid medium

Malic acid	5.0 g	Alcoholic solution bromothymol blue (5%)	2 ml
K_2HPO_4	0.5 g	Vitamin solution**	1 ml
$MgSO_4 \cdot 7H_2O$	0.2 g	KOH	4.0 g
NaCl	0.1 g	Agar	1.75 g
$CaCl_2$	20 mg	Distilled water	1000ml
Fe EDTA (1.64% w/v aqueous)	4 ml	pH	7.8
Trace elements*	2 ml		

*Trace element solution

$Na_2MoO_4 \cdot 2H_2O$	200mg
$MnSO_4 \cdot H_2O$	235mg
H_3BO_3	280mg
$CuSO_4 \cdot 5H_2O$	8mg
Distilled water	200ml

** Vitamin solution

Biotin	10 mg
Pyridoxin	20 mg
Distilled water	100 ml

F. Media for phosphate solubilizing organisms

Pikovskaya's medium

Glucose	10.0 g	$MnSO_4$	Trace
Tricalcium phosphate	5.0 g	$FeSO_4$	Trace
$(NH_4)_2SO_4$	0.5 g	Yeast extract	0.5 g
KCl	0.2 g	Agar	15.0g
$MgSO_4 7H_2O$	0.1 g	Distilled water	1000 ml

G. Media for enumeration of nitrifying bacteria (Alexander and Clark, 1965)

Most Probable Number (MPN) method for *Nitrosomonas*

Ammonium-Calcium carbonate medium

$(NH_4)_2SO_4$	0.5 g	$MgSO_4 7H_2O$	0.3 g
$K_2H\ PO_4$	1.0 g	$CaCO_3$	7.5 g
$FeSO_4 7H_2O$	0.03g	Distilled water	1000 ml
NaCl	0.3 g		

Reagent: Griess Ilosvay reagent. (a) 0.6g sulfanilic acid dissolved in 70 ml hot distilled water, cooled. 20 ml of concentrated HCl is added and volume made upto 100 ml.

(b) 0.6g of α-naphthylamine is dissolved in 12 ml distilled water, containing 1 ml concentrated HCl and volume made up to 100 ml

(c) 16.4 g sodium acetate dissolved in distilled water and volume made upto 100 ml

(three solutions a, b and c are stored in dark bottles and mixed in equal parts before use)

MPN for *Nitrobacter* - Nitrite calcium carbonate medium

KNO_2	0.006 g	$CaCO_3$	1.0 g
K_2HPO_4	1.0 g	$CaCl_2$	0.3 g
NaCl	0.3 g	Distilled water	1000 ml
$MgSO_4\ 7H_2O$	0.1 g		

Reagent: Zinc-copper-manganese dioxide mixture (1 g powdered zinc, 1g powdered manganese dioxide and 0.1g copper powder are mixed

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E. Media for cellulose decomposing microorganisms

1. Omeliansky's medium

$(NH_4)_2SO_4$	1.0 g	NaCl	Trace
K_2HPO_4	1.0 g	Distilled water	1000 ml
$MgSO_4 \cdot 7H_2O$	0.5 g		
$CaCO_3$	2.0 g		

2. Czapek's-Dox medium:

Czapek's-Dox medium where sucrose is replaced by other cellulose compounds

Carbon mineralisation

A. Composition of minus nitrogen nutrient solution

K_2SO_4	0.25 M (43.56 g)
K_2HPO_4	0.05 M (3.48 g)
$MgSO_4 \cdot 7H_2O$	0.01 M (2.46 g)

Hoglands micronutrient solution 10 ml.

B. Composition of Hoglands micro-nutrient solution

H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnCl_2$	0.11
$CuCl_2$	0.05 g
$NaMoO_4$	0.025 g
Distilled water	1000 ml

Take 2 ml of the above solution and dilute to one litre.