ANTIBIOTIC PRODUCING AND ANTAGONISTIC MICROORGANISMS IN THE FOREST SOILS OF KERALA

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Science in Agriculture

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Vellanikkara, Trichur

1988

DECLARATION

I hereby declare that this thesis entitled "Antibiotic producing and antagonistic microorganisms in the forest soils of Kerala" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara,
30th may 1988

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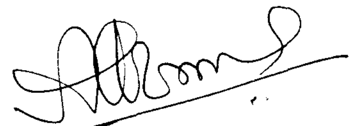

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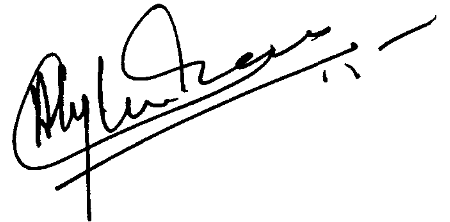
Members 1. Dr. ABI CHEERAN 

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ACKNOWLEDGEMENTS

The author wishes to express his deep sense of gratitude and indebtedness to:

Sri.A. Sukumara Varma, Chairman of the Advisory Committee and Associate Professor of Plant Pathology for inspiring guidance and helpful criticism at every stage of this investigation and in the preparation of the thesis,

Dr.Abicheeran, Professor and Head, Department of Plant Pathology for his constant care, encouragement, critical suggestions and help during the course of investigation,

Dr.P. Varadarajan Nair, Professor of Plant Pathology for his counsel and valuable help during the course of this investigation,

Dr.A.I. Jose, Professor and Head, Department of Soil Science and Agricultural Chemistry for his valuable suggestions and help rendered in the course of this investigation and preparation of the manuscript,

Shri.V.K.G. Unnithan, Associate Professor, Department of Agricultural Statistics for all the help rendered in the course of research work,

Dr.Koshi Abraham, Smt.Vilasini, Sri.M.V.Rajendran Pillai, Assistant Professors, Department of Plant Pathology for the help rendered during the research work,

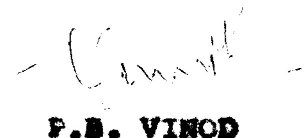
all the members of the Department of Plant Pathology for their generous assistance in various ways,

Department of Microbiology, College of Veterinary and Animal Sciences, Mannuthy; Department of Plant Pathology, Kerala Forest Research Institute, Peechi and Department of Immunology, Amala Cancer Research Institute, Trichur for the facilities extended in carrying out this investigation,

all my friends and classmates for the help and assistance extended during the research work,

Government of Kerala for deputing me for the M.Sc.(Ag) course,

and above all to the Almighty, for the blessings showered on me in completion of this thesis.



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Introduction

INTRODUCTION

In a natural ecosystem, biological balance results from a diverse network of interacting organisms in dynamic equilibrium. Each species is adapted to the prevailing environment, and is a source of food for others. Each species also has one or more mechanisms to endure or escape its competitors and natural enemies, and each ecological niche is occupied both in space and time. Generally plant disease outbreak occurs due to an ecological shock that causes biological imbalance. Infectious disease itself is an ecological force and will eventually restore balance within the ecosystem. Agriculture as commonly practised contributes to biological imbalance by replacing biological diversity with a single plant genotype, by placing crop plants in an environment to which they are poorly adapted, by exposing the crop to inoculum of pathogens but without benefit of normal endurance or escape mechanisms, and by creating biological voids with tillage, pesticides, and other practices.

The undisturbed moist evergreen forest soil is one of the most ideal habitats for the existence of many micro-organisms which live in close proximity and interact in a

unique way. The sum total of all the individual interactions establishes the native flora typifying the habitat. Detrimental effects of one species on its neighbours are quite common in soil, and they are detected by the decrease in abundance or metabolic activities of the more susceptible organism. There is a permanent struggle for existence in the habitat, and only those species most suitable for the specific environment survive. The categories of deleterious interactions are competition, antibiosis and parasitism or predation.

The evergreen, virgin forest soils of Kerala are a treasure house of antagonistic and antibiotic producing microorganisms, because their natural ecosystem has not been tampered by man's activities since evolution as a forest. No study has been carried out to unearth the various microorganisms present in the forest soils of Kerala. The present investigation is carried out for throwing some light on the soil microflora of the typical evergreen forest soils of Kerala, with a view to explore the presence of antagonistic and antibiotic producing micro-organisms for utilising them in the biological control of important soil borne plant pathogens like *Pythium myriotylum* Drechsler, *Phytophthora palmivora* (Butler) Butler and *Rhizoctonia solani* Kuhn.

This study was therefore undertaken with the following objectives in view:

i) Isolation of micro-organisms from the forest soils.

ii) Identification and characterisation of the isolates.

iii) Evaluation of different isolates for their antagonistic properties against the important soil-borne plant pathogens such as *Pythium*, *Phytophthora* and *Rhizoctonia*.

iv) Evaluation of the different isolates for their antibiotic producing ability.

Review of Literature

REVIEW OF LITERATURE

Baker and Cook (1974) stated "antagonistic potential resides in every soil microorganism and any random soil sample should yield antagonists to some microorganisms. Metabolites are secreted and one of these would certainly prove inhibitory to some other microorganisms." Many soil inhabitants produced inhibitory substances in laboratory media and when tested in pure culture they were found to supress numerous microorganisms. Species of Pencillum, Trichoderma, Aspergillus, Fusarium and many other fungi were found to excrete antibiotic substances. Actinomycetes were found to be active in producing antibiotics like streptomycine, chloramphenicol, cycloheximide and chlortetracycline. Antibiosis is especially common among streptomyces isolates. The most frequently encountered bacteria synthesising antibiotics were species of Bacillus and strains of Pseudomonas that liberate pyocyanin and related compounds (Alexander, 1977).

Quantitative estimation of soil microorganisms

Waksman and Curtis (1916, 1918) and Jensen (1943) made an attempt to estimate the microflora of soil and reported that the population varied from 3000 - 50,00,000 per g of soil

depending on soil types. Skinner *et al*. (1952) reported several hundred thousands to hundred millions of bacteria per g of dry soil. Waksman (1952) observed that the fungi were lesser in number than the actinomycetes and fungal population in soil ranged from few to as many as ten lakhs per g.

When the soil depth increased the microbial population was found to decrease. A gradual reduction in microflora in deeper layers of soil was observed by different workers (Aristovskaya, 1951, 1957; Waksman, 1952; Rose, 1954; Milosevic, 1958; Tsao *et al*., 1959; Rangaswamy and Venkatesan, 1963; Corke and Chase, 1964; Venkatesan, 1964 and Rangaswamy *et al*. 1967).

The soil microbial population was found to decrease as the organic carbon availability of the soil decreased (Waksman and Curtis, 1916, 1918; Stere, 1942; Laudelout *et al*., 1949; Aristovskaya, 1951, 1957; Rose, 1954; Blue *et al*., 1955; Zhukova, 1956; Jagnow, 1958; Tsao *et al*., 1959; Popova, 1963; Rangaswamy and Venkatesan, 1963 and Rangaswamy *et al*., 1967).

Antagonism is a phenomenon employed in biological control and it means a relation between organisms in which one organism, the antagonist creates adverse circumstances for the other for its growth. According to Park (1961),

categories of antagonism are antibiosis, competition or exploitation. Exploitation includes parasitism and predation. Jackson (1965) reported that metabolic products of some organisms had harmful effect on pathogens and termed it as antibiosis.

Antibiotic producing and antagonistic microorganisms

Many workers have isolated and studied the antibiotic and antagonistic properties of different soil microorganisms which include fungi, actinomycetes and bacteria. Of these the following microorganisms have been studied in detail.

Mucor spp.

Considering the antagonistic property of *Mucor* spp. only scanty information is available in favour of its antagonistic property. Codiguola and Gallino, (1974) reported that *M. hiemalis* exhibited antibacterial and antifungal activities. Domsch *et al*. (1980) found that *M. hiemalis* f. *hiemalis* was a hyperparasite of selerotia of *Sclerotinia sclerotiorum*, *S. borealis*, *S. trifoliorum* and *Claviceps purpurea*.

In a number of studies conducted by various workers like Durrell (1968); Dennis and Webster (1971); Mandelbrot and Erb (1972) and Hunter and Butler (1975), *Mucor* spp. was

found to be parasitised by other obligate hyperparasites.

Syncephalastrum sp.

The mycoparasitism of hyphae of Aspergillus niger by Syncephalastrum racemosum was shown by Pidoplichko (1953). Vyas and Jain (1976) reported that metabolites of S. racemosum, Penicillium obscurum and Monilia sp. were found to be active in the growth promoting activity but however, culture filtrates of some fungi showed strong growth inhibitory response as well.

Cunninghamella sp.

Many workers have observed that Cunninghamella elegans was inhibited by other fungal antagonists (Chu and Alexander, 1972), Sneh et al. (1977). Jesiorska (1974) observed that the growth of Chalara elegans could be inhibited in in vitro by C. elegans.

The production of a sporostatic factor by C. elegans has been reported by Garrett and Robinson (1969).

Trichoderma spp.

The antagonistic property of various species of Trichoderma has been established as evident from the reports of many workers.

Weindling (1932) reported that *Trichoderma viride* parasitised the mycelia of *Pythium* spp. and *Phytophthora parasitica*. The parasitism of mycelia of *Rhizoctonia solani* by *Trichoderma* spp. and *Penicillium vermiculatum* was reported by Boosalis (1956). He found that hyphae of the host fungus were invaded by penetration pegs developing from mycelium in contact with host hyphae. The hyperparasitism of *T. viride* on numerous fungal hosts including *Sclerotinia sclerotiorum* was observed by Pohjakallio and Makkonen (1957). Durrell (1968) observed the parasitism of the mycelia of *Fusarium solani*, *F. oxysporum*, *R. solani*, *Cochiliobolus sativus*, *Rhizopus oryzae* and several other fungi by *T. viride*. Dennis and Webster (1971) reported that hyphal contact between *T. hamatum* and several saprophytic or parasitic fungi was accomplished by curling of hyphae. The hyperparasitism and hyphal curling around the test organism by *T. polysporum* was reported by Chohan and Singh (1974). Reeves (1975) found that the hyphae of *Phytophthora cinnamomi* were lysed and oospore production induced by *T. viride*. Mew *et al*. (1980) observed that *Trichoderma* spp. coiled around the sclerotia of *R. solani* and made them inactive. Krishnamoorthi and Bhaskaran (1987) screened *T. viride*, *T. harzianum*, *Lactesaria urvalis*, *Bacillus subtilis* and *Pseudomonas fluorescens* for their antagonism against *Pythium indicum* and found that *T. viride* and *L. urvalis*

produced a thermostable component which destroyed the host hyphae and in addition exhibited physical parasitism like coiling end invasion of the host hyphae. Fedoseeva et al. (1983) reported that among the fungi tested, two isolates of T. lignorum showed lytic activity and inhibited Ustilago maydis.

The antimetabolite production by various species of Trichoderma was reported by several workers. Brian and Mc Gowan (1946) isolated a fusidic acid like antibiotic named viridin from T. viride, which possessed antifungal properties. He also reported similar antifungal metabolite production by Gliocladium virens. Shibata et al. (1964) reported that the metabolite from T. polysporum included trichodermin, trichodermal, pachybasin and chrysophenol. Park and Robinson (1964) demonstrated an uncharacterised acidic substance in ageing cultures of T. viride with morphogenic effect on fungal hyphae. Dennis and Webster (1971) reported that T. hamatum produced volatile and non-volatile metabolites having antifungal acitivity. Papavizas (1984) reported production of trichodermin by T. lignorum (T. viride) and also opined that Trichoderma and Gliocladium produced various enzymes such as endo and exoglucanase, cellobiase and chitinase. Manian and Paulsamy (1987) studied T. aurioviride isolated from soil and found that its culture filtrate antagonised mycelial growth and sclerotial initiation in R. solani.

The use of *T. viride* as biocontrol agent against *Pythium ultimum* in beets (Liu and Vaughan, 1965), and against *R. solani* (Roy, 1977) has been established. Herman *et al.* (1980) observed that treatment of seeds of raddish and pea with conidia of *T. hamatum* protected seed and seedlings from *R. solani* and *Pythium* spp. and this was as effective as fungicidal seed treatment. The use of *T. viride* against *Claviceps fusiformis* causing ergot of pearl millet (Mohan *et al.*, 1987) and *P. graminocolum* causing root rot of sugarcane seedlings (Padmanabhan and Alexander, 1987) has also been established.

Dennis and Webster (1971) reported the antagonistic properties of *T. harzianum* as coiling around or invading the hyphae of many test fungi. Mukhopadhyay and Indulikachandra (1986) studied the mode of antagonism of *T. harzianum* against *P. aphanidermatum* and found that it caused lysis and disintegration of protoplasm of the test fungi when grown on potato dextrose agar plates in dual culture. They also found that *T. harzianum* showed antibiotic activity towards *P. aphanidermatum*. The antagonistic property of *T. harzianum* against different fungi was also reported by Marchiaio (1972), Nordbring-Herts (1973), Mew and Rosales (1984), Venkatasubbaiah *et al.* (1984), Jharia and Khare (1986) and Padmakumari and Balakrishnan (1986).

Agarwal *et al.* (1977) revealed that *T. harzianum* was antagonistic against *S. rolfsii* and found that the culture filtrate inhibited the growth of the pathogen on potato dextrose agar.

The production of antimetabolites as a property of antagonism by *T. harzianum* was reported by Domsch *et al.* (1980) and Mukhopadhyay and Indulikachendra (1986). Domsch *et al.* (1980) observed that carbondioxide and ethanol produced by *T. harzianum* were responsible for inhibition of growth and sporulation of *Aspergillus niger* and *Pestalotia rhododenri*.

T. harzianum employed as agent of biological control of many crop diseases was reported by various workers. The control of *P. aphanidermatum* using *T. harzianum* was reported by Fajola and Alasoadura (1975), Mukhopadhyay and Indulikachandra (1986) and Mukhopadhyay (1987). Control of *R. solani* using *T. harzianum* was reported by Elad *et al.* (1980); Alegarsamy *et al.* (1987) and Mukhopadhyay (1987). Sivan and Chet (1986) found that *T. harzianum* from rhizosphere of cotton seedlings was found to be an antagonist against *F. oxysporum* on cotton, melons and wheat.

Komatsu (1976) reported that the antagonism exhibited by *T. koningii* was by coiling around the hyphae of *Lentinus edodes* and several other parasitic fungi.

Production of anti-fungal substances by *T. koningii* was reported by several workers like Brian and Hemming (1947) and Park (1961).

Kukhopadhyay (1987) investigated the bio-control efficiency of *T. koningii* and was exploited for the control of *P. aphanidermatum*, *R. solani*, *S. rolfsii* and *F. oxysporum* f. sp. *ciceri*.

Aspergillus spp.

The antagonistic properties of various species of *Aspergillus* were reported by several workers and were mainly attributed to the production of antimetabolites.

The inhibition of growth of *R. solani* by parasitising the hyphae by *A. niger* was reported by Gokulapalan and Nair (1984). The antagonistic property of *A. niger* was also reported by Padmakumari and Balakrishnan (1986) and they found that in dual culture, the antagonistic organism continued its growth and covered the whole plate while *R. solani* ceased its growth after contact with antagonist. Bora (1977) observed that *A. niger* has shown greatest antagonism against *R. solani* from egg plant, when its antagonistic property was estimated among other soil fungi.

The antibiotic production by *A. niger* was reported by Broadbent (1966). The antibiotic jawaherene was detected in the study.

Raistrick and Smith (1935) reported production of terrein by *A. terreus*. The production of geodin, terricin and terric acid was also reported (Marcus, 1947). Zaehner *et al*. (1963) reported that the antibiotic properties of the metabolites of *A. terreus* were due to flavipin, eridin, geodin, patulin, terric acids and sideramine ferrichysin.

Trevinoc and Espinosa (1981) applied conidial suspension of different species of *Aspergillus* with potato saccharose agar to cocoa litter around the base and on the stem up to 1.8 m against *Phytophthora palmivora*. Only *A. terreus* was found to retard the start of disease by 30 days. Roy (1984) reported that *A. terreus* isolated from soil inhibited growth of *R. solani* in *in vitro*.

A. versicolor was reported to produce many metabolites having antibiotic properties. Sterigmatocystin and averusin were produced by *A. versicolor* (Bullock *et al*., 1962, 1963). Pusey and Roberts (1963) found that averusin an anthraquinone was produced by *A. versicolor*. An antifungal substance versicolorin was also reported to be produced by *A. versicolor* (Dhar and Bose, 1968).

Penicillium spp.

Many species of *Penicillium* are well known for the production of antimetabolites and most of them are antibiotics. The antagonitic property of this group of microorganisms is largely due to antibiosis and various researchers have investigated for exploring their metabolite production.

Chand and Logan (1984) found that *Penicillium cyclopium* and *P. nigricans* were antagonistic to or parasitic on *R. solani* in *in vitro*. In the dual culture studies with *P. citrinum*, it has been reported to have strong antagonistic activities towards *Gaeumanomyces graminis* and *Pythium* sp. (Domsch, 1960), *R. bataticola* (Dhingra and Khare, 1973), *S. sclerotiorum* (Rai and Saxena, 1975), *Staphalococus aureus* and *Salmonella typhi* (Jefferys *et al*., 1953). Jharia and Khare (1986) observed the digestion of sclerotium and hyphae of *R. bataticola* by *Penicillium funiculosum* and *P. pinophilum*. Mukharjee *et al*. (1987) reported that *P. citrinum* was found to be the most effective among the fungal antagonists he tried against *Macrophomina phaseolina*.

The antibiotic production by *P. citrinum* has been observed by many workers. Hetherington and Raistrick (1931) found that citrinin an aromatic polycyclic compound was produced by *P. citrinum*. *P. citrinum* is reported to produce orcinol, protocatechuic acid and other hydroxybenzenes, citrinin and several related metabolites (Curtis *et al*., 1968). Citrinin has been reported to have fungistatic and hyphae narrowing properties (Robinson and Park, 1966).

Ciegler *et al*. (1971) established that penicillic acid is produced by a large number of fungi including *P. simplicissimum*. Waginer *et al*. (1980) reported that Penitren-A is a tremorgin produced by a number of species of *Penicillium* such as *P. simplicissimum*.

Paecilomyces spp.

Many workers have investigated the production of antibiotic substances by various species of *Paecilomyces* which are antagonistic against many fungi. The colonisation of sclerotia of *S. sclerotiorum* and *R. solani* was reported by various workers like Karhuvaara (1960), Makkonen and Pohjakallio (1960) and Maciejowska and Williams (1961).

Arai *et al.* (1973) reported that the peptide antibiotic leucinostatin produced by *P. lilacinus* was effective against some gram positive bacteria and a wide range of fungi. Samson (1974) reported the production of lilacinin by *P. lilacinus*. Mc Lennan and Ducker (1954) and Bilai *et al.* (1964) observed antibiotic activity of *P. rubrum* against bacteria.

Talaromyces sp.

The production of metabolites having antifungal properties by *T. wortmannii* was reported by Breen *et al.* (1955); Brian *et al.* (1957); Atherton *et al.* (1968) and Basu and Majumdar (1969). Antibacterial activity of *T. wortmannii* was also reported (Bilai *et al.*, 1964). In dual culture studies with *S. sclerotiorum*, Mc Laren *et al.* (1986) observed that *T. flavus* was a destructive hyperparasite which grew toward and coiled around the host hyphal cells.

Fusarium sp.

The antagonistic property of *F. oxysporum* by mycoparasitism was reported by Park (1963) against a number of fungi. Sneh *et al.* (1977) observed the antagonistic property of *F. oxysporum* against *Phytophthora cactorum* while

Marchisio and Mâsca (1984) reported strong antagonistic property of *F. oxysporum* in dual cultures of fungi isolated from root surface and rhizosphere of *Abies alba* Mill.

Streptomyces spp.

Streptomyces is the promising group of actinomycetes well known for the production of many different types of antibiotics. Many species among these groups of organisms produce specific antibiotics under ideal conditions. These groups of actinomycetes have been well studied by research workers to reveal their antibiotic properties.

Streptomycin isolated from *S. grieseus* by Schatz *et al*. (1944) was considered to be the first broad spetrum antibiotic discovered and found to be effective against the tuberile *Bacillus*. Chi (1967) found that *S. rimosus* was strongly inhibitory to growth of *F. solani*, *R. solani*, *Verticillium dahliae* and *V. albo-atrum* and slightly to *P. debaryanum*. Neweigy *et al*. (1982) and Logan *et al*. (1984) reported the selective nature of antagonistic property of *Streptomyces* spp. Mohamed (1985) reported that *Streptomyces* spp. inhibited the growth of *R. solani* and *S. rolfsii*. Kundu and Nandi (1984) found that *S. aranae* and *S. chibaensis* isolated from field soil under cauliflower cultivation showed antagonism against

R. solani in in vitro and also in natural soil. Rothrock and Gottilieb (1984) observed that S. hygroscopicus var. geldanus reduced saprophytic growth and also the population of R. solani was inhibited by geldanamycin, an antibiotic produced by S. hygroscopicus. Ainsworth (1971) stated that amphotericine was a polyene antibiotic obtained from Streptomyces sp. and was found to be antifungal. The degradation of hyphae of P. aphanidermatum upon contact with soil particularly with some actinomycetes in in vitro was reported by Domsch et al. (1980).

Merriman et al. (1974) reported that in biological control of Thanatephorus cucumeris, seed inoculations of wheat or carrot with S. griesgus increased the yield significantly.

Bacillus spp.

Among the bacteria, Bacillus group produces different antibiotics possessing inhibitory effect on other micro-organisms. Many workers have reported the antagonistic property of B. subtilis against many pathogenic fungi. Mitchell and Hurwitz (1965) established the effectiveness of B. subtilis as a biological control agent against

Phytophthora spp. and *R. solani*. Similar results were also reported by Aldrich and Baker (1970), Broadbent *et al.* (1971), Michael and Nelson (1972) and Kommedahl and Mew (1975). Henis and Inbar (1968) observed that the metabolites from *B. subtilis* inhibited the growth of *P. ultimum*. The antagonistic property of *B. subtilis* against *R. solani* was reported by Olsen and Baker (1968). *Bacillus* spp. were found to be the most important antagonists of *F. udum* which caused inhibition, lysis and higher number of chlamydospore formation (Zasserni and Tosi, 1985). Podile and Dube (1987) reported that *B. subtilis* was antagonistic to plant pathogen *V. dahliae*, *V. albo-atrum*, *F. oxysporum* f.sp. *udum*, *Phytophthora drechsleri* and *Rhizopus nigricans*. *B. subtilis* was found to have anti-fungal activities and plant promoting activities.

Production of antibiotic substances by *B. subtilis* was reported by various workers. Utkhede and Rahe (1980) found that six isolates of *B. subtilis* from sclerotia of *Sclerotium cepivorum* produced antibiotics, antagonistic to growth of pathogen. Vasudeva et el. (1958) reported production of the antibiotic bulbiformin by *B. subtilis*.

Merriman *et al.* (1974) reported that in biological control of *T. cucumeris*, seed inoculations of wheat or carrot with *B. subtilis* increased the yield significantly.

Tschen and Kuo (1985) noticed that application of antibiotic from *B. subtilis* culture filtrate to rice leaves inhibited growth of *R. solani* and prevented the development of disease. Seed treatment with *B. subtilis* appeared to be promising for the control of *Macrophomina phaseolina* (Mukharjee *et al*., 1987).

Materials and Methods

MATERIALS AND METHODS

Selection of site

The evergreen forests of Wynad and Idukki districts were selected for the present study. In Wynad district Ladysmith forest of Thariyode range and in Idukki, Cheriyakanam of Thekkady range were selected.

Collection of soil samples

The soil samples were collected during December, 1985. In each locality, six pits were dug for collecting soil samples at a distance of fifty meteres. Due consideration was given for the land topography while taking the pits. The soil samples were collected from three different depths 0-10, 11-20 and 21-30 cm and transferred to sterile chambers using soil auger. The available phanerogamic flora around 50 meters of the profile pit were collected for identification. The rainfall data for last ten years were taken.

Determination of pH and organic carbon content of soil

The soil samples of different depths collected from each locality were analysed for soil reaction and organic

carbon by employing the standard methods (Jackson, 1958).

Quantitative estimation of microflora

The quantitative assay of microflora was carried out by serial dilution plate technique (Stanier *et al.*, 1977). Ten g each of the soil sample was added to 100 ml sterile distilled water in 250 ml conical flasks and shaken for 5 min in orbital shaker. Ten ml of this soil dilution was then transferred to another flask containing 100 ml sterile distilled water to get 10^{-2} dilutions. Later 10^{-4} and 10^{-6} dilutions were prepared from this by serial dilution.

Estimation of fungal population

One ml of 10^{-4} soil dilution was pipetted into sterile petridishes to which 20 ml of melted and cooled Martin's rose bengal streptomycine agar media was poured. Three petridishes were kept as replications for each sample. The petridishes with the media were swirled thoroughly to get uniform distribution. After solidification, the dishes were incubated at room temperature for four days. The fungal colonies developed at the end of four days were counted using dark field colony counter and expressed as number of colonies per g of dry soil.

Estimation of actinomycete population

The estimation of actinomycete population was done with a soil dilution of 10^{-6} using Kenknight agar medium and the method followed was as in the estimation of fungal population. The dishes were incubated for seven days at room temperature and the actinomycete colonies were counted, using dark field colony counter and expressed as number of colonies per g of dry soil.

Estimation of bacterial population

Bacterial population was estimated using 10^{-6} soil dilution in nutrient agar medium. The method employed was the same as in estimation of fungal population. The dishes were incubated for 48 h at room temperature. The bacterial colonies developed were counted with the help of dark field colony counter and expressed as number of colonies per g of dry soil.

Qualitative estimation of microorganisms

Fungi

The young fungal colonies developed in dilution plates were transferred to potato dextrose agar medium (PDA). Pure cultures of fungi were obtained by single spore isolation

technique/single hyphal tip method and they were maintained in PDA.

Morphological characters of the fungi in pure culture were studied by growing them in petridishes, slants and slide culture techniques. On the basis of the morphological characters, they were identified.

Actinomycetes

The single colonies of actinomycetes developed in Kenknights agar were transferred to slants of the same medium and maintained in pure culture. They were provisionally identified on the basis of morphological characters.

Bacteria

The bacterial colonies developed in the dilution plate method were streaked in nutrient agar and single colony isolation was made. The pure cultures were maintained in NA as slant cultures. Bacterial isolates were identified by morphological and physiological characters.

The pure cultures of isolated fungi, actinomycetes and bacteria were sent to the Commonwealth Mycological Institute, Surrey, England and got identified. It was found to be in conformity with that of this author.

Isolation and pure culturing of the test organisms

Pythium myriotylum Drechsler

The isolate used in the study was obtained from naturally infected ginger rhizomes collected from the ginger (*Zingiber officinale*) plot of the College of Horticulture, Vellanikkara, by tissue isolation method. The isolate was purified by repeated hyphal tip plating and the organism was maintained on PDA by subculturing periodically.

Phytophthora palmivora (Butler) Butler

The isolate used in the study was obtained from naturally infected pepper (*Piper nigrum*) leaves, collected from the Pepper Research Station, Vellanikkara by tissue isolation method. The isolate was purified by repeated hyphal tip plating and the organism was maintained on oat meal agar by subculturing periodically.

Rhizoctonia solani Kühn

The isolate used in the study was obtained from a naturally infected rice plant collected from the rice fields of the Agricultural Research Station, Mannuthy. The fungus was isolated and grown in PDA from the sheath portions of

infected plants showing characteristic symptoms of attack, employing tissue isolation method. The culture was incubated under laboratory condition. The isolate was purified by repeated hyphal tip transfer and the organism was maintained on PDA by subculturing periodically.

Growth rate of antagonists and test organisms

Fungi

An aliquot of 15 ml of PDA was transferred into 90 mm petridishes. After solidification of the media, a 5 mm disc from actively growing zone of the fungus on PDA was lifted by a sterile 5 mm cork borer and transferred to the centre of the media in petridish. The plates were incubated at room temperatures (28 $\pm$ 2°C) and radial growth of the fungi was measured at intervals of 24 h up to 15 days to know their respective growth rates.

Actinomycetes

Actinomycete colonies were streaked on 90 mm petridishes with 15 ml Kenknights agar media and growth rate waa recorded every 24 h up to 20 daya.

Bacteria

For estimating the growth rate of bacterial colonies, the bacteria were streaked at one end of the plates poured with 15 ml NA in 90 mm petridishes and measurements of growth of the colonies were taken for four days at intervals of 24 h.

Test organisms

P. myriotylum and *R. solani* were grown in PDA and *P. palmivora* was grown in oat meal agar by adopting the method described in the case of fungi. Observation on radial growth was taken at intervals of 24 h up to 14 days.

Screening the microorganisms for antagonistic property against test fungi

Qualitatively estimated microorganisms were subjected to antagonistic studies against the three test organisms, *P. myriotylum*, *P. palmivora* and *R. solani* employing dual culture method (Johnson and Curl, 1972).

Fungi

The antagonistic study with the fungal isolates was done by the dual culture method. The organisms were

inoculated in dual culture after giving due consideration for the growth rate of both the test organism and the potential antagonist. An aliquot of 15 ml of PDA was transferred into 90 mm petridishes. After solidification of the media a 5 mm disc from an actively growing zone of the fungal isolate on PDA was removed by a sterile cork borer and transferred to one end of petridish. A disc of 5 mm of the test fungus was similarly transferred from another plate and placed at the opposite end, towards the periphery. The time of inoculation of the test organism was decided after taking into account its growth rate with respect to antagonist. When the test organism employed was _P. palmivora_ the method used was double agar technique (Johnson and Curl, 1972). The test organism was inoculated on the basal media of CMA, over which a thin film of PDA was poured and the antagonistic fungus inoculated.

The growth measurements were taken at intervals of 24 h up to ten days. The type of antagonism exhibited was recorded. Five replications were maintained for each antagonistic fungus. The test organism and the antagonist grown in monocultures served as control. The isolates possessing good antagonistic property were identified.

Actinomycetes

In the case of antagonistic study of the actinomycetes, dual culture method was employed with the double agar technique (Johnson and Curl, 1972). The antagonist was inoculated on nutrient glucose agar as basal medium and over that PDA or OMA was poured and test organism *P. myriotylum*, *R. solani* or *P. palmivora* was inoculated respectively. The time of inoculation of test fungi was delayed due to the slow growth of actinomycetes. The growths of antagonist and test organisms was recorded at intervals of 24 h. The types of reaction and antagonism exhibited were also recorded. Replications and control were maintained as in the case of fungi.

Bacteria

The antagonistic study with bacteria using the test organisms was done by the method as described in case of actinomycetes. The bacterial antagonist was streaked horizontally against the test organism towards the periphery of the dish in the double agar technique. The growth of antagonist and test organism was measured and recorded.

The types of reaction and antagonism exhibited were also recorded.

<u>Assay of culture filtrates of the antagonists</u>

The isolates showing good antagonistic properties were grown in liquid cultures and were utilised for conducting the culture filtrate studies. Fifty ml of the potato dextrose broth was taken in 250 ml Erlenmeyer flasks and sterilised at 15 lbs pressure for 20 min. The broth in the flasks was then inoculated with 5 mm mycelial discs of each of the fungus grown on PDA. They were then incubated at room temperature (28 $\pm$ 2°C) for 14 days in shake cultures.

Actinomycete culture was grown in 50 ml of broth contained in 250 ml flasks by inoculating a 5 mm disc of actinomycete taken from an actively growing culture in nutrient glucose agar. The culture was incubated for 21 days at room temperature (28 $\pm$ 2°C).

Bacterial antagonists were also grown in nutrient glucose broth by inoculating two loopfull of each of the isolates into 50 ml of broth contained in 250 ml flasks. They were also incubated for 14 days at room temperature (28 $\pm$ 2°C) in sheke cultures.

The cultures of fungi, actinomycetes and bacteria were filtered by coarse filtration using Buckner flasks. This

filtrate was again filtered through millipore filters (pore sise 450 nm) and stored in vials for conducting the culture filtrate assays and antibiotic sensitivity assays.

The culture filtrates stored in vials were assayed for their inhibitory action against the test organisms *P. myriotylum*, *P. palmivora* and *R. solani* by employing the poison food technique (Zentmayer, 1955). A quantity of 0.3 ml of the culture filtrate was poured into 90 mm sterile petridishes and 20 ml of PDA was poured in case of *P. myriotylum* and *R. solani* and 20 ml of OMA in case of *P. palmivora*. The petridishes were rotated well for mixing thoroughly. After solidification a 5 mm disc of an actively growing culture of the test organism was placed at the centre of the dish. Growth measurements were recorded on the day when the test organism reached 90 mm in control. Five replications were maintained in each case and control plates were also maintained by adding 0.3 ml of sterile distilled water.

The inhibitory properties of culture filtrates of the antagonists were essayed against the three test organisms, *P. myriotylum*, *P. palmivora* and *R. solani* and expressed

as per cent inhibition using the following formula suggested by Vincent (1927).

$$\frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100 = \text{Per cent inhibition}$$

Antibiotic Assay

The antibiotic production by the antagonists was assayed using their culture filtrates.

Escherichia coli (NCTC 10418) was used as the test organism. It was grown in peptone water for 6-8 h and was seeded with sterile cotton swab on solid bacto antibiotic assay medium No.3 with 15 g/l agar in petridishes. Sterile Whatman filter paper discs of 5 mm diameter (antibiotic sensitivity discs) were soaked in the sterile culture filtrates of the antagonists and after allowing the excess filtrate to flow off, the discs were placed on the bacteria seeded plates. It was inoculated overnight and the diameter of each zone of inhibition was observed and recorded. Standard curve was drawn in respect of a range of concentration of Tetracycline hydrochloride (Mc Coy, 1976). Comparison of the diameter of each zone of inhibition of the culture filtrates, with the standard curve gave an estimate of the concentration of the antibiotic in the culture filtrate. Average of five observations were taken for each culture filtrate.

Results

RESULTS

Locations of soil sample collection

The soil samples were collected from evergreen forest areas of Wynad and Idukki districts. The Ladysmith forest of Thariyode in Wynad and Cheriyakanom of Thekkadi in Idukki were selected. Soil samples were collected during 1985 as described in materials and methods.

The average annual rainfall in Wynad and Idukki for the period 1976 to 1985 was recorded as 1297.27 mm and 1763.86 mm respectively (Table 1). The Wynad region has a dry spell of two to four months while Idukki has only one to three months.

Florestic composition

A total of 64 species of plants distributed among 40 phanerogamic families has been identified from areas designated for the collection of soil samples. Of the 64 species of plants, 10 were common both in Idukki and Wynad tract but 25 species were restricted to Wynad alone. Thus a total of 35 phanerogamic species in Wynad which included 25 trees, five shrubs, three climbers and two herbs were distributed in 29 families (Table 2). Idukki area had a

Table 1. Annual rainfall data of Wynad and Idukki forest

Year	Rainfall in mm	
	Wynad forest	Idukki forest
1976	731.86	1402.96
1977	1417.27	2030.26
1978	1474.28	1100.42
1979	1634.12	1520.75
1980	1778.73	1441.00
1981	1629.30	2598.20
1982	1090.65	1710.00
1983	835.08	2136.55
1984	1122.09	1951.00
1985	1259.54	1747.50
Average	1297.27	1763.86

Table 2. Phanerogamic flora at the site of collection

Sl. No.	Phanerogamic flora	Nature of growth	Locality
I	Acanthaceae		
	1. Strobilanthus barbatus, Nees	Shrub	Wynad & Idukki
II	Ampelideae		
	2. Vitis pallida, Kondag mara	Climber	Idukki
III	Anacardiaceae		
	3. Mangifera indica, Linn	Tree	Idukki
IV	Anonaceae		
	4. Miliusia velutina, H.F. & Thoms	Tree	Wynad
V	Aristolochiaceae		
	5. Aristolochia indica, Linn	Shrub	Wynad
VI	Asclepiadaceae		
	6. Hemidesmus indicus	Herb	Idukki
VII	Bignoniaceae		
	7. Stereospermum chelonoides, DC; Wight	Tree	Wynad
VIII	Bixaceae		
	8. Hydnocarpus laurifolia (Dennest.) Steumr = Hyduocarpus wightiana, Blume	Tree	Idukki
IX	Burseraceae		
	9. Canasium strictum, Roxb	Tree	Idukki
X	Campanulaceae		
	10. Lobelia nicotianaefolia	Herb	Idukki
XI	Combretaceae		
	11. Terminalia paniculata, Roth	Tree	Idukki
XII	Dipterocarpaceae		
	12. Hopea glabra, Wight	Tree	Wynad

Contd.

Table 2. Continued

1	2	3	4
XIII	Ericaceae		
	13. Rhododendron arboreum, Wall	Tree	Wynad
XIV	Euphorbiaceae		
	14. Bredelia retusa, Spreng	Tree	Wynad
	15. Bischofia javanica, Blume	Tree	Idukki
	16. Macaranga roxburgi, Wight	Tree	Idukki
	17. Manihot glasiovii, Muell. Arg	Tree	Idukki
XV	Guttiferae		
	18. Garcinia xanthochymus, Hook	Tree	Wynad
	19. Mesua ferrea, Linn	Tree	Idukki
XVI	Lauraceae		
	20. Actinodaphana madraspatna, Bedd	Tree	Idukki
	21. Cinnamomum sulphuratum, Nees	Tree	Idukki
	22. Machilus macrantha, Nees Wight	Tree	Wynad
XVII	Lecythedaceae		
	23. Careya arborea, Roxb	Tree	Wynad
XVIII	Leguminaceae		
	24. Acrocarpus frasinifolius, Wight	Tree	Idukki
	25. Dalbergia latifolia, Roxb	Tree	Wynad
	26. Dalbergia paniculata, Roxb	Tree	Idukki
	27. Erythrina struta, Roxb	Tree	Idukki
	28. Mucuna gigantea, DC, Brit	Climber	Wynad
	29. Spatholobus roxburgi, Benth	Woody climber	Wynad
XIX	Lythraceae		
	30. Lageratraemia lanceolata, Wall. ex. Wight	Tree	Wynad & Idukki
XX	Malvaceae		
	31. Sida rhombifolia	Herb	Wynad

Contd.

Table 2. Continued

1	2	3	4
XXI	Meliaceae		
	32. Cedrila toona, Roxb	Tree	Wynad & Idukki
	33. Melia azadarach, Linn	Tree	Idukki
XXII	Moraceae		
	34. Artocarpus hirsuta, Lamk, Wight	Tree	Wynad & Idukki
	35. Artocarpus integrifolia, Linn, Roxb	Tree	Wynad & Idukki
	36. Ficus bengalensis, Linn, King	Tree	Idukki
	37. Ficus callosa, Willd, King	Tree	Wynad
	38. Ficus infectoria, Roxb, Wight	Tree	Wynad
XXIII	Myristicaceae		
	39. Myristica attenulata, Wall, King	Tree	Wynad
	40. Myristica beddomei, King	Tree	Idukki
XXIV	Myrtaceae		
	41. Eugenia conymbosa, Lam	Tree	Idukki
	42. Eugenia jambolana, Lam, Wight	Tree	Idukki
	43. Syzygium cumini	Tree	Wynad
XXV	Oleaceae		
	44. Olea dioica, Roxb, Wight	Tree	Wynad
XXVI	Palmaceae or Palmae		
	45. Calamus rotang, Linn	Climber	Wynad & Idukki
	46. Caryota urens, Linn	Tree	Wynad & Idukki
XXVII	Pandanaceae		
	47. Pandanus tectorius, Solander	Shrub	Wynad

Contd.

Table 2. Continued

1	2	3	4
XXVIII	Plumbaginaceae		
	48. Plumbago zeylanica	Herb	Wynad
XXIX	Polygalaceae		
	49. Xanthophyllum flarescens, Roxb	Tree	Wynad
XXX	Rhamnaceae		
	50. Ziziphus nigosa, Lamk, Wight	Shrub	Wynad
XXXI	Rubiaceae		
	51. Adena cordifolia (Roxb) Hook f. ex. Brandis	Tree	Idukki
	52. Canthium dicocum	Shrub	Idukki
	53. Coffea spp., Linn	Tree	Idukki
XXXII	Rutaceae		
	54. Atlantia malabarica	Tree	Idukki
	55. Clausena indica, Oliver	Tree	Wynad & Idukki
XXXIII	Sapindaceae		
	56. Nephelium longana, Camb	Tree	Idukki
	57. Schleichera trifuga, Willd, Bedd	Tree	Wynad
XXXIV	Sapotaceae		
	58. Palaquium ellipticum, Benth	Tree	Wynad & Idukki
XXXV	Sterculiaceae		
	59. Helictereus isora, Linn, Wight	Shrub	Idukki
XXXVI	Styraceae		
	60. Symplocos spicata, Roxb, Wight	Tree	Wynad
XXXVII	Tiliaceae		
	61. Grewia tilifolia, Vahl	Tree	Wynad & Idukki

Contd.

Table 2. Continued

1	2	3	4
XXXVIII	Thymelaeceae		
	62. *Lasiosiphon eriocephalus*, Bedd	Shrub	Wynad
XXXIX	Ulmaceae		
	63. *Celtis cinnamomea*, Lindl	Tree	Idukki
XXXX	Verbinaceae		
	64. *Clerodendron viscosum*, Vent = *Clerodendron infortunatum*, Gaertn	Shrub	Idukki

phanerogamic flora which included 31 trees, four shrubs, two climbers and two herbs, thus making a total of 39 species distributed in 25 families (Table 2). All these plants contributed a dense vegetation during the major part of the year.

In this tropical, moist, evergreen forest, most of the tree species were evergreen and the plant species which were decidous, shed their leaves only for a short period, thus giving the forest an evergreen appearance throughout the year.

Soil reaction and organic carbon status

In both the localities, Wynad and Idukki, the soils were lateritic in origin and they were typically forest soils. The soil reaction and status of organic carbon of both forests are given (Table 3).

Both the forest soils were acidic in reaction, but Wynad soil was found to be more acidic in reaction with pH range 4.9 - 5.5, while that of Idukki ranged from 5.4 - 6.7. The maximum pH obtained was in 0 - 10 cm layer of the soil in both the localities and pH decreased as the depth increased and minimum pH 4.9 - 5.2 cm was obtained in 21 - 30 cm layer of the soil in Wynad and that of Idukki was 5.4 - 6.0.

Table 3. Soil reaction and organic carbon status of the forest soils of Wynad and Idukki

Location	Depth of soil (cm)	Wynad		Idukki	
		pH	Organic Carbon %	pH	Organic carbon %
I	0-10	5.50	2.53	6.70	3.77
	11-20	5.00	1.93	6.50	2.62
	21-30	4.90	1.72	5.60	1.33
II	0-10	5.50	2.54	6.10	4.84
	11-20	5.20	1.75	6.10	1.70
	21-30	5.20	1.72	5.70	1.40
III	0-10	5.30	2.55	6.60	4.88
	11-20	5.00	1.88	6.50	2.92
	21-30	4.90	1.70	6.00	1.25
IV	0-10	5.10	2.37	6.50	2.95
	11-20	5.00	1.70	6.20	1.59
	21-30	4.90	1.85	5.40	1.37
V	0-10	5.40	2.48	6.70	2.35
	11-20	5.30	1.65	6.50	1.20
	21-30	5.20	1.51	6.40	0.47
VI	0-10	5.30	2.41	6.70	1.28
	11-20	5.10	1.74	6.50	1.20
	21-30	4.90	1.16	6.50	0.43

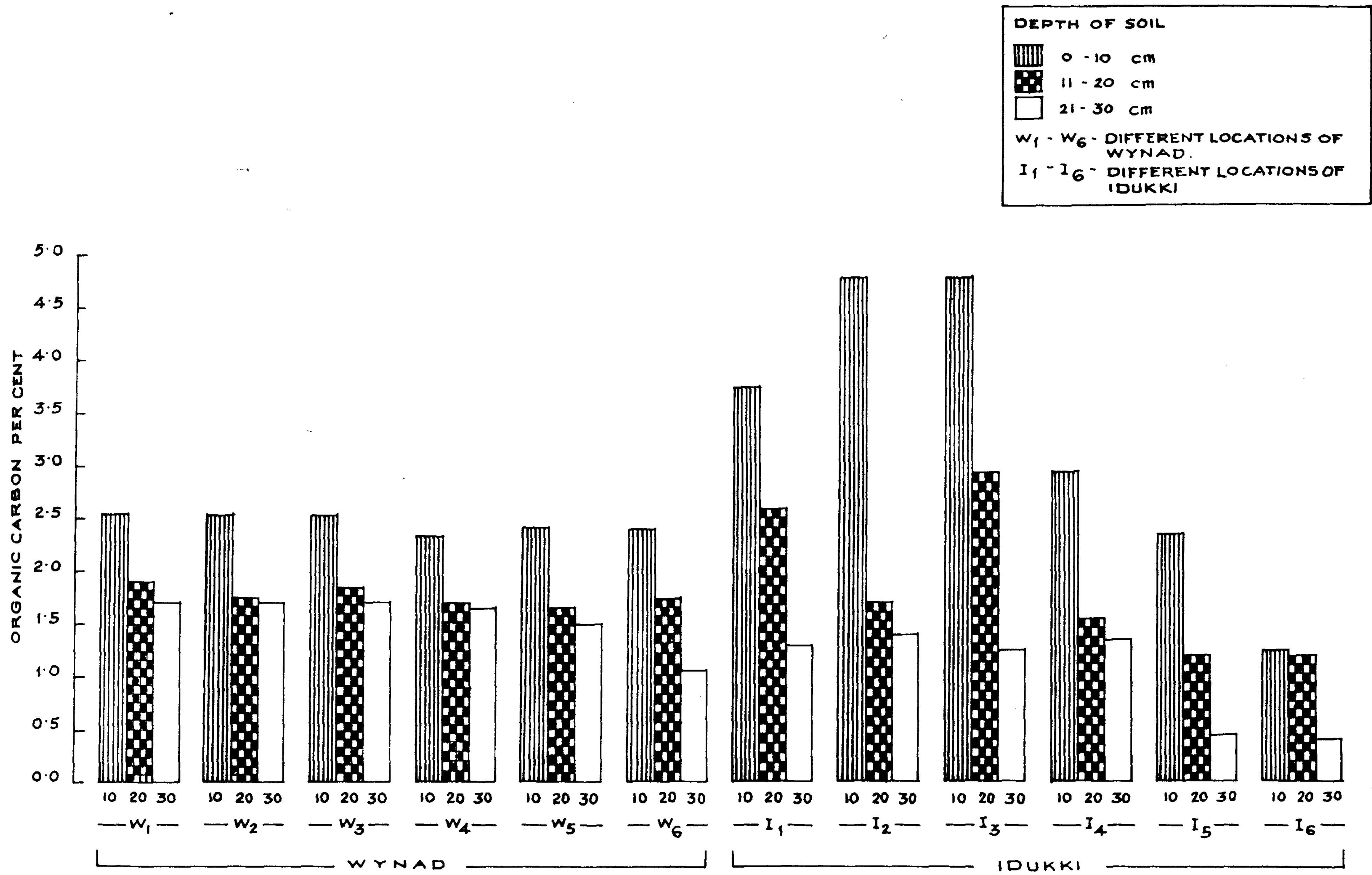


FIG.1.ORGANIC CARBON PERCENT IN FOREST SOIL OF WYNAD AND IDUKKI.

The organic carbon was generally low in Wynad soil (1.16 - 2.55 per cent) when compared to the soils of Idukki (0.4 - 4.88 per cent). In both case, the top most layer of soil of 0 - 10 cm recorded the maximum organic carbon (2.37 - 2.55 per cent in Wynad soils and 1.28 - 4.88 per cent in Idukki soils). The organic carbon was low in the lower layer of soil (21 - 30 cm) the values being 1.16 - 1.7 per cent in Wynad and 0.43 - 1.4 per cent in Idukki. The data showed a negative correlation between the depth of the soil and organic carbon per cent (Table 3, Fig. 1).

<u>Quantitative estimation of microflora</u>

The total populations of microorganisms as well as the population of fungi, actnomycete and bacteria were estimated from six localities of the two districts as described in materials and methods. In each locality soils from three depths namely 0 - 10, 11-20 and 21-30 cm were subjected to estimation. The data are presented in Table 4, 5, 6, 7 and 8 and Fig. 2, 3, 4 and 5.

<u>Total microbial population</u>

The maximum population of microorganisms was observed in the top layer of soil (0 - 10 cm) in both Wynad and

Table 4. Total microbiol populations in Wynad and Idukki forest soils

Locations	Depth of soil (cm)	Total microbial population in 10^6/g Soil on oven dry basis	Total microbiol population in 10^6/g soil on oven dry basis
		Wynad	Idukki
I	0-10	37.865	57.983
	11-20	5.002	11.118
	21-30	0.586	0.845
II	0-10	34.328	47.178
	11-20	10.650	11.867
	21-30	0.517	0.865
III	0-10	38.749	48.695
	11-20	6.867	14.505
	21-30	0.525	0.956
IV	0-10	35.272	44.491
	11-20	7.463	12.467
	21-30	0.621	0.649
V	0-10	34.240	40.140
	11-20	8.352	9.324
	21-30	0.591	0.467
VI	0-10	38.249	39.420
	11-20	9.317	7.455
	21-30	0.541	0.433

Table 5. Population of fungi in Wynad and Idukki forest soils in 10^4/g of dry soil

Locations	Wynad			Idukki		
	Soil depth in cm			Soil depth in cm		
	0-10	11-20	21-30	0-10	11-20	21-30
I	4.48	3.18	2.59	5.34	4.75	2.50
II	4.80	3.10	2.65	5.84	4.65	2.53
III	4.88	3.74	2.49	5.53	4.45	2.60
IV	4.23	3.25	2.05	5.06	3.72	2.89
V	4.25	3.18	2.09	5.02	2.39	1.69
VI	3.83	2.72	2.05	3.40	1.54	1.32
Total	26.48	19.18	13.91	30.19	21.50	13.53
Mean	4.41	3.19	2.32	5.03	3.58	2.25

Table 6. Population of actinomycetes in Wynad and Idukki forest soils in 10^6/g of dry soil

Locations	Wynad			Idukki		
	Soil depth in cm			Soil depth in cm		
	0-10	11-20	21-30	0-10	11-20	21-30
I	0.21	0.18	0.05	0.33	0.18	0.06
II	0.21	0.17	0.06	0.46	0.22	0.07
III	0.20	0.18	0.05	0.48	0.25	0.09
IV	0.23	0.18	0.04	0.46	0.24	0.11
V	0.20	0.17	0.05	0.49	0.25	0.10
VI	0.21	0.18	0.03	0.49	0.24	0.09
Total	1.26	1.06	0.28	2.71	1.38	0.52
Mean	0.210	0.176	0.047	0.451	0.230	0.087

Table 7. Population of bacteria in Wynad and Idukki forest soils in 10^{6}/b dry soil

Locations	Wynad			Idukki		
	Soil depth in cm			Soil depth in cm		
	0-10	11-20	21-30	0-10	11-20	21-30
I	37.61	4.79	0.51	57.60	10.89	0.76
II	34.07	10.45	0.43	46.66	11.60	0.77
III	38.50	6.65	0.45	48.16	14.21	0.84
IV	35.00	7.25	0.56	43.98	12.19	0.51
V	34.00	8.15	0.52	39.60	9.05	0.35
VI	38.00	9.11	0.49	38.88	7.20	0.33
Total	217.18	46.40	2.96	274.88	65.14	3.56
Mean	36.196	7.73	0.493	45.813	10.856	0.593

Table 8. Relationship of soil depth and microbial population in paired 't' test

Depth of soil	Idukki				Wynad				Idukki and Wynad				Remarks
	F	A	B	T	F	A	B	T	F	A	B	T	
a - b	4.176	2.32	14.27	14.48	5.04	7.5	19.90	19.95	7.51	0.2678	18.98	18.95	
a - c	13.039	17.98	16.56	16.84	26.83	22.36	42.16	42.36	16.34	8.253	20.54	20.426	
b - c	1.1965	22.74	11.04	11.20	5.90	22.41	8.89	9.08	5.5	30.36	6.6	11.853	

a - 0-10 cm
b - 11-20 cm
c - 21-30 cm

	5 %	1 %
t_5	2.571	4.032
t_{11}	2.201	3.106

F - Fungi
A - Actinomycetes
B - Bacteria
T - Total microbial population

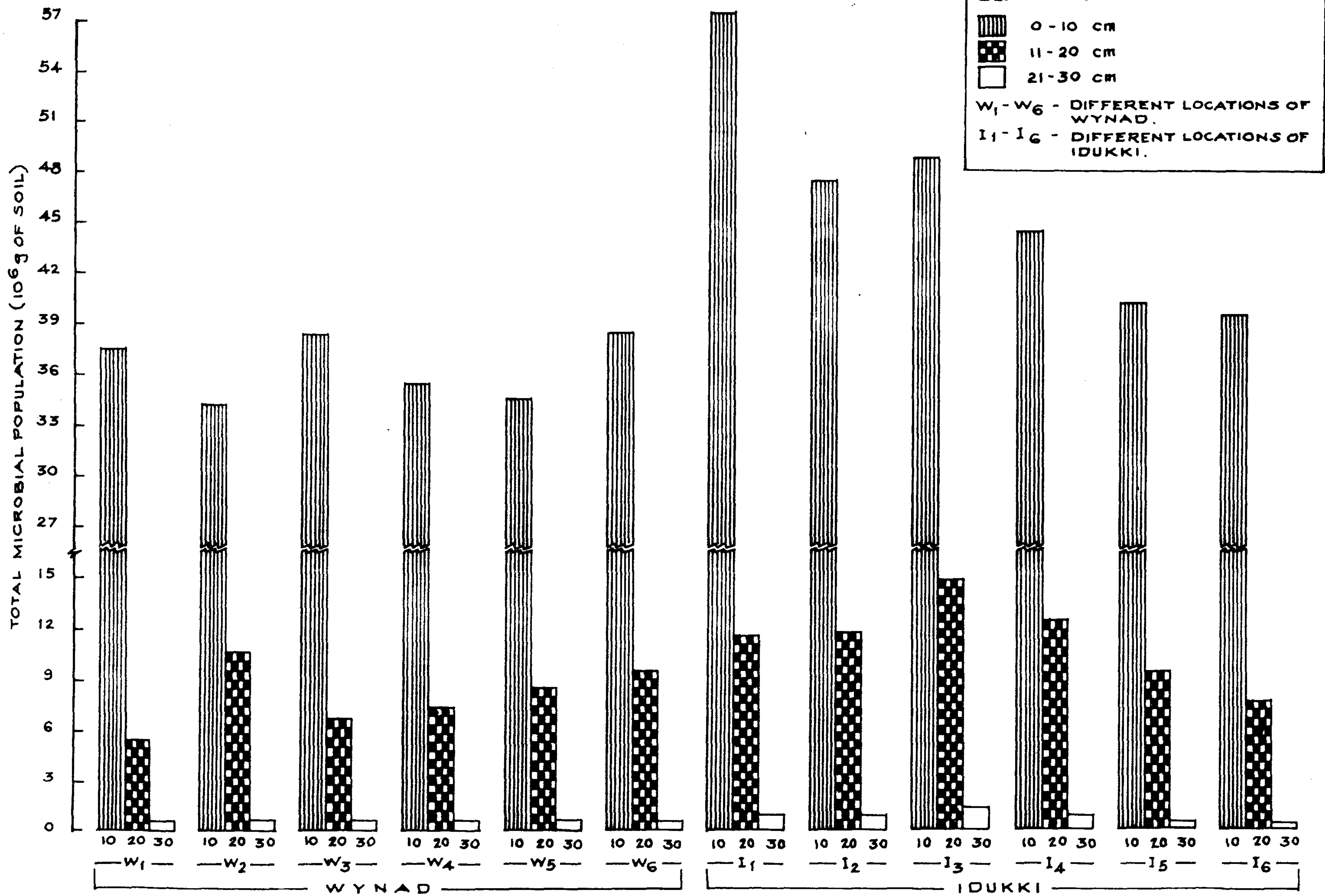


FIG. 2. TOTAL MICROBIAL POPULATION OF WYNAD AND IDUKKI FOREST SOILS.

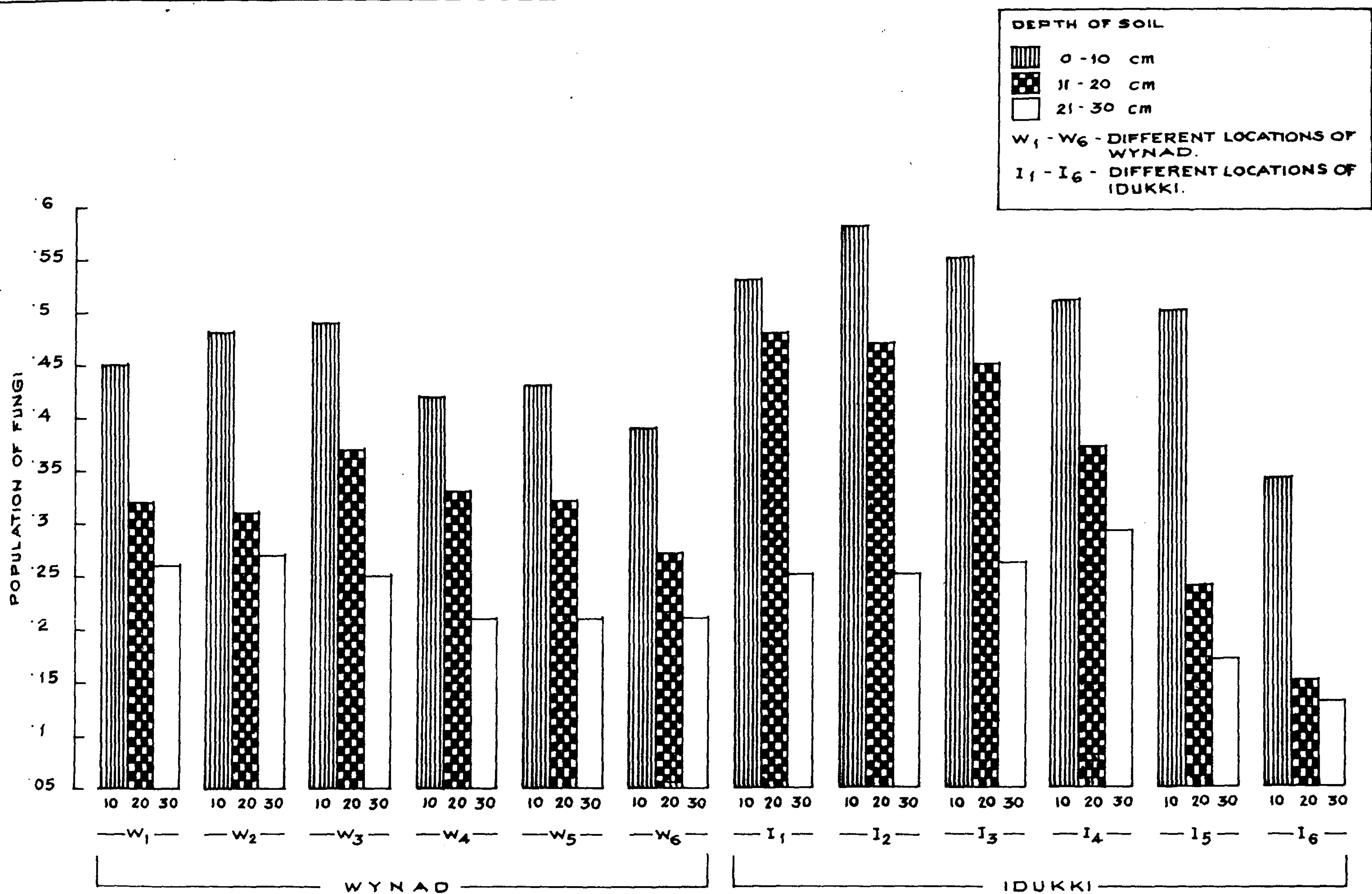


FIG. 3. POPULATION OF FUNGI IN WYNAD AND IDUKKI FOREST SOILS.

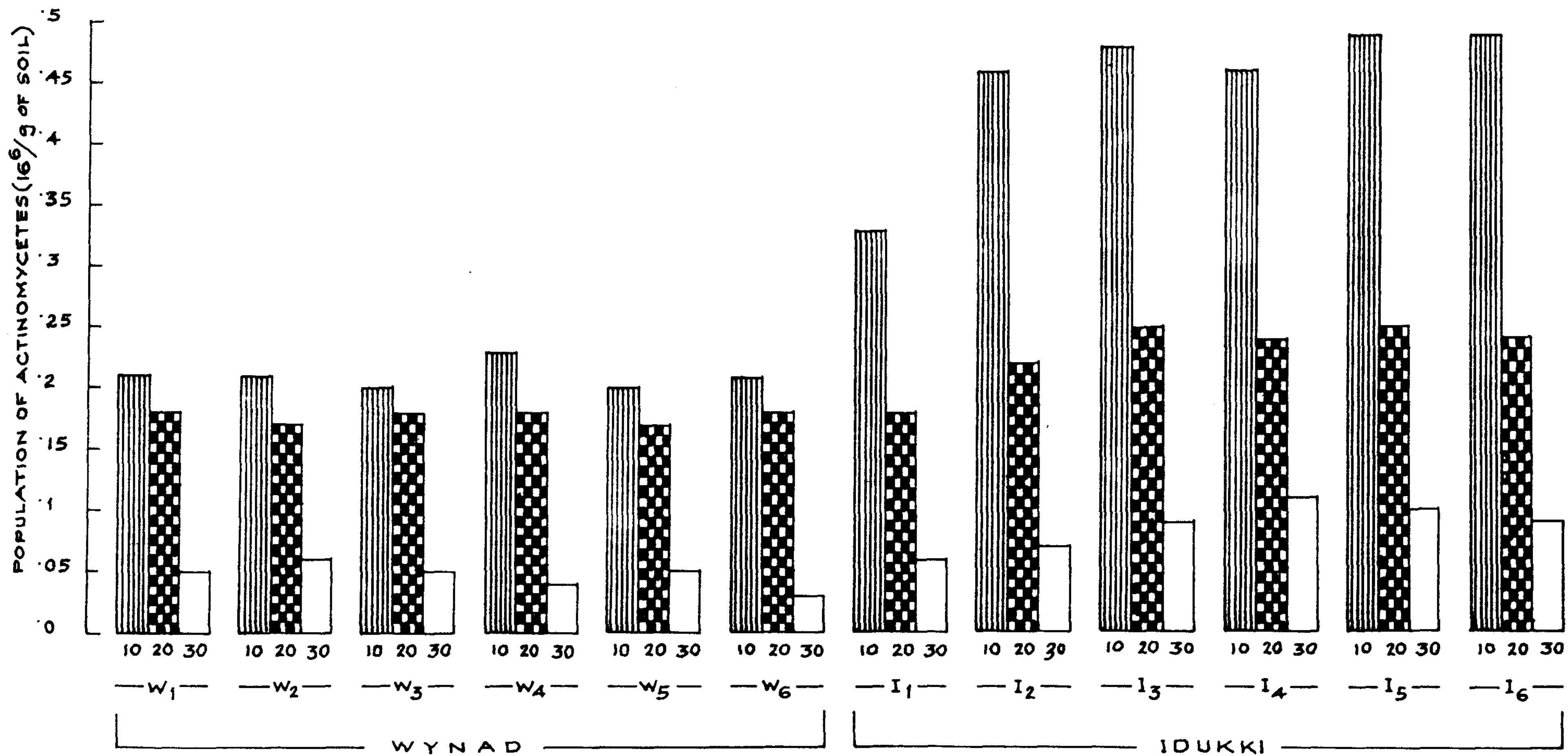


FIG. 4. POPULATION OF ACTINOMYCETES IN WYNAD AND IDUKKI FOREST SOILS.

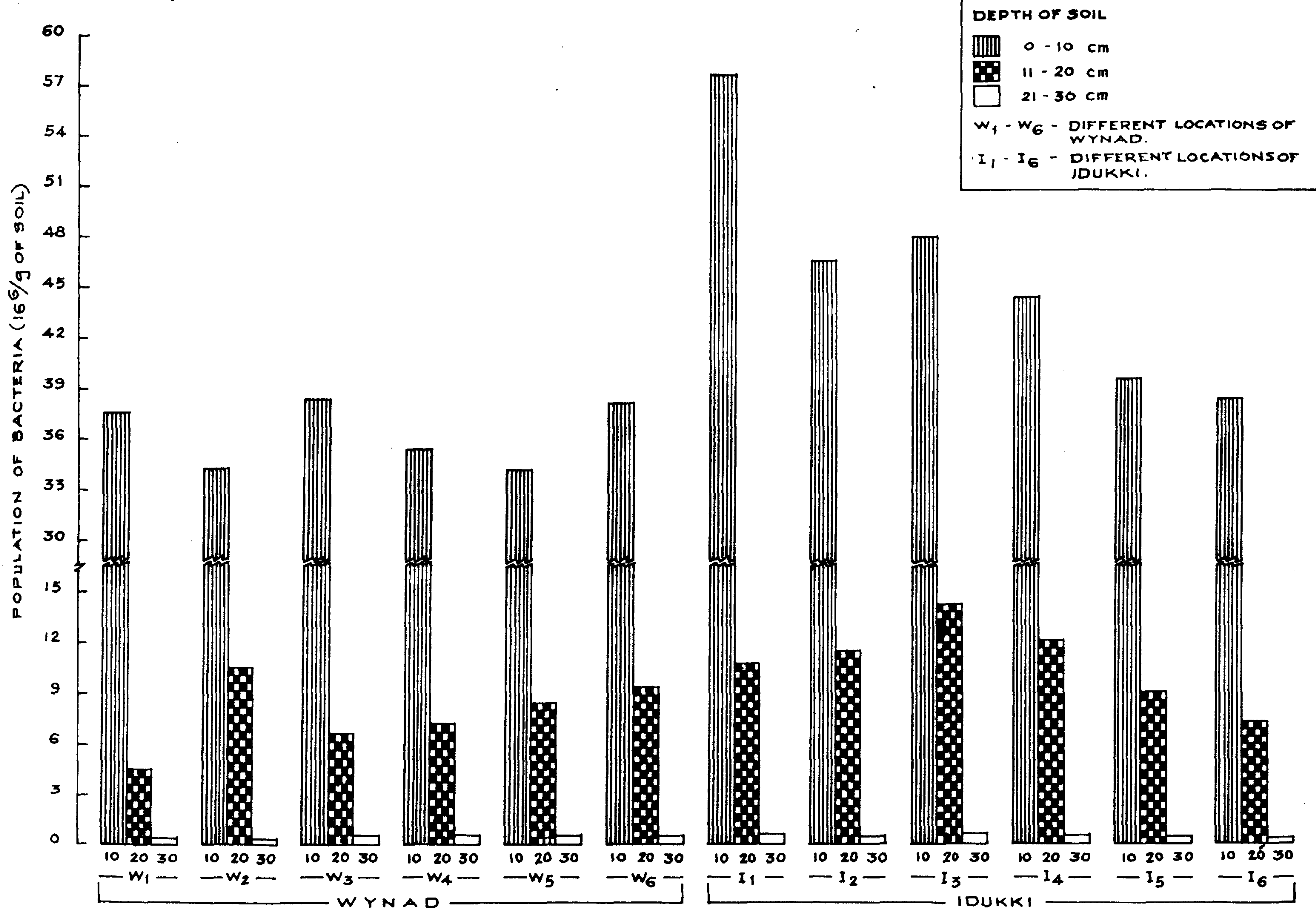


FIG. 5. POPULATION OF BACTERIA IN WYNAD AND IDUKKI FOREST SOILS.

Idukki districts. In general, the population was high in soils of Idukki being 39.42 - 57.98 x 10^6/g of soil while in Wynad it ranged from 34.24 - 38.74 x 10^6/g of soil (Table 4). When soil depth increased the microbial population decreased considerably in soils of both the districts. In Idukki, it ranged from 0.433 - 0.956 x 10^6/g of soil, while in Wynad microbial population was very low ranging between 0.517 - 0.621 x 10^6/g in 21 - 30 cm depth of soil layer. The same trend was observed in the middle layer (11 - 20 cm) in both Idukki and Wynad.

Statistical analysis by paired 't' test revealed that the depth of soil and total microbial population have direct relationship. When the depth increased, the microbial population significantly decreased in both the forest soils. The total microbial population differed significantly in all three depths of soil. The pooled analysis of data also showed significant difference (Table 8).

Population of fungi

The maximum fungal population was recorded in 0 - 10 cm depths of soil in both Wynad and Idukki.

The population of fungi in the surface layer (0 - 10 cm) of Wynad and Idukki ranged between 3.83 - 4.88 and 3.4 - 5.84 x 10^4/g of dry soil respectively, mean being 4.41 and 5.03 x 10^4/g of dry soil respectively (Table 5).

In the second layer (11 - 20 cm) the fungal population in Wynad and Idukki ranged between 2.72 - 3.74 and 1.54 - 4.75 x 10^4/g of dry soil respectively, mean being 3.19 and 3.58 x 10^4/g of dry soil respectively.

In the third layer (21 - 30 cm), when compared to other two layers, the fungal population decreased considerably in both the forest soils but the decrease was more pronounced in Idukki. The fungal population ranged from 2.05 - 2.65 x 10^4/g in Wynad as against 1.32 - 2.89 x 10^4/g of dry soil in Idukki.

When compared to Wynad soil, the decrease in fungel population from the second to third layer was high in Idukki. In the former, the mean population of fungi, decreased from 3.19 to 2.31 x 10^4/g of dry soil while in the latter it decreased from 3.58 - 2.55 x 10^4/g of dry soil (Table 5, Fig. 3).

The statistical analysis by paired 't' test revealed that there was significant difference in the fungal population

of different layers of the soil except between the second and third layers of Idukki soils where it was not significantly different (Table 8).

In three locations of Idukki, the fungal population was low in the second and third layers and difference between the fungal population in the layers was also negligible. But pooled analysis of the data for Wynad and Idukki showed that there was a significant difference of fungal population in all the three layers.

Population of actinomycetes

The actinomycete population was generally high when compared to that of the fungi. Maximum population was observed in the top most layer of soil in Wynad and Idukki districts. It ranged from 0.20 - 0.23 x 10^6/g of dry soil in Wynad with an average of 0.21 x 10^6/g of dry soil. In Idukki soils it was much higher which ranged from 0.33 - 0.49 x 10^6/g with an average of 0.45 x 10^6/g of dry soil. In the second layer (11 - 20 cm), the population ranged from 0.17 - 0.18 x 10^6/g of dry soil with a mean of 0.24 x 10^6/g of dry soil in Idukki. Almost similar trend was observed in the third layer (21 - 30 cm) in Wynad range being 0.03 to 0.06 x 10^6/g of dry soil with a mean of 0.047 x 10^6/g of

dry soil while that in Idukki it ranged from 0.06 - 0.11 x 10^6/g of dry soil with a mean of 0.087 x 10^6/g of dry soil (Table 6).

Statistical analysis revealed that there was significant difference in the actinomycete population in different layers of soil except the first and second layers in Idukki. In all other cases, the population was significantly higher in the top layer and than it significantly reduced in the sub soil layers. The pooled analysis of actinomycete population of Wynad and Idukki revealed that there was no significant difference between the first and second layers, but there was significant difference between the second and third layers.

Population of bacteria

Among the microorganisms, the bacterial population was found to be the maximum in all the three layers of soil in both the districts. Like fungi and actinomycetes the maximum bacterial population was found in the top layer of soil which ranged from 34.2 - 38.5 x 10^6/g of dry soil with a mean of 36.196 x 10^6/g of dry soil in Wynad. In Idukki the population was much higher than Wynad, ranging from 38.88 - 57.62 x 10^6/g

of dry soil, mean being 45.813 x 10^6/g of dry soil. But there was a sudden decrease in bacterial population in the second layer (11 - 20 cm) of both forest soils. In Wynad, it ranged from 4.79 - 10.45 x 10^6/g of dry soil, mean being 7.73 x 10^6/g of dry soil while in Idukki it was 7.20 - 14.21 x 10^6/g of dry soil, with a mean of 10.856 x 10^6/g of dry soil. In the third layer the decrease in population was much pronounced ranging from 0.43 - 0.56 x 10^6/g of dry soil, with a mean of 0.493 x 10^6/g of dry soil in Wynad and in Idukki it ranged from 0.33 to 0.84 x 10^6/g of dry soil, with a mean of 0.593 x 10^6/g of dry soil (Table 7 and Fig. 4).

Statistical analysis revealed that there was significant difference in the population of bacteria in different layers of soil in both the districts. There was significant decrease in bacterial population with the increase in depth of soil. The pooled analysis of data in Idukki and Wynad also showed similar results (Table 8).

The maximum population of bacteria was observed in the top layer of soil and a remarkable decrease in the population was found in the middle and it was still pronounced in the bottom layer.

Qualitative estimation of microflora

The qualitative estimation of the soil microflora was studied both in Wynad and Idukki forest. Of these 20 species of fungi were brought into pure culture and 18 were identified but two could not be identified due to lack of reproductive structures. These 18 species are distributed in 11 genera. Among these only 12 species were found in Idukki while 17 of them present in Wynad. Nine species were common in both the localities (Table 9).

Only one genus of actinomycete was observed belonging to *Streptomyces*. But it comprised of three morphological groups, with straight, flexuous and fascicled sporophores. Those with straight and flexuous sporophores were common in Wynad and Idukki and those with fascicled sporophores were isolated only from Idukki (Table 9).

Out of the four *Bacillus* spp., *B. subtilis* and *Bacillus* -1 are common both in Wynad and Idukki whereas *Bacillus*-2 and *Bacillus* 3 are present only in Idukki (Table 9).

Identification of soil microflora

The microorganisms isolated from the soil were brought in pure culture as described in materials and methods. The

Table 9. Qualitative estimation of soil microflora in Wynad and Idukki forests

Name of microorganism	Locations - from where they have been isolated		
	Wynad	Idukki	Both from Wynad and Idukki
Fungus			
1. *Mucor* sp.	*	*	*
2. *Syncephalastrum racemosum*	*	*	*
3. *Trichoderma koningii*	*	o	o
4. *Trichoderma harzianum*	*	*	*
5. *Trichoderma longibrachiatum*	o	*	o
6. *Microascus cinereus*	*	o	o
7. *Cunninghamella elegans*	*	o	o
8. *Absidia corymbefera*	*	*	*
9. *Aspergillus versicolor*	*	o	o
10. *Aspergillus melleus*	o	*	o
11. *Aspergillus sydowii*	*	o	o
12. *Aspergillus terreus*	*	o	o
13. *Aspergillus niger*	o	*	o
14. *Penicillum simplicissimum*	*	o	o
15. *Penicillum citrinum*	*	*	*
16. *Talaromyces wortmanni*	*	*	*
17. *Paecilomyces lilacinus*	*	o	o
18. *Fusarium oxysporum*	*	*	*
19. Unidentified fungal flora having sparse growth without any reproductive structures	*	*	*
20. Unidentified fungal flora having profuse growth without any reproductive structures	*	*	*

Contd.

Table 9. Continued

1	2	3	4
<u>Actinomycetes</u>			
1. *Streptomyces* sp. (with straight sporophores)	*	*	*
2. *Streptomyces* sp. (with flexuous sporophores)	*	*	*
3. *Streptomyces* sp. (with fascicled sporophores)	o	*	o
<u>Bacteria</u>			
1. *Bacillus subtilis*	*	*	*
2. Bacillus-1	*	*	*
3. Bacillus-2	o	*	*
4. Bacillus-3	o	*	o

0 - Absence

* - Presence

slide culture of all the fungi and actinomycetes were prepared and detailed morphological study was made and were identified. The fungal, Actinomycetes and bacterial cultures were sent to Commonwealth Mycological Institute, Kew, Surrey, England for identification and were confirmed.

Fungi

1. Absidia corymbefera (Cohn) Sacc & A. Trotter)
   Nottebrock, H; Scholar, H.J. & Wall, M. 1974.
   Sabourandia, 12, 64-74.
2. Aspergillus melleus Yukawa
   = A. quercinus (Bain) Thom & CL 1926
   = Sterigmatocystis quercina (Bain 1881)
   Thom, C. and Raper, K.B. 1945. A Manual of the Aspergilli pp. 276-8.
3. Aspergillus niger Van Tieghem
   Thom, C. and Raper, K.B. 1945. A Manual of the Aspergilli pp. 216-9.
4. Aspergillus sydowii (Bainier & Sartory) Thom & Church
   Thom, C. and Raper, K.B. 1945. A Manual of the Aspergilli
5. Aspergillus terreus Thom
   Thom and Church 1918. Am. J. Bot. 5:85-6.
   Sacchardo, P.A. 1931. Syll. Fung. 25:659.

Thom, C. and Raper, K.B. 1945. A Manual of the Aspergilli pp. 195-7.

6. Aspergillus versicolor (Vuillemin) Tiraboschi

   Ann. Bot. (Rome) 7:9, 1908.

   Saccardo, P.A. 1913. Syll. Fung. 22:1261.

   Thom, C. and Raper, K.B. 1945. A Manual of the Aspergilli pp. 190-2.

7. Cunninghamella elegans Lendner

   = Cunninghamella hertholletiae Stadd 1911

   Cutter, V.M. 1947. The Genus Cunninghamella Farlouria, 2, 321-345.

8. Fusarium oxysporum Schl. ex Fries.

   Syst. Mycol. 3:471:1732.

   Subramanian, C.V. 1954. J. Madras Univ. 13 24 - 34.

9. Microascus cinereus (Emile-Weil & Gaudin) Cursi

   Bull. Stay. Veg. Roma N. S. 11:60 (1930)

   Scopulariopsis cinerea Emile-Weil & Gaudin

   Sae 1931: Syll. Fung. 25:681.

10. Mucor sp. Mich. ex St. Am.

    Mucor Mich ex Fr.

    Sacc. VII, 190.

11. *Paecilomyces lilacinus* (Thom) Samson

= *Pencillium lilacinum* Thom 1910. *Sacc*. XXII, 1268.

Rapper, K.B. and Thom, C. 1949. *A Manual of the Penicillia*. pp.285-8.

12. *Penicillium citrinum* (Thom)

Raper, K.B. and Thom, C. 1949. *A Manual of the Penicillia* pp.345-50. Sacchardo, P.A. 1913. *Syll*. *Fung*. *22*:1266.

13. *Penicillium simplicissimum* (Oud.) Thom

= *Spicaria simplicissima* Oudemans 1903

Raper, K.B. and Thom, C. 1949. *A Manual of the Penicillia* pp.304-5 & 81, C.D.

Sacc. XVIII, 538.

14. *Syncephalestrum racemosum* Cohn ex Schrotter

Taxter, R. 1897 New or Peenlion Zygomycets II. *Syncephalastrum* and *Syncephalis* *Bot*. *Gaz*. *24*, 1-15.

Boedijn, K.B. (1988). *Notes on the Mucorales of Indonesia*. *SYDOWIA* *12*, 321-362.

15. *Talaromyces wortmanni* (Klöcker) C.R. Benjamin

= *Pencillium wortmanni* (Klocker 1903) Raper, K.B. and Thom, C. 1949. *A Manual of the Penicillia*. pp.583-6.

16. *Trichoderma harzianum* Rifai

Rifai, M.A. 1969. *A revision of the Genus Trichoderma*. *Mycol*. *pap*. 116, 1-56.

17. *Trichoderma koningii* Oudem

Rifai, M.A. 1969. *A revision of the Genus Trichoderma.* *Mycol. pap.* 116, 1-56.

18. *Trichoderma longibrachiatum* Rifai

Rifai, M.A. 1969. *A revision of the Genus Trichoderma.* *Mycol. pap.* 116, 1-56.

Actinomycetes

Streptomyces Waksman and Heurici, 1943.

(*Jowr-Bact.* *46*, 1943, 339)

Bacteria

Bacillus Cohn

(*Beitrage Z. Biol. d. Pflanzen, 1, Heft 2*, 1872, 146 and 175)

1. *Bacillus subtilis* Cohn, emend Prasmowski, 1880 (*Cohn, Beitr. Z. Biol. d. Pflasen, 1, Heft 2*, 1872, 174)
2. *Bacillus-1*. Almost identical to *B. subtilis*
3. *Bacillus-2*. Small celled *Bacillus* sp. with fast growth in nutrient agar.
4. *Bacillus-3*. Small celled *Bacillus* sp. with slow growth in nutrient agar.

Isolation of test organisms

Three soil borne fungi viz. *Pythium myriotylum* causing soft rot of ginger, *Phytophthora palmivora* causing foot rot (quick wilt of pepper) and *Rhizoctonia solani* causing sheath blight disease of rice have been isolated as described in materials and methods. The auxenic culture of *R. solani* was maintained in PDA at room temperature, while those of *P. myriotylum* and *P. palmivora* were maintained in OMA at 20-22°C in BOD incubator. These fungi were used as test organisms for further studies.

Growth rate of antagonists and test organisms

A good understanding of rate of growth of antagonists and test organisms is highly essential for judging the time of inoculation of these organisms in dual cultures for studying the antagonistic properties.

The growth rate of the antagonists which comprised of 18 fungi, three actinomycetes and four bacteria and the three test organisms were studied as described in materials and methods. The results are presented in Table 10a, b and c.

Table 10(a) Growth rate of antagonists and test organism (mm)

(a) Antagonistic fungi

Sl. No.	Name	Days after inoculation														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*Mucor* sp.	50	90	-	-	-	-	-	-	-	-	-	-	-	-	-
2	*Absidia corymbifera*	25	35	59	72	87	90	-	-	-	-	-	-	-	-	-
3	*Syncephalastrum racemosum*	25	60	90	-	-	-	-	-	-	-	-	-	-	-	-
4	*Cunninghamella elegans*	18	30	60	51	65	75	87	90	-	-	-	-	-	-	-
5	*Trichoderma harzianum*	35	55	76	87	90	-	-	-	-	-	-	-	-	-	-
6	*T. koningi*	38	60	86	90	-	-	-	-	-	-	-	-	-	-	-
7	*T. longibrachiatum*	32	58	76	88	90	-	-	-	-	-	-	-	-	-	-
8	*Aspergillus milleus*	3	6	9	11	13	15	17	20	21	23	25	26	28	30	32
9	*A. niger*	10	27	35	40	44	49	55	60	66	71	76	81	85	90	
10	*A. sydowii*	4	9	13	18	26	30	35	42	52	63	72	80	88	90	
11	*A. terreus*	2	5	9	11	14	16	18	21	23	25	28	30	33	35	37
12	*A. versicolor*	29	35	39	42	45	50	54	59	63	67	71	75	79	83	87
13	*Penicillium citrinum*	2	5	8	10	12	15	18	20	22	24	27	29	32	35	38
14	*P. simplicissimum*	2	5	8	13	18	21	23	26	28	31	34	36	38	60	42
15	*Paecilomyces lilacinus*	2	10	16	21	26	30	36	41	48	54	61	67	72	78	85
16	*Talaromyces wortmanni*	3	5	7	9	10	13	15	17	20	23	25	28	31	33	36
17	*Microascus cinereus*	2	5	8	10	13	17	21	25	28	31	34	38	41	45	51
18	*Fusarium oxysporum*	1	4	8	13	19	26	30	35	39	45	52	57	63	69	76

Table 10 b, c & d. Growth rate of antagonists and test organisms (mm)

(b) Antagonistic actinomycete

Sl. No.	Name	Days after inoculation																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	*Streptomyces* sp. (with straight sporophores)	2	5	9	13	17	22	27	29	31	33	35	38	40	42	45	47	49	52	55	59
2.	*Streptomyces* sp. (with flexuous sporophores)	1	4	7	11	16	20	24	28	32	36	39	42	44	46	47	48	49	50	51	52
3.	*Streptomyces* sp. (with fascicled sporophores)	2	4	6	9	12	15	19	23	27	30	33	36	39	41	43	45	46	47	48	49
(c) Antagonistic bacteria																					
1.	*Bacillus subtilis*	45	90	90	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Bacillus*-1	45	90	90	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	*Bacillus*-2	40	82	90	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	*Bacillus*-3	26	59	82	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(d) Test organisms																					
1.	*Pythium myriotylum*	38	82	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Phytophthora palmivora*	5	13	25	31	41	48	54	63	69	75	79	83	87	90	-	-	-	-	-	-
3.	*Rhizoctonia solani*	21	43	65	75	81	86	90	-	-	-	-	-	-	-	-	-	-	-	-	

Fungi

The growth rate of the fungi including 18 antagonists and the three test organisms varied widely. All the mucoraceous fungi were found to be fast growing. Of these the unidentified Mucor sp. covered the entire 90 mm petri-dish within two days followed by S. racemosum within three days. However, C. elegans and A. corymbefera were found to be slow growing and they took eight days and six days respectively for covering the 90 mm petridish (Table 10a).

The growth rate of three Trichoderma spp. was not having much variation and they covered the entire 90 mm petridish within four to five days (Table 10a).

The different species of Aspergilli vary in their growth rate. A. milleus and A. terreus were found to be slow growing fungi with a radial growth of 32 and 37 mm dia respectively after 15 days whereas, A. versicolor grew 87 mm within 15 days and A. niger and A. sydowii covered the 90 mm petridish within 14 days (Table 10a).

In general Pencillium group of fungi was found to be very slow growing except T. wortmanni which covered 85 mm growth within 15 days. All other fungi of this group

including *M. cinereus*, growth rate was very slow being 36 - 51 mm in 15 days (Table 10a).

The *Fusarium* sp. was found to be moderately growing fungus which grew 76 mm within 15 days (Table 10a).

Actinomycetes

The growth rates of three species of *Actinomycetes* were studied. All of them were very slow growing organisms and recorded only 43-47 mm growth within 15 days. Due to their very slow growing habit the petridishes were incubated for another five more days and the growth recorded was 49-59 mm (Table 10b).

Bacteria

The growth rates of four types of *Bacillus* sp. have been studied and found that all of them were fast growing. The isolate *Bacillus-3* was found to have slow growth initially but reached 90 mm growth within four days. The other three types reached the same growth within a period of three days (Table 10c).

When the growth rates among the Eumycophyta were compared, most of the *Mucor* spp. were found to be growing

very fast and mejority of <u>Pencillium</u> spp. growing very slow only. Among tha protophyta all the <u>Streptomycets</u> spp. were found to be very slow growing organisms while the <u>Bacillus</u> spp. growing rapidly.

Considering the growth rate of the test organisms <u>P</u>. <u>myriotylum</u> was found to be a fast growing fungus which covered the 90 mm petridish after third day of inoculation while <u>R</u>. <u>solani</u> took seven days and <u>P</u>. <u>palmivora</u> 14 days for the same (Table 10d).

<u>Screening the microorganisms for antagonistic property against the test fungi</u>

The qualitatively estimated microorganisms were brought in to axenic culture and were tested for antagonistic properties against the soil borne pathogens viz. <u>P</u>. <u>Myriotylum</u>, <u>P</u>. <u>palmivora</u> and <u>R</u>. <u>solani</u> by adopting the dual culture method as described in materials and methods. The microorganisms which were tested for antagonistic property were called as 'antagonists' and the soil borne fungi against which they were tested, called test organisms. The growth rate of the antagonists as well as test organisms was known (Tables 10a, b, c and d) and slow growing organisms

were inoculated sufficiently earlier than the fast growing organisms when they were grown in dual culture. The reactions of the organisms in dual culture were observed and results presented (Tables 11 to 35).

Mucor sp.

P. myriotylum

The antagonist was inoculated on the same day with test organism in dual culture. As the antagonist and test organisms were having almost same growth rate, on the second day both the organisms grew over each other and covered the entire petridish.

The reaction obtained in dual culture was mere intermingling and no other antagonistic properties observed (Table 11).

P. palmivora

Test organisms was slow growing compared to the antagonist and so inoculated four days prior to the antagonist. The test organism in the dual and mono culture grew only 45 mm after six days. The antagonist grew 90 mm within two days.

The reaction shown in dual culture was only mere intermingling and no antagonistic property observed (Table 11).

R. solani

When compared to _P. palmivora_, _R. solani_ was found to be a fast growing organism and it was inoculated one day prior to antagonist in the dual culture method. The antagonist completely covered the petridish within two days while test organism grew only 56 mm during the same period in dual culture.

Here also reaction was mere intermingling and over growth and no antagonistic property observed (Table 11).

The _Mucor_ sp. is not having any antagonistic property against any of the test organisms and the reation observed was intermingling and overgrowth.

Absidia corymbefera

P. myriotylum

Test organism was inoculated one day aftar the antagonist because of the slow growth rate of the antagonist.

The test organism and the antagonist recorded normal growth rate on first day in dual culture. On the second day antagonist showed normal growth rate but the test organism grew only 5 mm. When the antagonist grew over the test organism by 7 mm on the third day, the test organism maintained its rate of growth 5 mm per day but growth rate of antagonist reduced to 8 mm on the third day. No further growth of the antagonist was observed even after nine days. However, test organism grew three mm more on the fourth day and remained stationary even after nine days (Tabla 12).

In dual culture, the antagonist and test organism showed limited or no growth after the normal initial growth for two days. No antagonistic properties have been observed.

P. palmivora

The test organism being a slow grower, was inoculated two days prior to antagonist in dual culture. The growth rate of test organisms and antagonist was found to be normal through out the period of observation and they freely intermingled and overgrew each other. No antagonistic properties were shown (Table 12).

R. solani

Test organism and antagonist were inoculated on the same day. In dual culture the growth rate of both organisms on the first and secondly was normal. On the third day, antagonist grew 50 mm and the test organism 40 mm, both contacted each other. Thereafter no further growth of both organisms was observed (Table 12). In dual culture antagonist and test organism were mutually inhibited on contact even after nine days growth (Plate 1).

A. corymbefera is not having any antagonistic property against *P. myriotylum* and *P. palmivora* but it has shown mutual inhibition on contact in the case of *R. solani*.

Syncephalastum racemosum

P. myriotylum

Antagonist and test organism were inoculated on the same day in dual culture as both of them were equally fast growing. On the third day both the organisms completed their growth in the petridish and freely intermingled each other (Table 13). No antagonism was observed.

Plate 1. *Absidia corymbefera* x *R. solani* in dual culture after 9 days growth
(1) Dual culture (2) Control

Plate 2. *Trichoderma harzianum* x *P. myriotylum* in dual culture on the second day
(1) Dual culture (2) Control

Plate 1

Plate 2

P. palmivora

The antagonist was inoculated one day after the test organism. Both the organisms grew almost at the same rate of their growth in mono culture and intermingled each other. The antagonist covered the 90 mm petridish on the third day but the test organism reached 69 mm on the nineth day only, though it has intermingled freely with antegonist.

The two organisms did not show any antagonistic properties in dual culture and reaction was mutual intermingling of two organisms (Table 13).

R. solani

The antagonist and test organisms were having more or less same growth rate and they were inoculated on the same day in dual culture. Initially, both organisms had same rate of growth in mono culture and dual culture. On the second day, antagonist grew 50 mm, while test organism had a growth of 40 mm. It was observed that there was no mutual intermingling but the growth of test organism was inhibited on contact (Table 13).

Cunninghamella elegans

P. myriotylum

Due to the slow growth rate of the antagonist, inoculation of test organism in dual culture was done one day after antagonist. Upto fourth day both the organisms grew freely as in mono culture. Thereafter antagonist reached a growth of 65 mm and the test organism 85 mm and no further growth was observed in either of the organisms. The two organisms grew by intermingling (Table 14).

The growth behaviour indicated mutual intermingling and no antagonistic property was observed.

P. palmivora

The test organism was inoculated one day prior to antagonist, in the dual culture. Both the organisms grew on the same pattern in the mono culture and dual culture.

In dual culture, intermingling was noticed on the fourth day. The test organism did not grow after the sixth day while growth of antagonist ceased from seventh day onwards (Table 14).

The reaction observed was mutual intermingling without any antagonistic property.

R. solani

The test organism was inoculated on the same day in dual culture because of almost equal growth rate. These two organisms showed almost same growth rate both in mono culture and dual culture. They grew and intermingled each other on the third day without showing any antagonistic property (Table 14).

Trichoderma harzianum

P. myriotylum

The antagonist and test organism were inoculated on the same day in dual culture. Initially, growth of both the organisms was the same in mono and dual culture. On the second day, while the antagonist maintained same rate of growth in mono and dual culture, the test organism rather grew slowly in the dual culture (35 mm) as against its growth in mono culture (82 mm) (Table 15, Plate 2). The antagonist further grew at a reduced rate over the test organism causing its die-back.

The growth of test organism was reduced considerably to 26 mm on third day, 8 mm on fifth day and thereafter at a slow rate reaching 3 mm on ninth day (Table 15, Plate 3).

The results indicated the antagonistic property of T. harzianum against P. myriotylum. On contact of the hyphae of the antagonist, die-back and disintegration of test organism were resulted, while the former continued its growth at a reduced rate.

P. palmivora

Test organism was inoculated three days prior to the antagonist due to the fast growing nature of the latter. In mono and dual culture, initial growth of both the organisms was same. On second day onwards, antagonist had same growth rate in mono and dual culture whereas the test organism had a radial growth of 35 mm in dual culture as against 41 mm in mono culture. On the third day, growth of test organism decreased to 17 mm and on fourth day further decreased to 4 mm (Table 15). The antagonist inhibited the test organism on contact. Even after contact with test organism, antagonist grew at a reduced rate in dual culture, resulting in the die-back and disintegration of test organism.

Plate 3

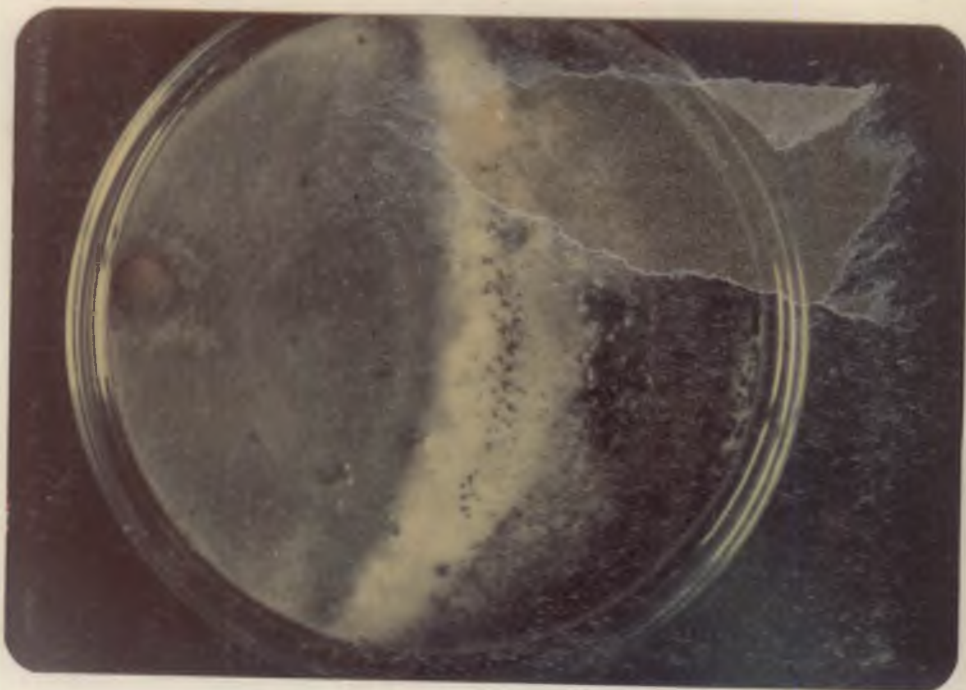
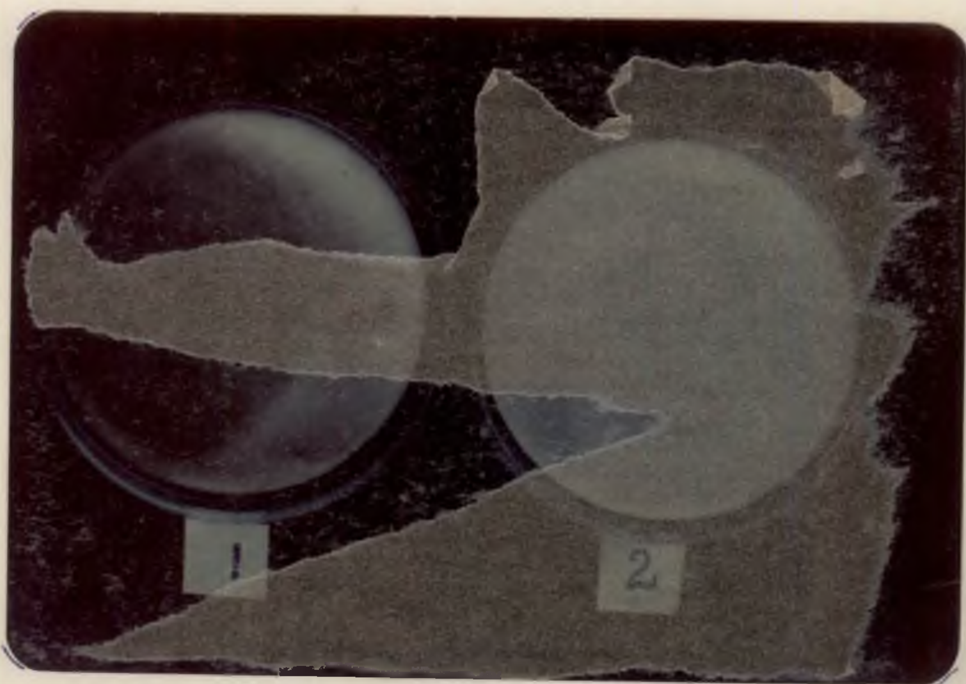


Plate 4



R. solani

The antagonist and test organism were inoculated on the same day in dual culture. On the first day, antagonist and test organism maintained almost the same growth on mono culture and dual culture. On the second day, growth of antagonist was the same in mono and dual culture, while test organism grew only 35 mm in dual culture as against 43 mm in mono culture (Table 15). The antagonist and test organism contacted each other, and the former grew over tha latter resulting in the die-back and disintegration of the latter. The disintegration continued upto seventh day and test organism remained as a narrow strip of 2 mm while antagonist grew 88 mm (Table 15).

The result showed that when antagonist grew over the test organism, die-back and disintegration of the latter occurred.

Trichoderma koningii

P. myriotylum

The antagonist and the test organism were having the same growth, and hence inoculated on the same day. Initially both the organisms had same growth in mono culture and dual

culture. On the second day, they showed a decreased growth rate in dual culture as against normal growth in mono culture. When the test organism and antagonist came in contact on third day, the antagonist grew over the test organism resulting in die-back of the latter with a reduction in its radial growth from 40 mm to 25 mm (Table 16, Plate 4b). On fourth and fifth day, the same trend was observed and due to the overgrowth of the antagonist on test organism, the disintegration and die-back of test organism occurred with only 10 mm growth of test organism remaining in duel culture on fifth day. The antagonist grew further at a reduced rate and reached a growth of 87 mm, causing disintegration of test organism and reducing its growth to only 3 mm (Table 16). The result indicated a definite antagonistic property of T. koningii on P. myriotylum by disintegration and die-back.

P. palmivora

The test organism was found to be a slow growing organism when compared to antagonist and so inoculated four days prior to the antagonist. On the first day in mono and dual culture, the antagonist grew 38 mm while growth of test organism was 41 mm (Table 16). On the second day, the antagonist reached 50 mm but the growth of the test organism

reduced to 40 mm establishing contact between them. On third day, the antagonist overgrew 20 mm on the test organism resulting disintegration of the latter. On the subsequent days antagonist overgrew the test organism at a reduced rate causing disintegration, recording the final growth of 6 mm for test organism and 84 mm for the antagonist (Table 16).

The result showed the antagonistic property of *T. koningii* on *P. palmivora* by disintegration and die-back of the latter on mutual contact.

R. solani

The test organism was inoculated one day prior to antagonist in the dual culture. On the first day both the organisms had same growth in mono and dual culture, but on the second day in dual culture, test organism had a reduced growth of 40 mm and antagonist 50 mm, resulting contact with each other. On third day, the antagonist overgrew the test organism by 15 mm and the region of overgrowth was completely disintegrated. On fourth day the antagonist grew further at a reduced rate over the test organism and it continued up to sixth day. By that time, the antagonist has grown 84 mm resulting disintegration of test organism and reducing it to

6 mm growth. There was no further growth of antagonist and disintegration of test organism (Table 16).

The results indicated the antagonism of *T. koningii* against *R. solani* by overgrowth, disintegration and die-back of hyphae.

Trichoderma longibrachiatum

P. myriotylum

The antagonist and test organism were placed on the same day in dual culture as they had almost the same growth rate. Initially both the organisms maintained their respective growth rates in monoculture and dual culture. On the second day the antagonist had a growth of 58 mm in mono and dual culture, but the test organism had only 32 mm in dual culture as against 82 mm in monoculture (Teble 17).

The antagonist contacted the test organism and inhibited its growth. On the third day, the antagonist overgrew the test organism and caused die-back, disintegration and reduced growth of test organism by 8 mm   On subsequent days the antagonist grew at reduced rate over the test ogranism and finally reached a growth of 88 mm as against only 2 mm growth of the test organism (Table 17). Just like

the other two species of *Trichoderma*, *T. longibrachiatum* also showed clear antagonistic property against *P. myriotylum* by overgrowth resulting in die-back and disintegration of test organism.

P. palmivora

The test organism was inoculated three days prior to antagonist, because of comparitively slow growth of the former. On the first day, both the organisms had same growth rate in mono and dual culture. On the second day, the antagonist grew at the same rate as in monoculture, but test organism had very slow growth rate, and contact of the organisms was established. On third day, the antagonist overgrew the test organism by 12 mm and the overgrown region of test organism was completely disintegrated. The antagonist further grew over the test organism diminishing the growth of test organism to 10 mm on fourth day and 2 mm on fifth day (Table 17).

The result indicated the antagonism of *T. longibrachiatum* against *P. palmivora* by overgrowth, disintegration and die-back of hyphae.

R. solani

Both the organisms were inoculated on the same day in dual culture. Initially they showed normal growth rate in dual culture. On second day, antagonist grew at normal rate while test organism showed diminished growth rate. They contacted each other on second day with 58 mm growth for antagonist and 32 mm for test organism (Table 17). On subsequent days antagonist grew at reduced rate over the test organism with growth of the latter being 15 mm, 6 mm, 4 mm, 3 mm, 2 mm and 1 mm on third, fourth, fifth, sixth, seventh and eighth days after inoculation respectively. The region of overgrowth was marked by disintegration and die-back of hyphae of test organism (Table 17, Plate).

The result indicated the antagonistic property of T. longibrachiatum against R. solani by overgrowth, disintegration and die-back of hyphae.

Aspergillus mellеus

P. myriotylum

The test organism was inoculated five days after the antagonist in dual culture because of the slow growth of the letter. Both the organisms grew at same rate in mono and

Plate 5. *Trichoderma longibrachiatum* x *R. solani* in dual culture after eight days of inoculation
(1) Dual culture (2) Control

Plate 6. *Aspergillus niger* x *P. myriotylum* in dual culture after five days
(1) Dual culture (2) Control

Plate 5

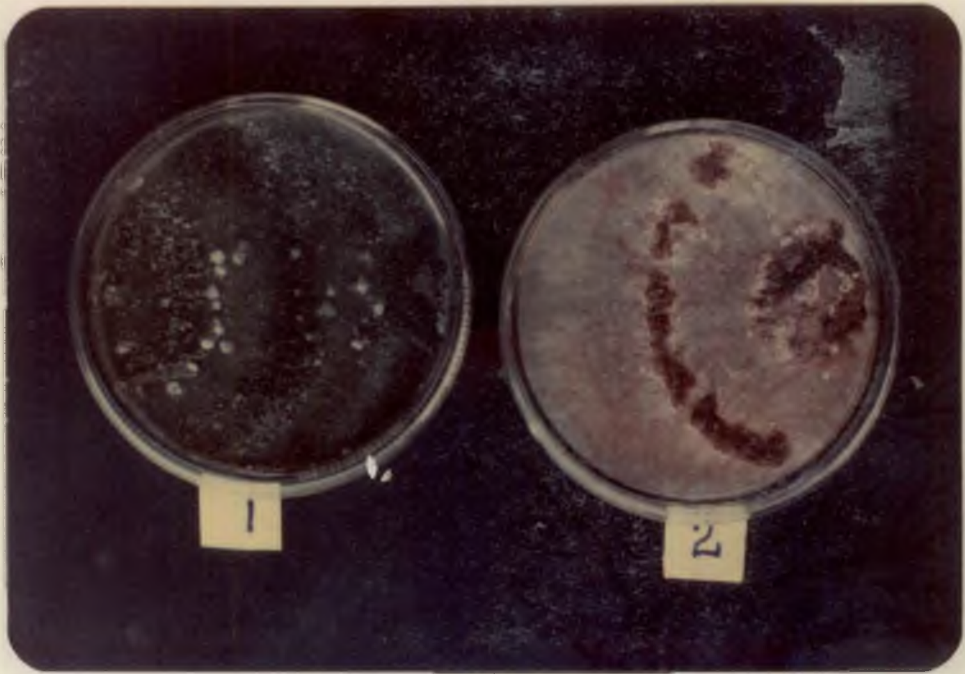
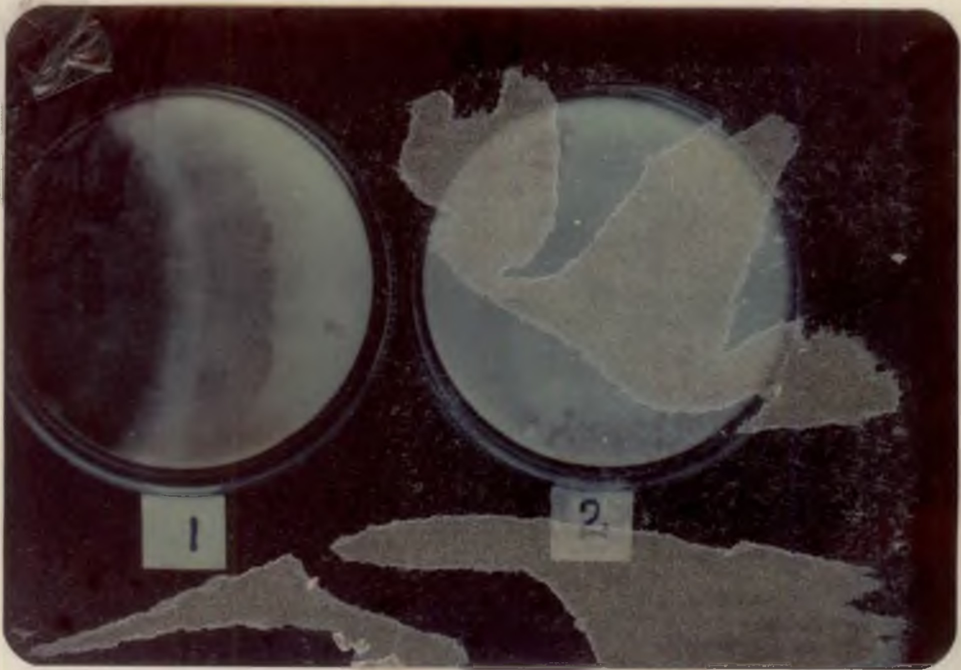


Plate 6



dual culture on the first and second day and showed intermingled overgrowth of the two organisms. On the third day, slightly reduced growth rate was recorded with 20 mm for antagonist and 82 mm for test organism and there was no further growth (Table 18). The result indicated free intermingling and overgrowth of the two organisms without any disintegration or die-back.

P. palmivora

The test organism was inoculated two days after the antagonist due to slow growth of the latter. In dual culture growth rate of test organism and antagonist was the same as that of mono culture without showing any inhibition (Table 18).

The result showed mere overgrowth and intermingling without disintegration or die-back.

R. solani

The test organism was inoculated five days after antagonist in dual culture because of slow growth of the latter. During first three days both antagonist and test organism had same growth in the mono and dual culture, but from fifth day, a decreased growth rate was observed in both the organisms in dual culture. Both antagonist and test

organism contacted each other and no further growth was observed (Table 18).

The result indicated that the antagonist and the test organism showed a character of mutual inhibition on contact in dual culture.

Aspergillus niger

P. myriotylum

The test organism was inoculated two days after antagonist in the dual culture because of comparatively slow growth of the latter. Initially both the organisms had the same growth in mono and dual culture. On the second day onwards, antagonist grew at its normal rate in the dual culture whereas the test organism recorded more than fifty per cent reduction in the growth viz., 40 mm as against 82 mm in mono culture on second day and 30 mm against 90 mm in monoculture on third day (Table 19). Antagonist still grew at the normal rate while disintegration at the growing point of test organism was noticed @ 10 mm per day on fourth and fifth day and after that it remained constant.

The antagonist showed clear inhibition of test organism at a distance, with a clear zone of 15 mm in the culture dish

(Plate 6). Thereafter, the antagonist grew further and disintegration of test organism was observed (Plate 7).

The result indicated the antibiotic property of *A. niger* against *P. myriotylum*. However, there was no total destruction of the test organism.

P. palmivora

The test organism was inoculated on the same day in dual culture because of more or less same growth rate. The growth of antagonist was 10 mm on the first day and test organism attained 5 mm (Table 19). Upto fifth day, both antagonist and test organism were having the same growth rate in mono and dual cultures attaining a growth of 44 mm and 41 mm respectively. On the sixth day the antagonist grew normally, reaching 49 mm while growth of test organism reduced from 41 mm to 30 mm causing disintegration from the growing tip (Plate 8). On subsequent days antagonist grew further, but the disintegration of test organism continued upto eighth day diminishing its growth to only 15 mm (Table 19). It showed the inhibition and disintegration of test organism without contact of antagonist.

The result indicated definite antagonistic property of *A. niger* against *P. palmivora*, by production of biotic

Plate 7. *Aspergillus niger* x *P. myriotylum* in dual culture after eight days

Plate 8. *A. niger* x *Phytophthora palmivora* in dual culture on the sixth day

Plate 7

Plate 8

substance which inhibit and disintegrate the growth of test organism.

R. solani

The test organism was inoculated one day after antagonist. Upto two days both the organisms had same growth in mono and dual cultures. Later the antagonist grew 40 mm in mono and dual culture on the third day while test organism showed a reduced growth of 48 mm in dual culture as against 65 mm in monoculture (Table 19). The antagonist inhibited the test organism at a distance. Antagonist continued its growth at the same rate upto the ninth day and no further growth after nine days (plate 9). Disintegration of growth of test organism from the tip started on fourth day onwards @ 3 - 5 mm per day and it was @ 10 mm on eighth day recording the growth of 22 mm and remained constant thereafter (Table 19) (Plate 10).

The results clearly indicated inhibition of growth of R. solani by A. niger at a distance and further disintegration of hyphae.

Aspergillus sydowii

P. myriotylum

Because of the slow growth rate of the antagonist, the test organism was inoculated six days after antagonist in the

Plate 9. *Aspergillus niger* x *R. solani* in dual culture after nine days
(1) Dual culture (2) Control

Plate 10. *A. niger* x *R. solani* in dual culture after eight days

Plate 9

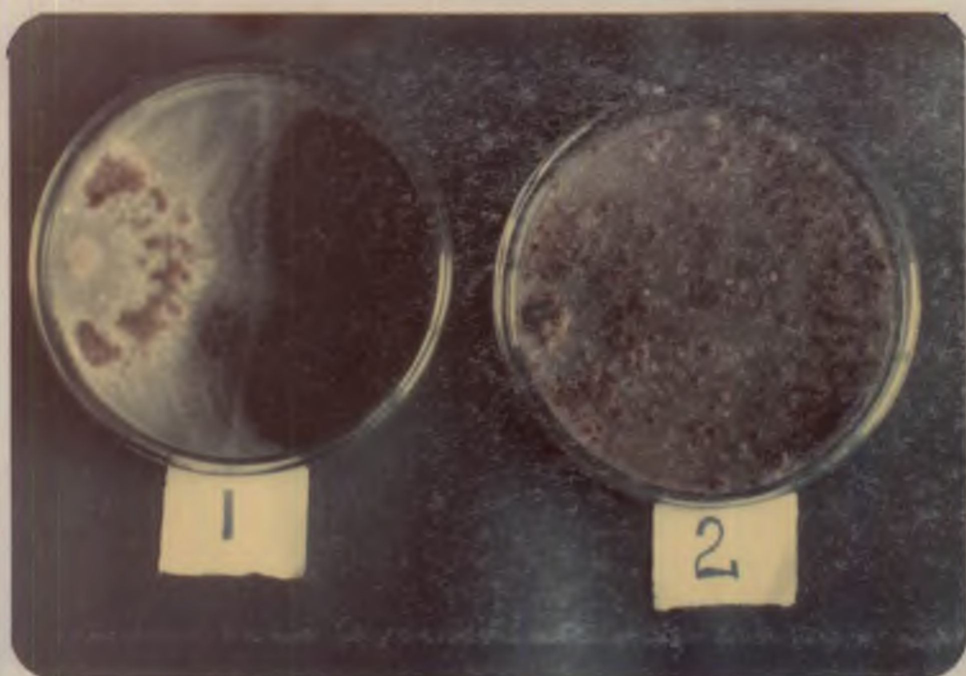
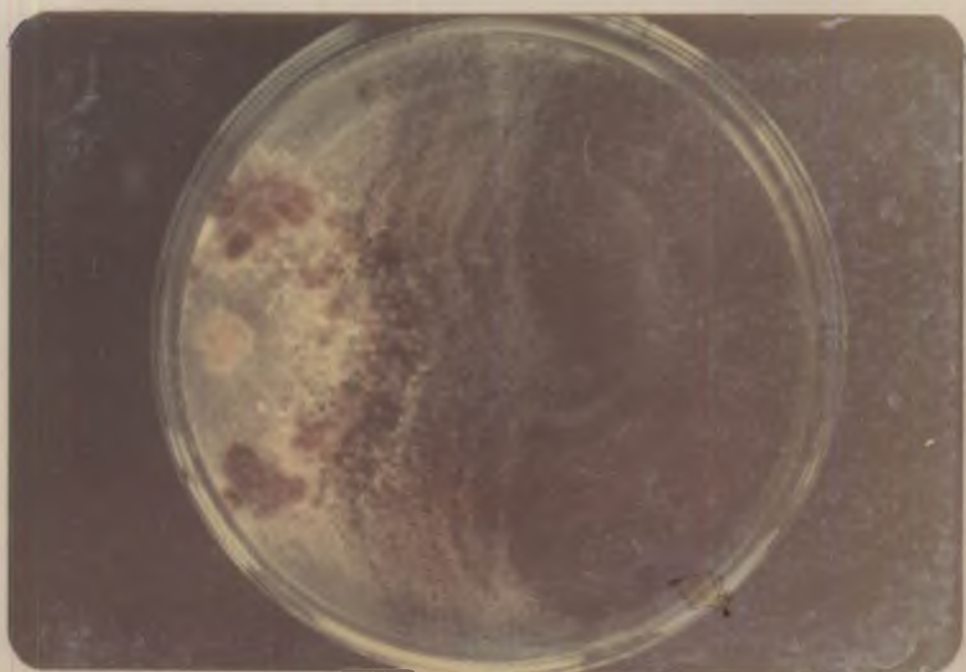


Plate 10



dual culture. The antagonist and test organism were having normal growth in the dual culture upto the third day when test organism completed its growth in the dish. Latter the antagonist grew in the normal rate over the test organism without its disintegration and die-back (Table 20).

The results indicated mutual intermingling by over-growing of the antagonist and test organism and absence of antagonism.

P. palmivora

The test organism was inoculated in the same day as the antagonist because of the same growth rate. The antagonist and test organism grew at normal rate in the dual culture as in monoculture upto eighth day. On eighth day intermingling and overgrowth were observed and further no more growth of either of the organisms was noticed in dual culture though their increase in growth was visible in monoculture (Table 20).

The results indicated no antagonistic property of A. sydowii against P. palmivora and showed only intermingling and overgrowth.

R. solani

The test organism was inoculated four days after antagonist due to slow rate of growth of the antagonist. The antagonist and test organism had normal rate of growth during first two days in dual culture. Later, the antagonist grew at normal rate while test organism at a reduced rate by 10 mm on third day (Table 20). By the time hyphae of both the organisms met each other. Thereafter antagonist continued its normal growth causing disintegration of test organism and reached 80 mm on eighth day leaving only 10 mm of the test organism and thereafter both remained constant (Table 20).

Data revealed that *S. sydowii* can inhibit and disintegrate the growth of *R. solani* by overgrowing and showed its antagonistic property.

Aspergillus terreus

P. myriotylum

The test organism was inoculated five days after the antagonist. The growth rate of both the organisms was the same in monoculture and dual culture. On the second day, the test organism overgrew the antagonist and on the third

day it covered the petridish when the antagonist grew at a reduced rate being 18 mm and 21 mm respectively on second and third days. The antagonist had the same reduced rate of growth in the monoculture also. Even after the complete occupation of test organism the antagonist grew further as in the monoculture and it was 33 mm on eighth day (Table 21).

The result revealed that in dual culture, *A. terreus* and *P. myriotylum* can grow by intermingling without any interference, showing no antagonistic property.

P. palmivora

The test organism was inoculated five days after the antagonist in the dual culture. There was no difference in growth of the two organisms in monoculture and dual culture through out the period of their growth. The test organism overgrew the antagonist on eighth day and remained constant in mono and dual culture (Table 21). The antagonist and test organism grew freely without any interference and overgrowth was observed only very late due to the slow growth rate of both the organisms.

The result clearly showed that *A. terreus* had no antagonism against *P. palmivora*.

R. solani

The test organism was inoculated five days after the antagonist, due to slow growth of the latter. The antagonist and test organism initially grew at same rate in mono and dual cultures. On the second day, while the antagonist had its normal growth, test organism showed a lesser growth rate. From third day onwards, it did not grow further (Table 11). The antagonist ceased its growth in dual culture after the second day onwards. But in monoculture, both the organisms were having further growth after third day.

The result revealed that these two organisms were having mutual inhibition and a 12 mm clear inhibition zone was created (Plate 11). This indicated that the biotic substances produced by these two organisms were inhibitory to each other.

Aspergillus versicolor

P. myriotylum

The test organism was inoculated one day after the antagonist in dual culture due to the slow growth of the latter. The growth rate of both the organisms on the first day was the same in mono and dual culture. While the

Plate 11. *Aspergillus terreus* x *R. solani* in dual culture after 5 days

Plate 12. *Penicillium citrinum* x *P. myriotylum* in dual culture on the tenth day
(1) Dual culture (2) Control

Plate 11

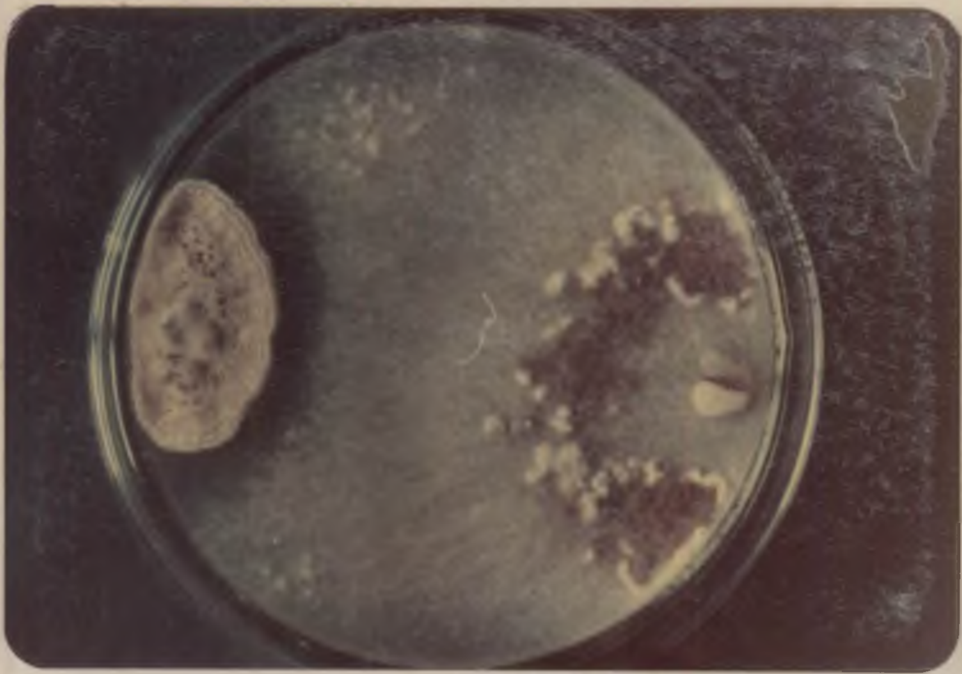


Plate 12

antagonist grew at normal rate in dual culture on second day, test organism grew only 44 mm as against 88 mm in the monoculture (Table 22). The antagonist grew 42 mm and 45 mm in mono and dual culture on the third and fourth day respectively, whereas test organism grew only 45 mm in dual culture as against 90 mm in monoculture. On subsequent days, the growth of both organisms remained constant in the dual culture.

The result indicated mutual inhibition on contact and a clear demarcation between the growth of antagonist and test organism was visible.

P. palmivora

The test organism was inoculated two days after the antagonist in dual culture due to the slow growth of the latter. The antagonist and test organism showed same growth rate in mono and dual culture. But on fourth day, both the organisms came in contact, and overgrew each other without any interference (Table 22).

The result revealed the reaction of overgrowth and mutual intermingling without any antagonistic effect.

R. solani

Test organism and antagonist were inoculated on same day in dual culture because of the same growth rate. The first and second day growth rate was same for both the organisms in mono and dual culture. On the third day, these organisms came in contact and no further growth for either of the organisms in dual culture was noticed (Table 22).

The result revealed that these two organisms were having mutual inhibition on contact and a demarcation can be seen at the point of contact.

Penicillium citrinum

P. myriotylum

The test organism was inoculated five days after the antagonist due to the slow growth of the latter. Initially the growth of both the organisms was same in mono and dual culture. On the second day, test organism reached 82 mm in monoculture and 60 mm in dual culture while antagonist grew 18 mm in both. There was no further growth of test organism opposite to the antagonist, but a lateral growth of almost about 80 mm was observed. The antagonist grew 24 mm in monoculture and 22 mm in dual culture on fifth day. There

was no further growth in dual culture, but reached 38 mm in monoculture on the tenth day. On fourth day after inoculation in dual culture, the test organism started die-back and disintegration @ 5 mm at the middle portion and it continued till the tenth day, reducing it to 25 mm in the central region (Table 23). The disintegration of the test organism by producing lesions was 60 mm width in centre. Dense growth of test organism was observed towards the periphery of petridish and thinner towards the middle and a clear lesion was seen at a distance of 15 mm from the periphery on both sides (Plate 12, 13). This indicated the production of powerful antifungal biotic substance after eight days of growth of the antagonist. The antimetabolite diffused in the media and caused death of test organisms in the central region where it diffused first.

P. citrinum produced powerful antifungal metabolites which inhibited P. myriotylum at a distance and caused destruction.

P. palmivora

In dual culture, test organism was inoculated five days after the antagonist due to slow growth of latter. The antagonist and test organism grew at the same rate upto the

Plate 13. *Penicillium citrinum* x *P. myriotylum* in dual culture on the tenth day (enlarged)

Plate 14. *Pencillium citrinum* x *P. palmivora* in dual culture on the tenth day

Plate 13

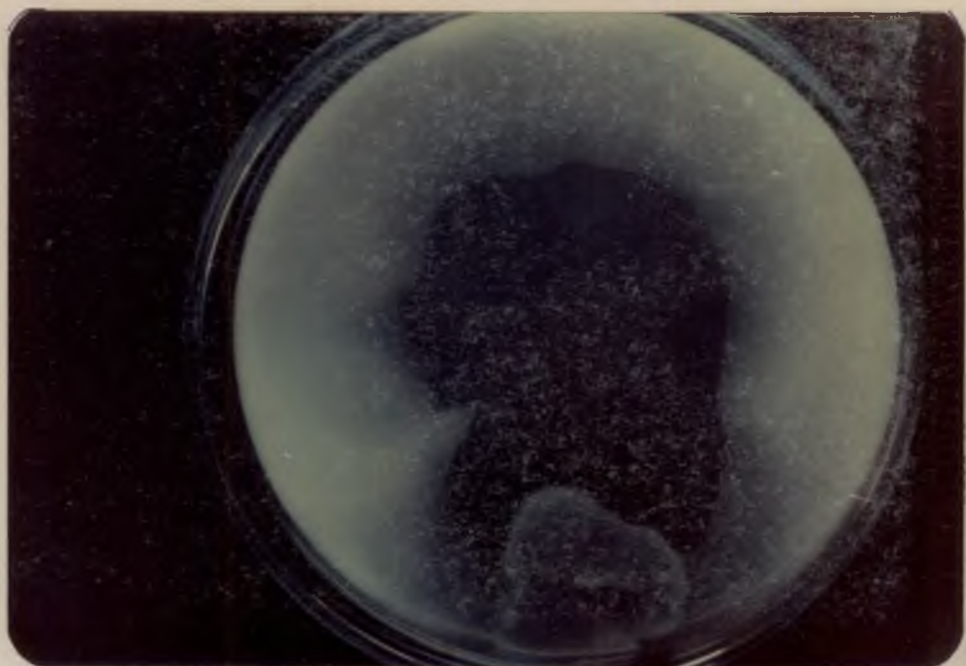


Plate 14

seventh day in monoculture and dual culture. On eighth day onwards there was no growth for the antagonist while only 2 mm growth was observed in the case of test organism (Table 23). After reaching 29 mm growth for antagonist and 50 mm for test organism, a clear inhibition zone of 11 mm in the centre was observed (Plate 14).

The observation clearly indicated that P. citrinum produced very powerful antifungal biotic substances which inhibited the growth of P. palmivora, at a distance.

R. solani

In dual culture, test organism was inoculated five days after the antagonist due to slow growth of the latter. Both organisms had the same growth rate upto the third day in mono and dual culture and attained 20 mm growth for antagonist and 65 mm for test organism. On the fourth day, the antagonist grew 2 mm more in mono and dual culture and both the organisms thereafter remained constant in dual culture (Table 23). A clear inhibition zone of 3 mm was developed in between the two organisms in dual culture.

The results indicated that P. citrinum produced some toxic metabolites which inhibited the growth of R. solani at a distance.

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Penicillium simplicissimum

P. myriotylum

In dual culture, the test organism was inoculated five days after the antagonist due to slow growth of the latter. On the first day, both the antagonist and the test organism had normal growth in mono and dual culture. On the second day, the antagonist showed same growth in mono and dual culture whereas the test organism grew only 60 mm in dual culture as against 81 mm in monoculture. Antagonist and test organism did not grow further on the third day but a 7 mm clear zone of inhibition was seen in dual culture (Plate 15). From fourth day onwards, inhibition of growth with die-back and disintegration of hyphae of the test organism @ 10 mm per day was observed upto the seventh day, recording a growth of 20 mm and remained constant thereafter (Table 24). The die-back and disintegration was not observed in the periphery of the petridish (Plate 16, 17).

The result indicated the production of antibiotic substance by *P. simplicissimum* diffusing into the media and causing die-back and disintegration of hyphae of *P. myriotylum*.

Plate 15. *Penicillium simplicissimum* x *P. myriotylum* in dual culture on third day

Plate 16. *Penicillium simplicissimum* x *P. myriotylum* in dual culture after seven days
(1) Dual culture (2) Control

Plate 15

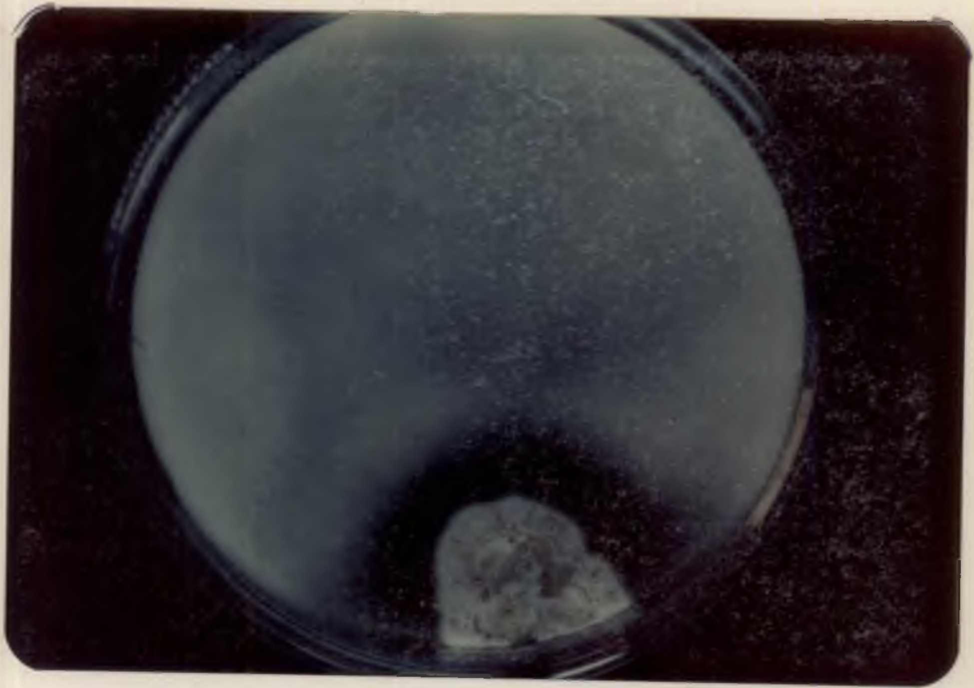
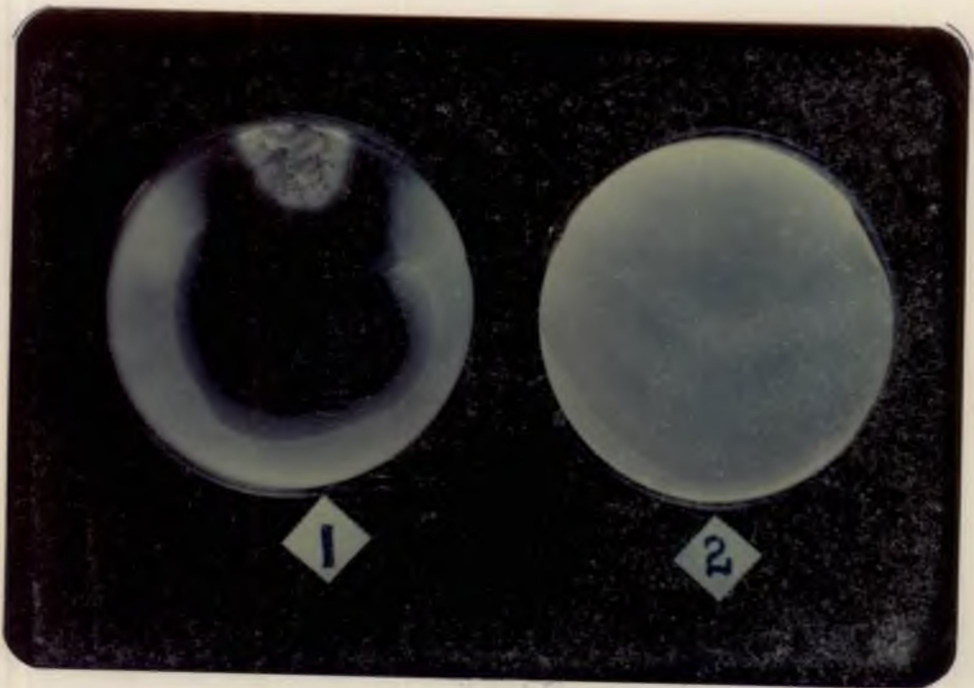


Plate 16



P. palmivora

In dual culture, test organism was inoculated five days after antagonist due to slow growth of the latter. The growth rate of antagonist and test organism was the same in mono and dual culture for the first three days. On the third day, antagonist reached a growth of 23 mm and remained constant thereafter (Table 24). But the test organism showed almost the same growth rate both in mono and dual culture upto eighth day and reached 60 mm and remained constant. The mycelium of test organism in dual culture was very sparse and a 7mm inhibition zone between test organism and antagonist was observed (Plate 18).

The result indicated that the test organism and antagonist showed mutual inhibition at a distance.

R. solani

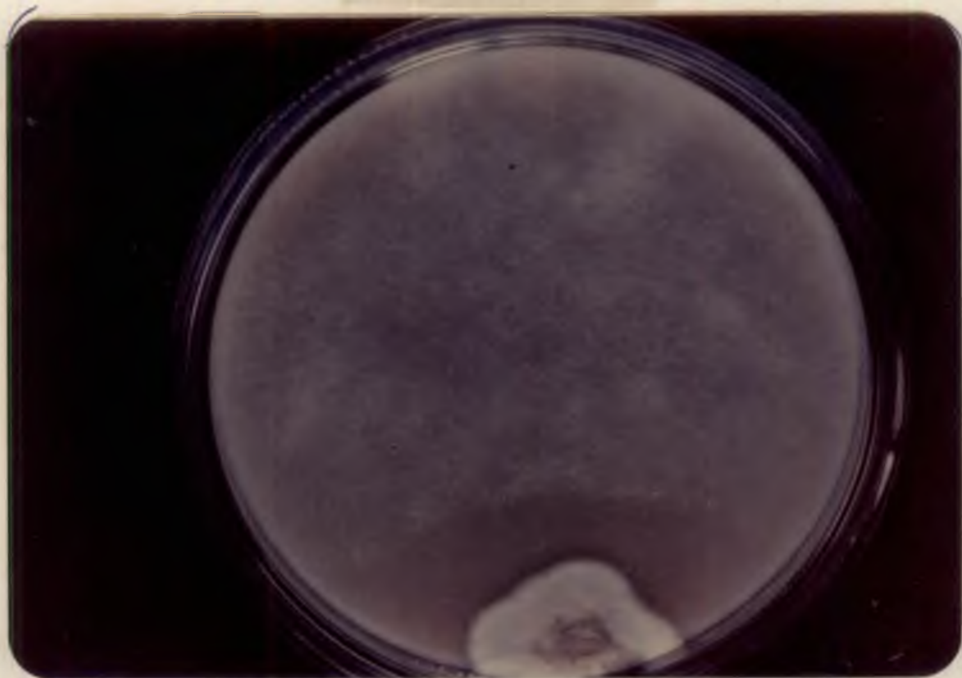
The test organism was inoculated five days after the antagonist in the duel culture due to slow growth of the latter. The test organism and the antagonist grew at more or less the same rate for the first two days in monoculture and dual culture. On the third and fourth day, the antagonist grew almost at the same rate attaining 28 mm and remained

Plate 17. *Penicillium simplicissimum* x *P. myriotylum* in dual culture after seven days (enlarged)

Plate 18. *Penicillium simplicissimum* x *P. palmivora* in dual culture after eight days

Plate 17

Plate 18



constant in dual culture while its growth reached 42 mm on the tenth day in monoculture. In duel culture, test organism grew at normal rate initially but on the third day 10 mm reduction of growth wes noticed when compared to the growth in monoculture, thus reached 55 mm and remained constant (Table 24). In dual culture a clear inhibition zone of 7 mm between the test organism and antagonist with sparse growth of hyphae of test organism towards the end was observed. The production of sclerotia by the test organism in the dual culture was very scanty when compared to mono culture (Plate 19 ~~10~~).

The result indicated that test organism and antagonist showed mutual inhibition at a distance.

Paecilomyces lilacinus

P. myriotylum

In dual culture, test organism was inoculated five days after the antagonist because of slow growth of the latter. Initially, the antagonist and test organism had the same growth in mono and dual culture. On the second day, while the antagonist had normal growth rate in mono and dual culture, test organism showed reduced growth of 50 mm in

Plate 19. *Penicillium simplicissimum* x *R. solani* in dual culture on tenth day
(1) Control (2) Dual culture

Plate 20. *Penicillium simplicissimum* x *R. solani* in dual culture on the tenth day (enlarged)

Plate 19

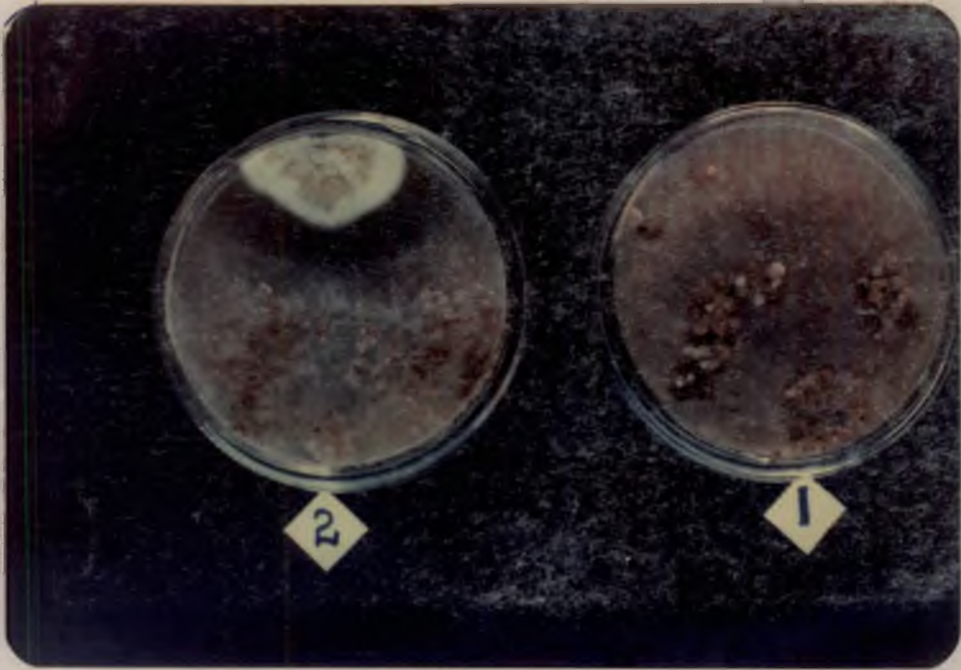


Plate 20

dual culture as against 82 mm in monoculture (Table 25). on the third day, the antagonist grew almost at the normal rate and came in contact with test organism, which did not grew further and growth of both the organisms in dual culture remained constant.

The data clearly revealed that after establishment of the antagonist in dual culture, the growth rate of the test organism reduced considerably and on contact, the test organism and antagonist showed mutual inhibition.

P. palmivora

The test organism was inoculated five days after the antagonist in dual culture due to slow growth of the latter. Till the seventh day, the antagonist and the test organism showed almost the same growth rate in mono and dual culture with mutual intermingling in dual culture from the fifth day onwards. The test organism showed no further growth after the seventh day (Table 25).

The data showed the character of mutual intermingling of *P. lilacinus* and *P. palmivora* in dual culture without any antagonistic property.

R. solani

In dual culture, that organism was inoculated five days after the antagonist due to slow growth of the latter. The growth of antagonist and test organism in mono and dual culture for seven days was almost at the same rate. On the third day onwards, both the organisms showed intermingled growth character and continued till test organism completely covered the petridish (Table 25).

The result indicated that the antagonist and the test organism freely grew in dual culture as in the case of monoculture and showed intermingled growth character in dual culture.

Talaromyces wortmanni

P. Myriotylum

The test organism was inoculated five days after the antagonist in dual culture due to the slow growth of the latter. On the first day, the antagonist and the test organism were having almost similar growth in mono and dual culture. But on the second day, the antagonist grew normally in mono and dual culture whereas the test organism showed a reduction of 17 mm in its growth rate in dual culture

(Table 26). On the third day, antagonist further grew and contacted the test organism which was stationary in its growth. There was no further growth for both the organisms in dual culture after their mutual contact (Table 26).

After establishment of antagonist in dual culture, the test organism showed a slow growth rate and on contact they exhibited mutual inhibition.

P. palmivora

In dual culture, the test organism was inoculated five days after the antagonist due to the slow growth of the latter. Upto the seventh day the antagonist and test organism had almost similar growth rate, in mono and dual culture. On the eighth day, the test organism showed a reduced growth of 55 mm in dual culture as against 63 mm in monoculture and on ninth day, contact with antagonist was established. Later there was no further growth for either of the organisms (Table 26).

The observations revealed that the antagonist and test organism were having mutual inhibition on contact.

R. solani

In dual culture, test organism was inoculated five days after the antagonist due to slow growth of the latter.

Initially, antagonist and test organism showed the same growth rate in mono and dual culture. On the fourth day, the antagonist and test organism grew 20 mm and 70 mm respectively and came in contact in dual culture. Later, there was no further growth for either of the organisms (Table 26).

The result clearly revealed that T. wortmanni and R. solani were having mutual inhibition on contact.

Microascus cinereus

P. myriotylum

In dual culture, test organism was inoculated five days after the antagonist due to fast growing nature of the former. On the first day, both the organisms showed similar initial growth character in mono and dual culture. But on the second day, the antagonist grew at normal rate whereas test organism had a reduced growth rate in dual culture, when compared to monoculture and established contact between them in duel culture. The growth of antagonist remained stationary on subsequent days but test organism continued to overgrow till the fourth day and further both the organisms remained stationary in dual culture (Table 27).

The data revealed that the antagonistic organism showed inhibition of its growth on contact with test organism, but the test organism overgrew in a limited area over the antagonist.

P. palmivora

The test organism was inoculated five days after the antagonist in dual culture due to slow growth of the latter. The antagonist and test organism grew almost at the same rate in mono and dual culture and contacted each other on the seventh day in dual culture. They continued their growth by intermingling in dual culture with almost the same rate of growth in monoculture (Table 27).

The result showed that both the organisms could grow very freely in dual culture with intermingling growth character.

R. solani

The test organism was inoculated five days after antagonist in dual culture due to slow growth of the latter. Both the organisms showed the same rate of growth for the first two days in mono and dual culture. For the next two

days, the test organism grew at a reduced rate and on the fourth day they contacted each other, and further, both of them ceased to grow (Table 27).

The result showed mutual inhibition of the test organism and the antagonist on their contact.

Fusarium oxysporum

P. myriotylum

The test organism was inoculated seven days after antagonist in dual culture, due to the slow growth rate of the latter. On the first two days, the growth in dual culture for both the antagonist and test organism was the same as that in monoculture. At that time they started intermingled growth and on the third day also they further grew by intermingling. Later on, tho antagonist grew over the test organism and the test organism became stationary in dual culture (Table 28).

The result indicated that there was no antagonistic property for these organisms and they showed intermingled growth character in dual culture.

P. palmivora

In dual culture, test organism was inoculated one day after the antagonist due to slightly reduced rate of growth of the latter. The antagonist and test organism grew at the same rate in mono and dual culture, throughout the period under observation. On the eighth day onwards they showed mutual intermingled growth (Table 28).

The data revealed that both these organisms could freely grow in dual culture by intermingling, without any antagonism.

R. solani

In dual culture, test organism was inoculated four days after the antagonist, due to slow growth of the latter. The first two days, the antagonist and test organism showed same growth rate in mono and dual culture. On the third day, while the antagonist showed normal rate of growth, the growth rate of test organism was seen reduced considerably. On the fourth day, the test organism and antagonist came in contact and no further growth for both of them was observed after the fourth day (Table 28).

The result revealed that both the organisms inhibited on contact.

Streptomyces sp. (with straight sporophores)

P. myriotylum

In dual culture, the test organism was inoculated seven days after the antagonist due to slow growth rate of the latter. The growth of test organism and antagonist was same in mono and dual culture on the first day after inoculation, but rate of growth of test organism was reduced in dual culture on the second day (Table 29). On the third day, the test organism continued its growth at a reduced rate and the antagonist and test organism came in contact. The flattening of hyphae of test organism on the sides nearest to the antagonist was observed in an area of 12 mm (Table 29, Plate 21).

Streptomyces sp. (with straight sporophores) was not having any strong antagonistic property but on contact it showed adverse effects on growth of the test organism by way of flattening of hyphae on the sides nearest to the antagonist.

P. palmivora

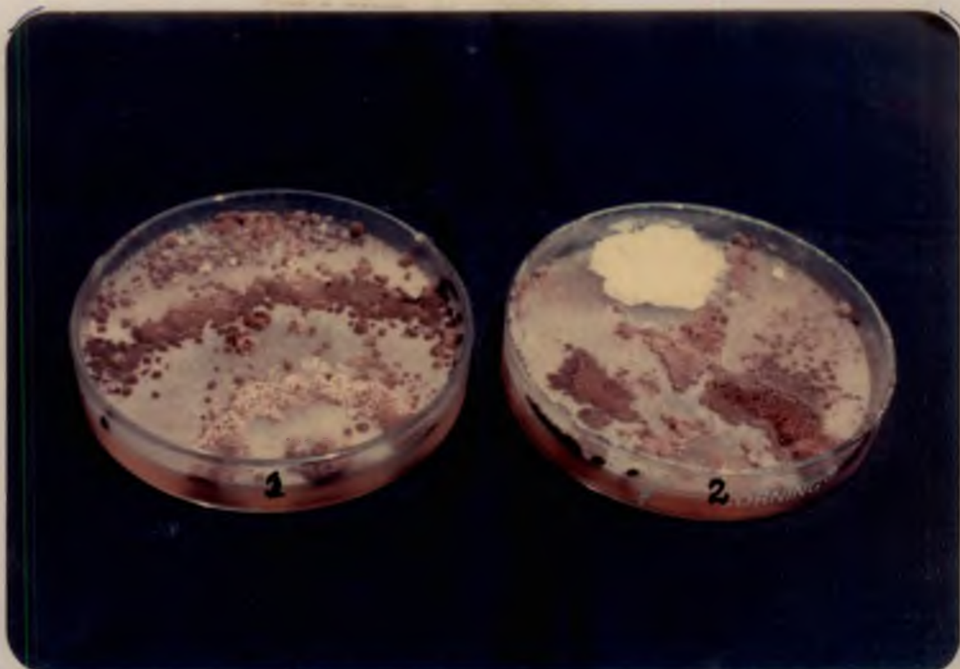
The test organism was inoculated seven days after the antagonist, in the dual culture due to slow growth of the latter. The antagonist and test organism grew almost at the same rate throughout the observation period in mono and dual

Plate 21. *Streptomyces* sp. (with straight sporophores) x *P. myriotylum* in dual culture on the seventh day
(1) Dual culture (2) Control

Plate 22. *Streptomyces* sp. (with straight sporophores) x *R. solani* in dual culture on the ninth day
(1) Control (2) Dual culture

Plate 21

Plate 22



culture. On the seventh day, the two organisms contacted in dual culture and thereafter they grew by intermingling without any adverse effect (Table 29).

The date revealed that the two organisms could grew together in dual culture without any adverse effect showing mutual intermingling.

R. solani

The test organism was inoculated seven days after the antagonist, in the dual culture due to slow growth of the latter. For the first two days, the growth of both the organisms in dual and monoculture was almost the same. Thereafter upto fifth day they grew at a reduced rate, when compared to monoculture. On the fifth day, the antagonist and test organism came in contact and no further growth for both of them wea seen (Table 29, Plate 22).

The observation clearly indicated thet the *Streptomyces* sp. (with straight sporophores) and *R. solani* were having mutual inhibition on contact.

Streptomyces sp.
(with flexuous sporophores)

P. myriotylum

The test organism was inoculated seven days after the antagonist in dual culture. The growth of both the organisms

in dual culture on the first day was found to be normal, but on the second day the test organism showed a reduced growth. On the third day also the reduced growth rate of test organism was noticed in dual culture. At this stage, antagonist and test organism came in contact and no further growth for both them (Table 30).

The data revealed that the *Streptomyces* sp. (with flexuous sporophores) and *P. myriotylum* expressed the character of mutual inhibition on contact.

P. palmivora

In dual culture, test organism was inoculated seven days after the antagonist due to the slow growth of the latter. Throughout the period under observation, both the organisms showed same growth rate in mono and dual culture. On the sixth day, these organisms came in contact with each other in dual culture. Thereafter they freely grew as in case of monoculture and intermingled each other (Table 30).

The growth character of these two organisms clearly showed that they had no antagonistic effect, but can grow by intermingling without any adverse effect.

R. solani

The test organism in dual culture was inoculated seven days after the antagonist. For the first two days, growth of both organisms in mono and dual culture was the same. But on the second day, the test organism showed reduced growth and on the fourth day they came in contact and no further growth for either of the organisms was observed in dual culture (Table 30).

The data revealed that *Streptomyces* sp. (with flexuous sporophores) and *R. solani* in dual culture showed the character of mutual inhibition on contact.

Streptomyces sp.
(with fascicled sporophores)

P. myriotylum

In dual culture, the test organism was inoculated seven days after the antagonist due to slow growth of the latter. On the first day, growth of both the organisms was the same in dual and monoculture but on the second day the test organism showed a reduced growth rate. On the third day both the organisms came in contact and there was no further growth for either of them in dual culture (Table 31).

The above observation clearly showed that *Streptomyces* sp. (with fascicled sporophores) and *P. myriotylum* inhibited each other on contact.

P. palmivora

In dual culture, the test organism was inoculated seven days after the antagonist due to slow growth of the latter. Throughout the period of observation, the two organisms grew at the same rate in mono and dual culture. On the seventh day, both the organisms came in contact and further grew over each other in dual culture at the same rate of growth in monoculture (Table 31).

The result indicated that these two organisms could grew without any interference in dual culture, showing the character of mutual intermingling.

R. solani

In dual culture the test organism was inoculated seven days after the antagonist due to slow growth of the latter. For the first two days, both the organisms grew at same rate in mono and dual culture and on third day, test organism showed a reduced growth rate. At this stage, the two organisms came in contact, and there was no further growth

for either of the organisms in dual culture (Table 31).

The data revealed that *Streptomyces* sp. (with fascicled sporophores) and *R. solani* mutually inhibited their growth on contact in dual culture.

Bacillus subtilis

P. myriotylum

The test organism and the antagonist were having almost same growth rates and thus inoculated on the same day in dual culture. On the first day, both the organisms grew 45 mm each in dual culture but the growing tip of the test organism had a set back eventhough its rate of growth in dual culture was slightly more than that of monoculture (Plate 23). On the second day, the antagonist grew further and disintegrated the growth of test organism and reduced to 30 mm and occupied the rest of the space in the petridish (Plate 24). The same growth pattern of the antagonist and test organism was continued on the next day also and then the growth size of test organism was diminished to 10 mm and rest occupied by antagonist. Thereafter, growth of both the organism remained stationary (Table 32)(Plate 25).

Plate 23. *Bacillus subtilis* x *P. myriotylum* in dual culture after one day

Plate 24. *Bacillus subtilis* x *P. myriotylum* in dual culture on the second day

Plate 23

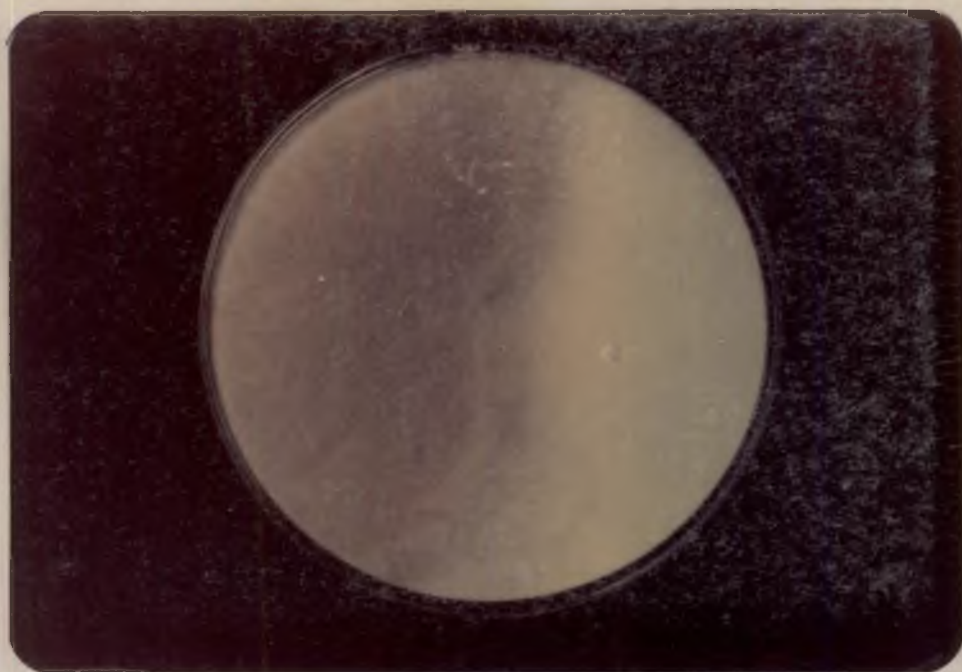


Plate 24

The result indicated disintegration and die-back of *P. myriotylum* by *B. subtilis* in dual culture when the latter continued its growth.

P. palmivora

Due to the slow growth of the test organism, it was inoculated five days prior to the antagonist in dual culture. The test organism grew at normal rate in mono and dual culture. But in dual culture antagonist grew at considerably reduced rate being 10 mm as against 45 mm in monoculture on the first day and 90 mm on the second day. There was no further growth of the antagoniat and the test organism till the end of the observation (Table 32, Plate 25).

The result revealed that the antagonistic bacteria in the presence of the test organism reduced its own growth rate, while the second day onwards, it completely checked the growth of the test organism and a zone of inhibition developed between the two colonies, showing mutual antagonism. Thus inhibition at a distance and mutual antagonism were the net result.

R. solani

The test organism was inoculated one day prior to antegonist in the dual culture. The test organism had normal

Plate 25. *Bacillus subtilis* x *P. palmivora* in dual culture after five days

Plate 26. *Bacillus* spp. x *R. solani* in dual culture after five days
(1) *Bacillus*-2 (2) *Bacillus subtilis*
(3) *Bacillus*-3 (4) *Bacillus*-1
(5) Control

Plate 25

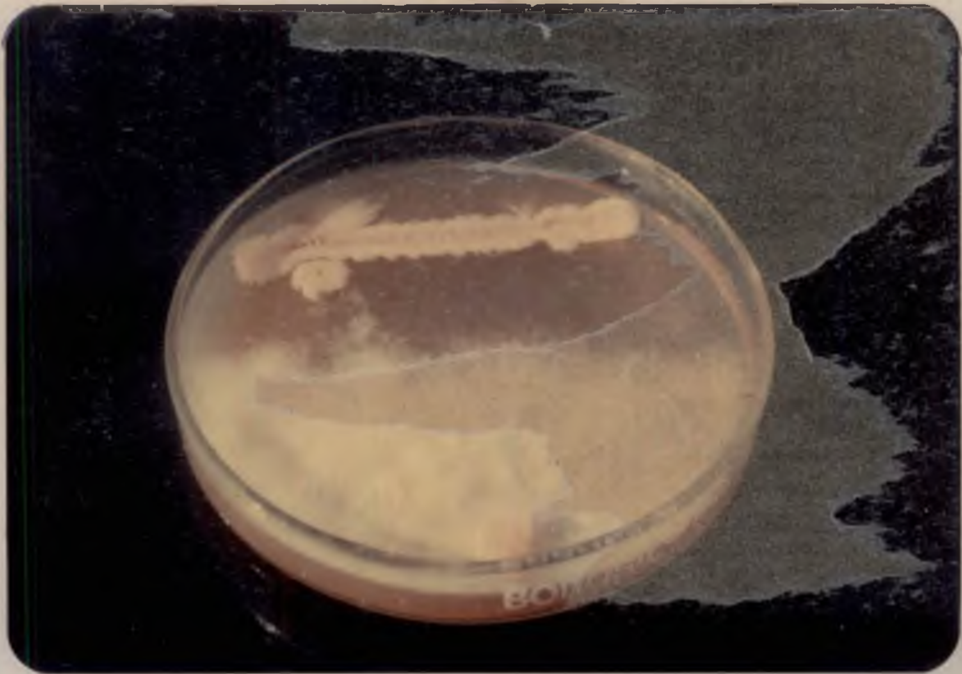
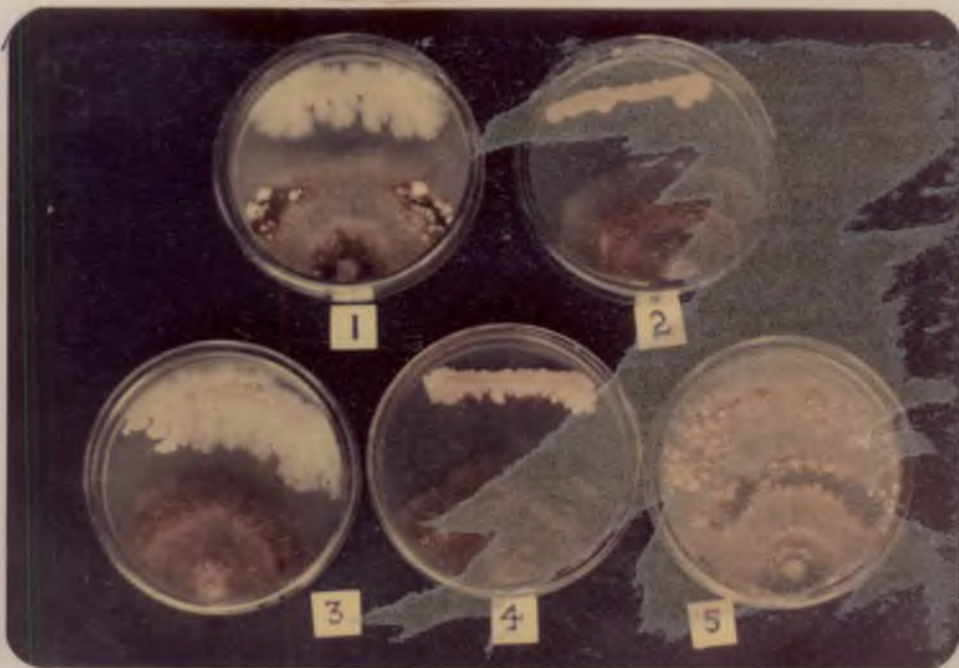


Plate 26



growth rate in dual culture on the first day but antagonist had a very slow growth rate being 45 mm in monoculture and 10 mm in dual culture. On the second day onwards there was no further growth for both of the organisms till the observations were completed (Table 32) (Plate 26).

The data clearly revealed that in dual culture the antagonist and the test organism could inhibit each other and a zone of inhibition developed between them.

<u>Bacillus</u>-1

<u>P. myriotylum</u>

The antagonist and the test organism were inoculated on the same day in dual culture due to almost the same growth of these two organisms. On the first day, both the organisms grew 45 mm in dual culture and contacted each other. On the contacting point, the thickness of mycelium of test organism was found to be reduced. The growth rate in dual culture was slightly higher than in monoculture for the test fungus. But on the second day onwards disintegration of growing tip of hyphae of the test organism was noticed, reducing its growth from 45 mm to 32 mm and further to 10 mm, the remaining space in the petridish being occupied by the antagonist. No

further growth of either of the organisms was observed (Table 33).

The result indicated disintegration and die-back of *P. myriotylum* by the bacterium which was identical to *B. subtilis* and latter continued its growth in dual culture.

P. palmivora

The test organism was inoculated five days prior to the antagonist in dual culture; due to the fast growing character of the letter. On the first day, the antagonist showed a very reduced growth of 10 mm in dual culture as against 45 mm in monoculture. But the test organism grew at normal rate, in mono and dual culture, on the first day. On the second day onwards, there was no further growth for either of the organisms and it continued till the end of observation (Table 33).

The result indicated that the antagonistic bacterium was having reduced growth rate in presence of the test fungi. Later both the organisms inhibited each other and a clear zone of inhibition was developed.

R. solani

The test organism was inoculated one day prior to that of the antegonist in dual culture due to fast growing nature

of the latter. On the first day, the test organism showed a normal growth rate in mono and dual culture, but the antagonist grew at a considerably reduced rate being 10 mm in dual culture as against 45 mm in monoculture. On the second day onwards, there was no further growth for the antagonist and the test organism and it continued till the end of observation (Table 33) (Plate 26).

The result revealed that the antagonistic bacterium and test fungus showed mutual inhibition and a clear zone of inhibition was produced.

Bacillus-2

P. myriotylum

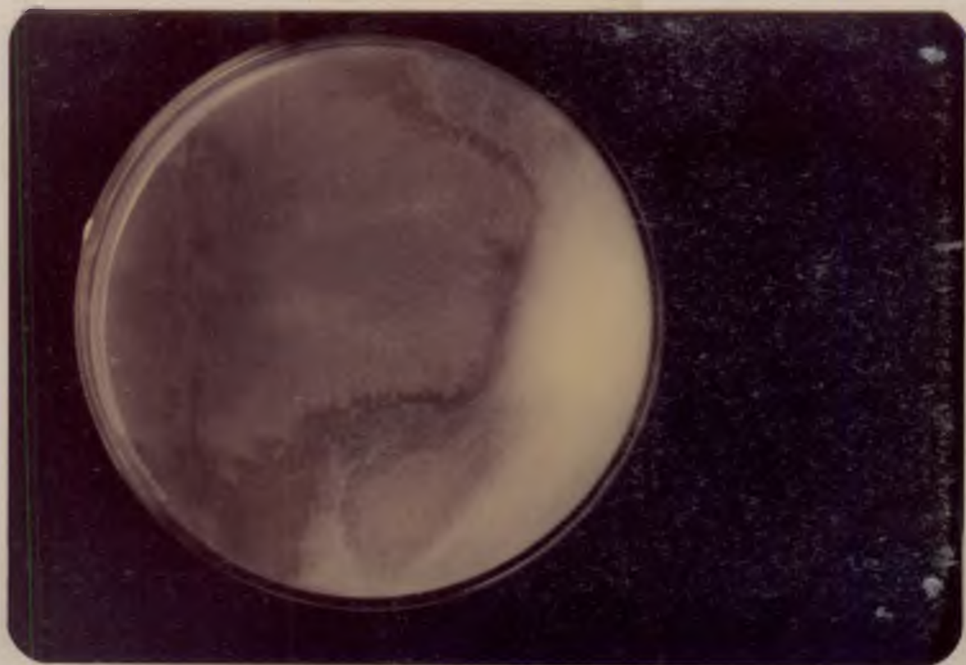
The test organism and the antagonist were inoculated on the same day in dual culture. On the first day, the antagonist grew at normal rate in mono and dual cultures, but test organism showed a faster growth rate in dual culture than in monoculture (Plate 27). On the second day, the antagonist advanced its growth disintegrating the mycelium of test organism and reduced its growth from 50 mm to 15 mm. Later, there was no further growth of either of the organisms in dual culture (Table 34) (Plate 28).

Plate 27. _Bacillus_-2 x _P_. _myriotylum_ on the first day

Plate 28. _Bacillus_-2 x _P_. _myriotylum_ in dual culture after two days

Plate 27

Plate 28



The result indicated disintegration and die-back of *P. myriotylum* by the bacterium which was small celled *Bacilli*. The antagonist continued its growth initially and later ceased to grow.

P. palmivora

The test fungus was inoculated in dual culture, five days before the antagonist, due to the slow growth of the former. On the first day, the test fungus had same rate of growth in mono and dual culture, but the antagonist showed a reduced growth rate in dual culture. On the second day antagonist grew only 12 mm in dual culture as against 82 mm in monoculture while test fungus grew 52 mm in dual culture as against 54 mm in monoculture. Neither of the organisms grew further from the third day onwards (Table 34, Plate 29).

The data revealed mutual antagonism and a zone of inhibition was developed between the two organisms.

R. solani

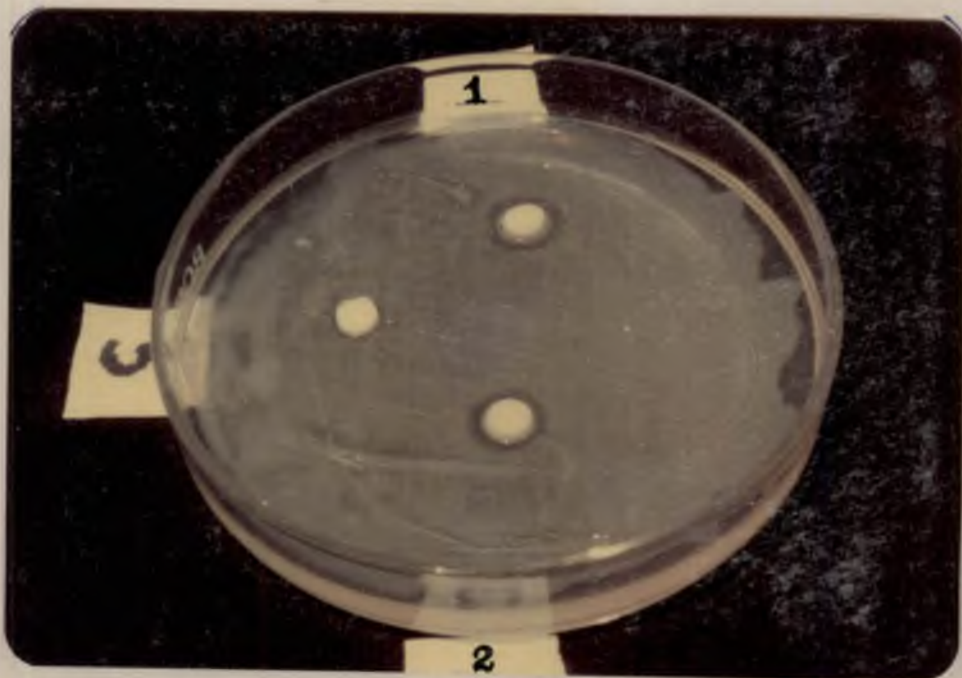
The test organism was inoculated one day prior to the antagonist in the dual culture due to fast growing nature of the latter. On the first day, the test organism showed the same growth rate, in mono and dual culture, but the antagonist

Plate 29. *Bacillus*-2 x *Phytophthora palmivora* after three days

Plate 30. Zone of inhibition by cell free culture filtrates of antagonists in *E. coli*
(1) *T. longibrachiatum*
(2) *A. versicolor*
(3) *A. sydowii*

Plate 29

Plate 30



showed a very reduced growth of 10 mm in dual culture as against 40 mm in monoculture. On the second day, both the test organism and the antagonist showed reduced growth of 55 mm and 20 mm respectively as against 65 mm and 82 mm respectively in monoculture. No further growth for either of the organisms in dual culture was observed (Table 34, Plate 26). Later, no growth was observed for the test fungus and antagonistic bacterium in dual culture (Table 34, Plate 26).

The result indicated mutual inhibition, between the two organisms and development of a clear zone.

Bacillus-3

P. myriotylum

Due to similar growth rate of test organism and the antagonist, they were inoculated on the same day in dual culture. The test organism and the antagonist grew almost at same rate in mono and dual culture on the first day. The antagonist grew at the same rate in mono and dual culture on the second day also but test organism had a considerably reduced growth of 31 mm in dual culture as against 82 mm in monoculture. The reduction in growth of test organism

continued on subsequent days and reached 10 mm on fourth day and remained stationary when its growth in monoculture already completed in the petridish. The antagonist had a reduced growth rate on the third and fourth day in dual culture when compared to monoculture and it attained 80 mm on the fourth day, thus disintegrating the test organism and remained stationary afterwards (Table 35).

The result indicated that die-back and disintegration of the test organism occured on contact with antagonist while the latter continued to grow.

P. palmivora

Due to the slow growth of the test organism, it was inoculated three days prior to the antagonist in dual culture. On the first day, the test organism showed same growth in mono and dual culture but the antagonist grew at a slow rate having 10 mm in dual culture as against 26 mm in monoculture. The growth of the antagonist remained constant during further period of observation. The test organism grew at the same rate in mono and dual culture during the second and third day but with a reduced growth on the fourth day. After reaching 50 mm on the fourth day it remained stationary (Table 35).

The result revealed that the antagonist could not grow at the normal rate and inhibited in the presence of test organism *P. palmivora* and on the fourth day onwards it checked the further growth of test organism and a zone of inhibition developed between the two showing mutual antagonism at a distance.

R. solani

The test organism was inoculated one day prior to the antagonist in dual culture due to fast growth of the latter. On the first day, both the organisms had the same growth rate, in mono and dual culture, but on the second day both of them had reduced growth in dual culture in contrast to their growth in monoculture. The antagonist reached 30 mm and test organism 48 mm growth in dual culture on second day. Thereafter no growth was observed in either of the organisms in dual culture while their growth increased in monoculture (Table 35).

The result revealed mutual inhibition at a distance with a clear zone developed in between the two.

Based on the reactions with the test organisms in dual culture, the antagonists are grouped and presented (Table 36).

Cell free culture filtrate studies

The microorganisms which showed conspicuous antagonistic characters in the dual culture against any of the test organisms were selected and their cell free culture filtrates were employed in poisoned food technique, as described in materials and methods, to estimate the inhibiting actions on test organism. The results are presented (Table 37, Plates 30-33).

The antagonist *T. koningii* inhibited the growth of test organisms by 22 per cent in case of *P. myriotylum*, 20 per cent of *P. palmivora* and 24 per cent of *R. solani* (Table 37).

Cell free culture filtrate of *T. harzianum* gave inhibition percentages 20, 22 and 16 in case of *P. myriotylum*, *P. palmivora* and *R. solani* respectively (Table 37).

T. longibrachiatum inhibited the growth of *P. myriotylum*, *P. palmivora* and *R. solani* at the rate of 24, 13 and 26 per cent respectively (Table 37).

Cell free culture filtrate of *A. sydowii* has shown slight antibiotic property, only against *R. solani* which was 20 per cent (Table 37).

Table 37. Effect of cell free culture filtrates of antagonists on the growth of test organisms (Poison food technique)

Name of antagonist	*Pythium myriotylum*		*Phytophthora palmivora*		*Rhizoctonia solani*	
	Growth measured three days after inoculation (mm)	Per cent inhibition	Growth measured 14 days after inoculation (mm)	Per cent inhibition	Growth measured seven days after inoculation (mm)	Per cent inhibition
1. *T. koningii*	70	22	72	20	68	24
2. *T. harzianum*	72	20	70	22	76	16
3. *T. longibrachiatum*	68	24	78	13	67	26
4. *A. sydowii*	90	--	90	--	72	20
5. *A. terreus*	90	--	90	--	20	76
6. *A. niger*	Nil	100	Nil	100	Nil	100
7. *P. simplicissimum*	Nil	100	27	70	29	68
8. *P. citrinum*	Nil	100	12	87	36	60
9. *B. subtilis*	Nil	100	20	78	27	70
10. Control	90	--	90	--	90	--

Plate 31. Zone of inhibition by cell free culture filtrates of antagonists in *E. coli*
(1) *F. oxysporum*
(2) *T. harzianum*
(3) *A. niger*
(4) *A. terreus*
(5) Control

Plate 32. Zone of inhibition by cell free culture filtrates of antagonists in *E. coli*
(1) *P. simplicissimum*
(2) *P. citrinum*
(3) *Talaromyces wortmanni*
(4) *T. koningii*
(5) Control

Plate 31

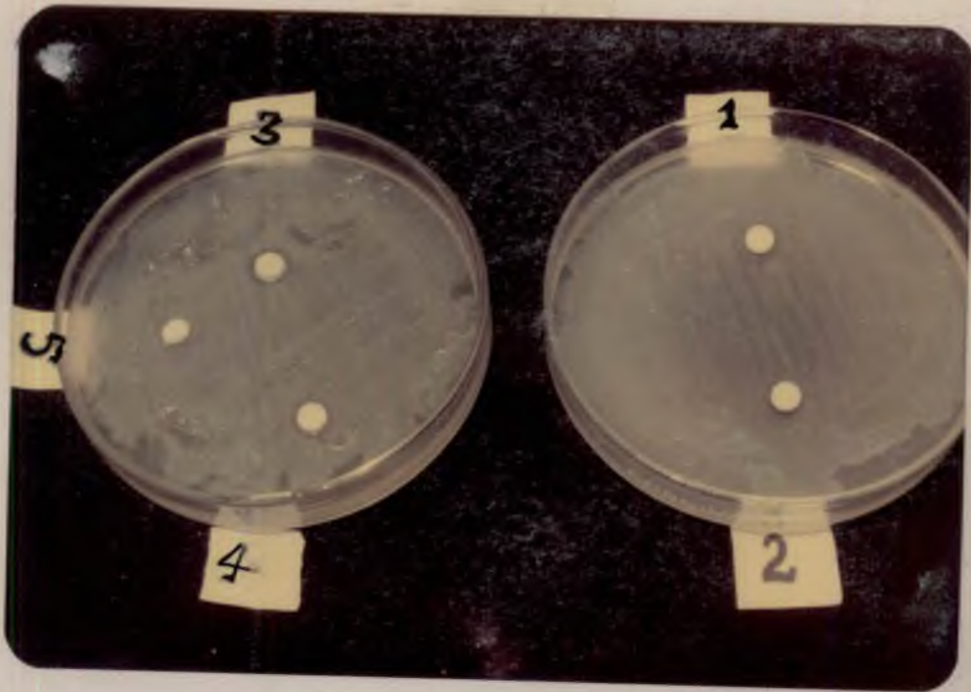


Plate 32

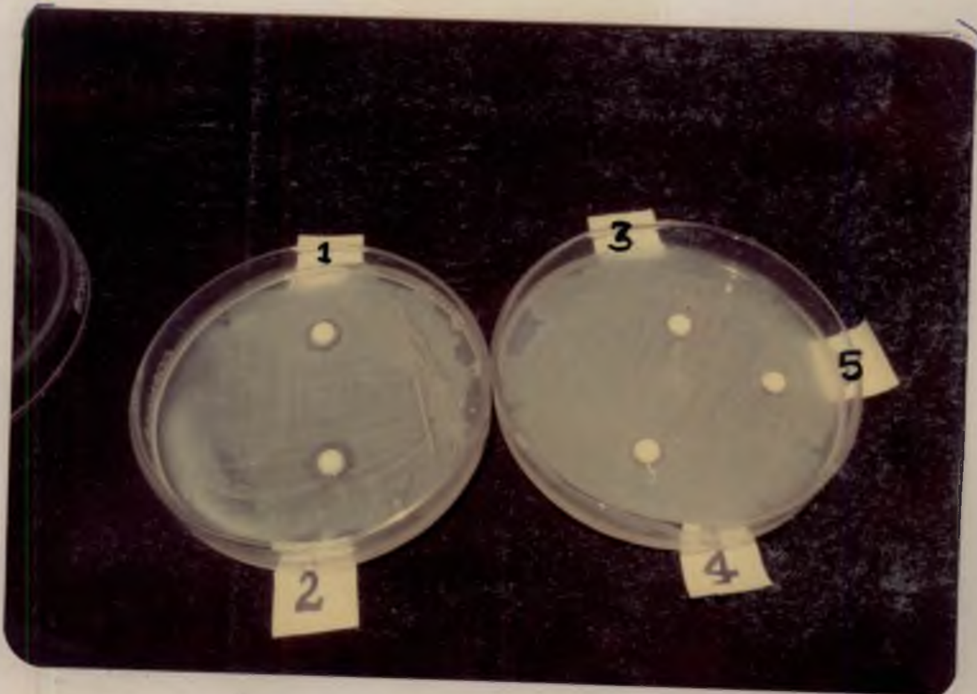
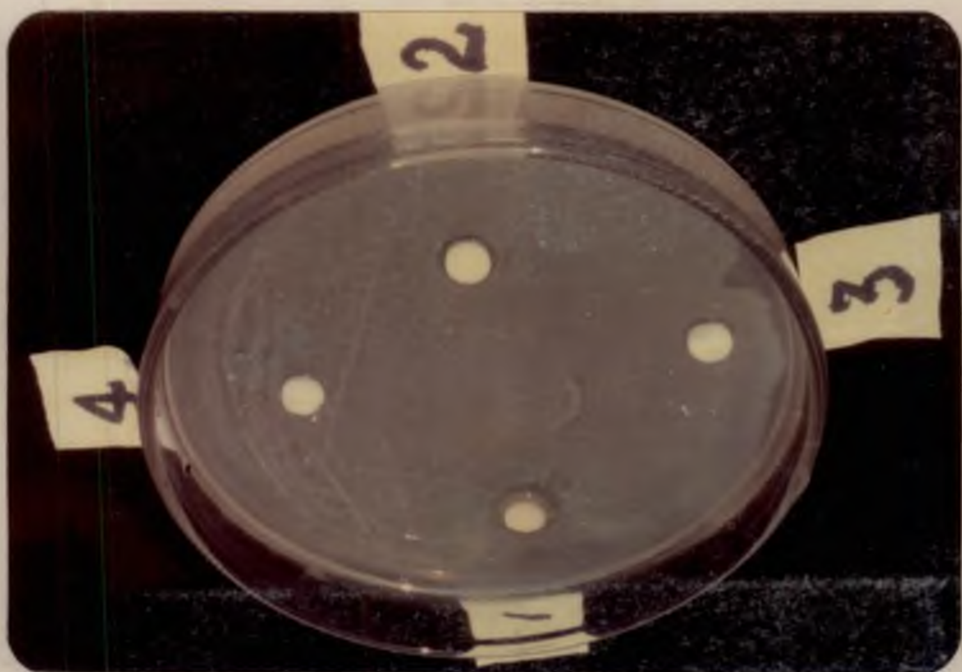


Plate 33. Zone of inhibition by cell free culture filtrates of antagonists in *E. coli*
(1) *Streptomyces* sp.
(2) *Bacillus subtilis*
(3) *Pacilomyces lilacinus*
(4) Control

Plate 33



A. terreus has no inhibitory action against *P. myriotylum* and *P. palmivora* while it inhibited *R. solani* considerably, having 78 per cent inhibition (Table 37).

The powerful toxic antibiotic produced by *A. niger* gave 100 per cent inhibition of all the three test organisms (Table 37).

P. simplicissimum also produced powerful toxic metabolite which has inhibited 100 per cent growth of *P. myriotylum*, 70 per cent of *P. palmivora* and 68 per cent of *R. solani* (Table 37).

P. citrinum also produced more or less same inhibitory metabolite like *P. simplicissimum* and gave inhibition of growth of 100 per cent of *P. myriotylum*, 87 per cent of *P. palmivora* and 60 per cent of *R. solani* (Table 37).

As regards inhibition by the antagonist *B. subtilis*, it was 100 per cent against *P. myriotylum*, while it was 78 and 70 per cent in respect of *P. palmivora* and *R. solani*.

Antibiotic assay

The antagonists which showed at least few antagonistic reactions in dual culture against any of the three test

organisms, *P. myriotylum*, *P. palmivora* and *R. solani*, and also tha isolates which were known to produce antibiotics were subjected to antibiotic assay as described in materials and methods.

A total of 14 microorganisms were screened against *Eschenchia coli* and the inhibition zone developed was compared with that of standard antibiotics as described in materials and methods. The results obtained are presented (Table 38) (Fig. 6) (Plates 30, 31, 32, 33).

Of the 14 microorganisms tested, the cell free culture filtrate of *P. citrinum* has shown the maximum antibiotic property having 11 mm inhibition zone which is equivalent to 325 ppm tetracycline hydrochloride. *Streptomyces* sp. (with straight sporophores) was second in the order giving an inhibition zone of 10 mm which is equivalent to 250 ppm of tetracycline hydrocholoride.

Cell free culture filtrates of three organisms viz. *T. longibrachiatum*, *P. simplicissimum* and *A. versicolor* produced an inhibition zone of 9 mm which is equivalent to 150 ppm of tetracycline hydrochloride. Cell free culture filtrate of *A. niger* produced 8.4 mm inhibition and that was equivalent to 90 ppm of tetracycline hydrochloride. Cell

Table 38. Antibiotic assay of cell free culture filtrates of antagonists

Sl. No.	Name of antagonists	Zone of inhibition produced by culture filtrate (mm)	Equivalency to Tetracycline hydrochloride (ppm)
1	T. koningii	7	50
2	T. harzianum	6	50
3	T. longibrachiatum	9	150
4	A. versicolor	9	150
5	A. sydowii	8	50
6	A. terreus	7	50
7	A. niger	8.4	90
8	P. simplicissimum	9	150
9	P. citrinum	11	325
10	T. wortmanni	6.6	50
11	P. lilacinus	6	50
12	F. oxysporum	7.4	50
13	Streptomyces sp.	1.0	250
14	B. subtilis	8	50
15	Control	0	

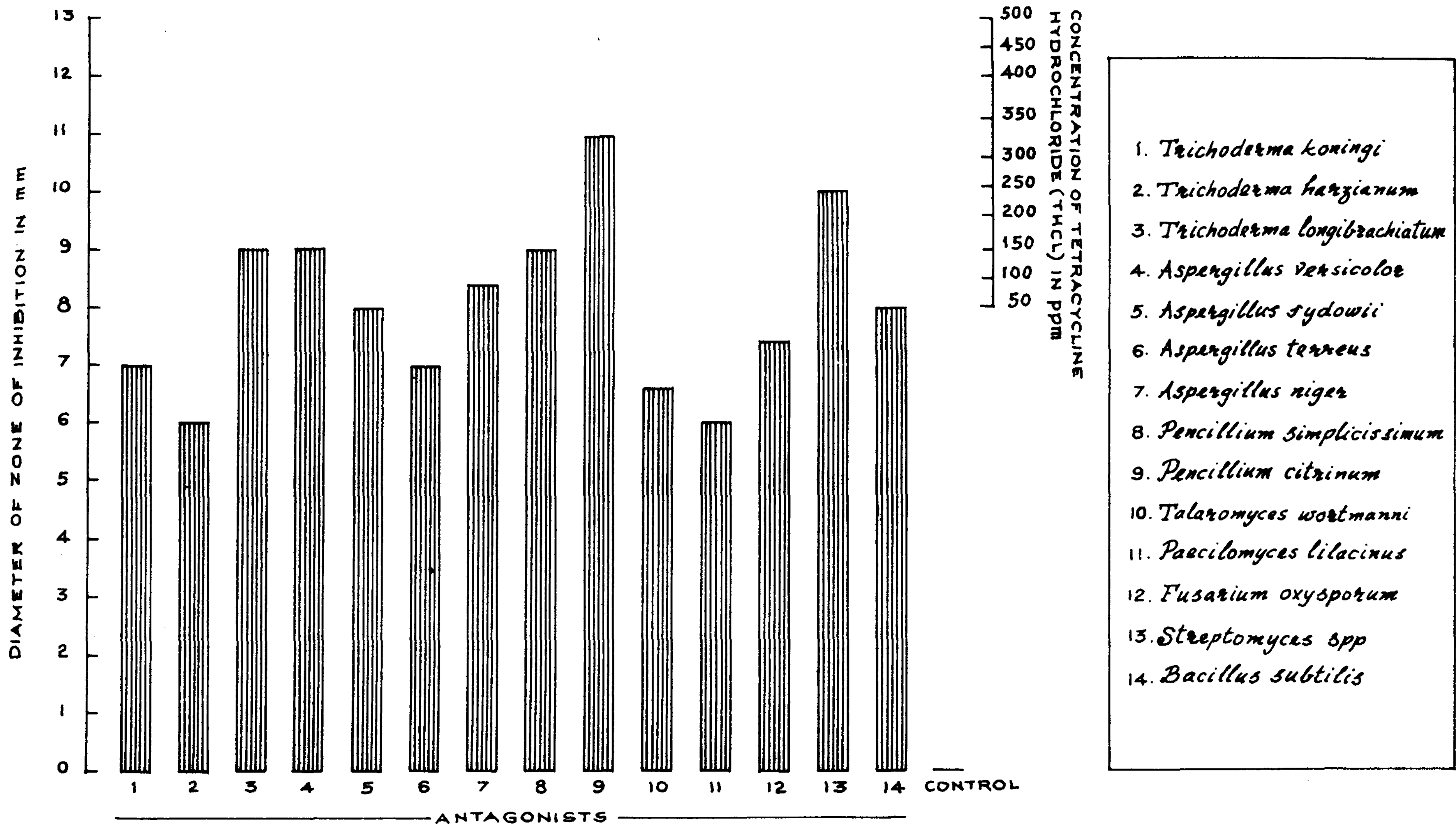


FIG. 6. GRADING AND COMPARISON OF ZONE OF INHIBITION PRODUCED BY THE CELL FREE CULTURE FILTRATE OF ANTAGONISTS IN *E. coli* WITH TETRACYCLINE HYDROCHLORIDE.

free culture filtrates of two organisms viz. *A. sydowii* and *B. subtilis* produced 8 mm inhibition zone which is equivalent to 50 ppm of tetracycline hydrochloride.

Six organisms namely *T. koningii*, *T. harzianum*, *A. terreus*, *T. wortmanni*, *P. lilacinus* and *F. oxysporum* produced very less antibiotic substances in culture filtrates and their zone of inhibition ranged from 6.0 - 7.4 mm which is equivalent to less than 50 ppm of tetracycline hydrochloride.

Discussion

DISCUSSION

In natural environment, a number of relationships exist between individual microbial species and between individual cells. The inter-relations and interactions of various microbial groups making up the soil community, however, are in a continual state of change and this dynamic state is maintained at a level characteristic of the flora. The composition of microflora of any habitat is governed by the biological equilibrium created by the association and interactions of all individuals found in the community (Alexander, 1977).

The undisturbed, moist evergreen forest soil is one of the most ideal substrates for the existence of the native microflora. Apart from the rich organic content of the soil, the undisturbed condition of the site since its evolution as a natural forest, makes the environment so congenial for the survival of the fittest microbes by competition, antibiosis and predation or parasitism. Thus, the ecological axiom is expected to be maintained in such soils.

In Kerala, no attempt has been made to explore the native microflora of the undisturbed forest soils. Keeping the above factors in mind the sites were selected for isolating antagonistic and antibiotic producing micro-organisms. The locations selected for soil sample collection in the study were having divergent vegetation comprising mostly of evergreen shrubs and trees. There were 35 and 39 phanerogamic spp. identified in 50 m^2 area around the site of soil sample collection in Wynad and Idukki districts respectively. This dense growth of vegetation and continuous wet period for a major part of the year made this area ever-green humid forest.

The Idukki soils were generally rich in organic carbon when compared to the Wynad soil. In both the places, the organic carbon decreased with increase in depth of soil, and maximum accumulation of organic carbon was noticed in the first layer (0-10 cm) of top soil. This may be due to the fact that the soils are undisturbed and accumulation of organic matter will be always in the surface soil (Table 3).

When depth of soil increased microbial population decreased both in Wynad and Idukki soils in all the collection sites. The microbial population was highest in the first

layer of top soil (0-10 cm) of Idukki which ranged from 39.42 to 57.983 million, while in Wynad it ranged from 34.242 to 38.74 million. In the next layer (11-20 cm) the microbial population further decreased both in Wynad and Idukki soils and again further reduction was noted in the bottom layer (21-30 cm). It was only 0.43-9.56 million in Idukki and 0.517-0.621 million in Wynad. The date clearly revealed that when depth increased, the microbial population significantly decreased in both the forest soils (Table 4 and 8). This finding is in full agreement with those of earlier workers (Alexander, 1977). The reduction in microbial population as a result of increase in depth of soil is due to the fact that when depth increases the organic carbon decreases and soil reaction also decreases slightly and there is every possibility of less aeration in deeper layers. The combined effect of these three factors creates a condition which is not congenial for the growth and reproduction ofthe microbes resulting in the decrease in microbial population. Organic carbon being the only source of energy for the soil microbes, is the major factor that determines the microbial population. This observation is in conformity with the findings of earlier soil microbiologists (Waksman and Curtis, 1916, 1918; Starc, 1942;

Laudelout *et al*., 1949; Aristovskaya, 1951, 1957; Rose, 1954; Blue *et al*., 1955; Zhukova, 1956; Jagnow, 1958; Tsao *et al*., 1959; Popova, 1963; Rangeswami and Venkatesan, 1963 and Rangaswami *et al*., 1967).

The maximum fungal population was observed in the top soils of both Wynad and Idukki districts. The population decreased with increase in depth except in Idukki where there was no significant difference between the second (11-20 cm) and third layer (21-30 cm). But in pooled analysis there was significant difference in fungal population in different depth of soils, as depth increased the population decreased (Table 5 and 8). A gradual reduction in microflora in deeper layers was observed in the present investigation and is fully supported the earlier findings of Aristovskaya (1951, 1957), Waksman (1952), Rose (1954), Milosevic (1958), Tsao *et al*. (1959), Rangaswami and Venkatesan (1963), Corke and Chase (1964), Venkatesan (1964) and Rangaswami *et al*. (1967).

The actinomycete population in general was found to be maximum in top layer, both in Wynad and Idukki soils. But in Idukki, there was no significant difference between the first (0-10 cm) layer and second layer (11-20 cm). The

pooled analysis of actinomycete population of Wynad and Idukki revealed that there was no significant difference between the first two layer, but there was significant difference between the second (11-20 cm) and third layer (21-30 cm) (Table 6 and 8). The reduction of actinomycetes population was observed with increase in depth but drastic reduction was noticed beyond a depth of 20 cm. This may probably be due to the comparatively low pH in lower layers and actinomycetes prefer generally higher pH. These findings are also in conformity with the findings of earlier workers (Waksman and Curtis, 1916, 1918; Jensen, 1943; Jones, 1943; Aristovskaya, 1951, 1957; Rose, 1954; Vojnovic and Sevic, 1954; Jagnow, 1956; Zhukova, 1956; Milosevic, 1958; Szabo et al., 1958; Teplyakova and Makshimova, 1958; Tsao et al., 1959; Popova, 1963; Rangaswami and Venkatesan, 1963; Corke and Chase, 1964; Venkatesan, 1964 and Rangaswami et al., 1967).

Like the fungi and actinomycetes, the bacterial population also decreased with increase in depth. But the reduction of population was very much pronounced than the other two groups. This progressive decline in population of bacteria with increase in soil depth is mostly in line

with the result of earlier workers (Waksman and Curtis, 1916, 1918; Landolout *et al*., 1949; Marszewska-Zimiecka and Golebiowska, 1949; Rose, 1954; Vojnovic and Sevic, 1954; Blue *et al*., 1955; Milosevic, 1958; Tsao *et al*., 1959; Rangaswami and Venkatesan, 1963; Corke and Chase, 1964; Venkatesan, 1964 and Rangaswami *et al*., 1967).

In Kerala, the major soil borne diseases are caused by the different species of fungi like *Pythium*, *Phytophthora* and *Rhizoctonia*. In the present investigation the micro-organisms obtained in the soil dilution plates were screened against the major soil borne plant pathogens, *Pythium myriotylum*, *Phytophthora palmivora* and *Rhizoctonia solani* for antagonistic properties by the standard methods.

In the dual culture method, 18 identified fungal species were screened against the three soil borne plant pathogens. The five dual culture reactions described by Johnson and Curl (1972) were observed and from these, those showing antagonistic properties were screened, as per their behaviour in the dual culture.

During the present investigation, the following five reactions have been observed for determining the antagonistic properties.

(1) Intermingling and overgrowth

(2) Mutual inhibition on contact

(3) Flattening of colony of the test organism on the sides nearest to the antagonist

(4) Mutual inhibition at a distance

(5) Inhibition at a distance and disintegration of test organism.

Eleven spp. of fungi and three spp. of actinomycetes have shown the character of intermingling and overgrowth in dual culture. Of these, Mucor sp. and Cunninghamella elegans showed this character in dual culture against all the three test organisms. But Absidia corymbefera, Syncephalastrum racemosum, Aspergillus melleus, A. sydowii, A. terreus, Microascus cinereus and Fusarium oxysporum showed their ability to live along with the test organisms P. myriotylum and P. palmivora by intermingling and over growth. But the above soil microorganisms have some reaction against R. solani (Table 36).

Paecelomyces lilacinus showed the character of intermingling and overgrowth in the case of P. palmivora and R. solani in dual culture. But it has shown some reaction against P. myriotylum.

A. versicolor and three spp. of Streptomyces can grow freely by intermingling and overgrowth along with P. palmivora but they have shown some reaction against P. myriotylum and R. solani (Table 22). Earlier workers like Porter (1924), Newhook (1951) and Johnson and Curl (1972) have also reported this type of reaction in dual culture.

A. sydowii and A. terreus, which have shown some reaction against R. solani, were also tested for their antibiotic properties against the three test organisms by using cell free culture filtrates. The result showed that the cell free culture filtrates have some inhibitory reaction against R. solani but no effect on P. myriotylum and P. palmivora. This cell free culture filtrate technique further confirms the results of dual culture studies. This finding is in agreement with the earlier work of Roy (1984). The character of intermingling and overgrowth in dual culture cannot be considered a property of antagonism, as these organisms have no adverse effect on the growth of the test organisms.

Another reaction in dual culture is mutual inhibition on contact. Eight species of fungi and three types of Streptomyces sp. have shown this reaction against more than

one test organism (Table 36). Both the test organisms and the antagonists showed normal growth rate in dual culture, but when they came in contact, further growth was stopped. _Talaromyces wortmannii_ has shown this reaction against all the three test organisms. However, two types of _Streptomyces_ sp. (flexuous sporophores and fascicled sporophores) and _A. versicolor_ have shown this reaction against _P. myriotylum_ and _R. solani_. But they have shown intermingling and overgrowth character against _P. palmivora_. _Paecelomyces lilacinus_ has shown the character of mutual inhibition on contact with _P. myriotylum_ but it has established a free intermingled growth in the case of _P. palmivora_ and _R. solani_.

Five fungi _viz_. _A. corymbifera_, _S. racemosum_, _M. cinereus_, _A. melleus_, _F. oxysporum_ and _Streptomyces_ sp. (straight sporophores) have shown the reaction of mutual inhibition on contact in dual culture with _R. solani_. Except the _Streptomyces_ sp. (straight sporophores) all these fungi have shown intermingling and overgrowth character with _P. myriotylum_ and _P. palmivora_ in dual culture.

Streptomyces sp. (straight sporophores) has shown three different reactions against the three test organisms. It has shown intermingled overgrowth with _P. palmivora_ but mutual

inhibition on contact with *R. solani* and a good antagonistic property, dieback and disintegration of test organism, with *P. myriotylum*.

One organism may show good antagonistic property against one or a few pathogens but against others it may not have any adverse effects. There are ample evidences for such behaviour of the soil microorganisms. The selective nature of the antagonistic property of *Streptomyces* sp. has been reported by earlier workers (Neweigy *et al*., 1982; Kundu and Nandi, 1984; Logan *et al*., 1984 and Mohamed, 1985).

Of course, the character of mutual inhibition on contact by test organism and antagonist is an antagonistic property. But it may not have much value in the biological control of soil borne pathogens, because, till the contact of the antagonist and the test organism, they will have free growth and even on contact there is no disturbance of the test organism except checking the further growth.

The dual culture reaction, mutual inhibition at a distance clearly showed that the antagonist and test organisms were producing some biotic substances diffusing into the media and acted inhibitory to each other. Of the organisms tested, *A. terreus* has shown this property only against

R. solani, while, against other two test organisms the reaction was intermingling and overgrowth (Table 21). This indicates that biotic substances produced by A. terreus and R. solani are diffusible in agar medium and are inhibitory to each other. None of this biotic substances was self inhibitory, because in monoculture both the organisms grew normally (Table 21). The cell free culture filtrate studies using the antagonist A. terreus also showed that it was having high inhibitory action (78 per cent) against R. solani. This finding also supports the results of dual culture technique (Table 37).

The antagonistic property of A. terreus against R. solani has been reported by earlier workers (Zaehner et al., 1963; Roy, 1984). The present investigation confirms the findings of the earlier workers.

Penicillium citrinum and P. simplicissimum have shown the reaction of mutual inhibition at a distance with P. palmivora and R. solani (Table 36). But they have shown some other antagonistic property against P. myriotylum. The ability of P. citrinum and P. simplicissimum to produce antibiotic substances inhibitory to P. palmivora and R. solani was further established in the cell free culture filtrate

techniques employed in the present investigation. There were 70 and 87 per cent inhibition in case of *P. palmivora* and 68 and 60 per cent in case of *R. solani* by the culture filtrates of *P. simplicissimum* and *P. citrinum* respectively (Table 37).

It is a well known fact that the *Penicillium* spp. can produce biotic substances which are inhibitory to many fungi which include *Phytophthora* and *Rhizoctonia* spp. (Raicu and Stan, 1975; Odigie and Ikdtun, 1982; Harol and Konde, 1983; Logan *et al*., 1984 and Jharia and Khare, 1986).

Bacillus subtilis and other three types of *Bacillus* spp. isolated during the present investigation also showed mutual inhibition at a distance in the case of *P. palmivora* and *R. solani* (Table 36). But these bacteria have shown different antagonistic property against *P. myriotylum*. In cell free culture filtrate studies also *B. subtilis* has shown 78 and 70 per cent inhibition in case of *P. palmivora* and *R. solani* respectively (Table 37). The antagonistic properties of *Bacillus* spp. and *B. subtilis* against *R. solani* have been established by earlier workers (Olsen and Baker, 1968; Neweigy *et al*., 1982; Venkitasubbiah, 1985). *Bacillus* spp. and *B. subtilis* have shown the antagonistic properties

against different spp. of Phytophthora as reported by Utkhede (1984) and Podile and Dube (1987).

In the biological control of soil borne plant pathogens, the usefulness of B. subtilis especially against R. solani and Phytophthora spp. is a well established fact (Mitchell and Hurwitz, 1965; Olsen and Baker, 1968; Aldrich and Baker, 1970; Broadbent et al., 1971; Michael and Nelson, 1972; Merriman et al., 1974 and Kommedahl and Mew, 1975).

The dual culture reaction of inhibition at a distance and disintegration of the test organism was shown by three fungal antagonists viz. A. niger, P. citrinum and P. simplicissimum (Table 36). Of these antagonists, A. niger has shown the above reaction towards P. myriotylum and R. solani while it has shown another type of reaction towards P. palmivora. The other two antagonists P. citrinum and P. simplicissimum showed the reaction of inhibition at a distance and disintegration of test organism against P. myriotylum. They also showed the reaction of mutual inhibition at a distance against P. palmivora and R. solani (Table 36).

The cell free culture filtrate studies using the antagonists A. niger, P. citrinum and P. simplicissimum also

showed 100 per cent inhibition against *P. myriotylum* indicating the production of antibiotics by the antagonists, which inhibited the test organism completely. The culture filtrate of *A. niger* has also shown 100 per cent inhibition of *R. solani* and *P. palmivora* while that of *P. simplicissimum* and *P. citrinum* have shown 70 and 87 per cent inhibition of *P. palmivora*, and 68 and 60 per cent inhibition of *R. solani*. This result of cell free culture filtrate studies strongly supports the results of dual culture technique (Table 37).

The antagonists *A. niger*, *P. simplicissimum* and *P. citrinum* have produced some diffusible metabolites into the medium which have strong antibiotic and some lytic activity against the test organism *P. myriotylum*. The antagonists have produced the antibiotics which diffused into the substrate slowly and thus inhibiting only the growth of test organism nearer to antagonist, while growth of test organism in the periphery was not affected. The disintegration of hyphae of the test organism was also noticed indicating the lytic property of the metabolites. The behaviour of the antibiotics produced by *A. niger* towards *P. myriotylum* and *R. solani* was similar, whereas towards *P. palmivora* it was different. *P. simplicissimum* and

P. citrinum also behaved in a similar manner towards P. myriotylum, but their behaviour towards P. palmivora and R. solani was mutual inhibition at a distance without causing any lysis.

The antibiotic producing ability of A. niger, P. citrinum and P. simplicissimum is a well established fact and the properties of inhibition and lysis of the pathogenic fungi were reported by many workers (Domsch, 1960; Broadbent, 1966; Robinson and Park, 1966; Raicu and Stan, 1975; Bora, 1977; Fedoseeva et al., 1983; Gokulapalan and Nair, 1984; Sy et al., 1984).

The dual culture reaction characterised by dieback and disintegration of the fungal test organism after meeting the antagonist, was shown by all the three species of Trichoderma viz. T. harzianum, T. koningii and T. longibracheatum, against the three test organisms P. myriotylum, P. palmivora and R. solani (Table 36). The antagonist when contacted the test organism, parasitises it and slowly caused the dieback and disintegration. The biotic substances produced during the growth of these antagonists, inhibited the test organisms and at the same time parasitised the hyphae of test organism resulting in disintegration. This is evident from the fact

that the antagonist continued its growth even after meeting the hyphae of the test organism causing the dieback and disintegration. The hyphal parasitism and production of inhibitory substances by different species of *Trichoderma*, resulting in dieback and disintegration of test fungi like *Pythium*, *Phytophthora* and *Rhizoctonia* were reported by many workers (Weindling, 1932; Boosalis, 1956; Durrell, 1968; Mew *et al*., 1980; Elad *et al*., 1983; Logan *et al*., 1984; Sivan *et al*., 1984; Mukhopadhyay and Indulika Chandra , 1986; Mukhopadyay,1987; Manian and Paulsamy, 1987). The present investigation also clearly demonstrated the ability of these three spp. of *Trichoderma* to cause dieback and disintegration of the above soil borne plant pathogens.

The cell free culture filtrate studies using *T. harzianum*, *T. koningii* and *T. longibracheatum* also showed evidence of production of biotic sustances which inhibited the growth of test organism to some extent (Table 37). The inhibition percentage observed on the test organisms by the above three species of *Trichoderma* ranged between 13-26 per cent.

The present investigation has proved that the above three species of *Trichoderma* have the ability to show their

antagonism against *Pythium*, *Phytophthora* and *Rhizoctonia* and that it is mainly due to the parasitism and to some extent due to production of biotic substances which are toxic to test organisms.

A. niger has shown the antagonistic property of dieback and disintegration against the test organism *P. palmivora* (Table 36). This indicates that *A. niger* can parasitise on *P. palmivora* causing disintegration and the biotic substances produced by this antagonist are found to be toxic to the test organism. The bioassay studies using cell free culture filtrates clearly demonstrated 100 per cent inhibition, not only against *P. palmivora* but also against *P. myriotylum* and *R. solani* (Table 37). The production of antibiotic substances having lytic activity by *A. niger* against *P. palmivora* is a well established fact and has been reported by earlier workers (Broadbent, 1966; Raicu and Stan, 1975; Bora, 1977; Trevinoo and Espinosa, 1981; Fedoseeva *et al*., 1983; Gokulapalan and Nair, 1984; Sy *et al*., 1984; Padmakumari and Balakrishnan, 1986). The ability of producing strong antibiotic substances by *A. niger* against the three important soil borne pathogens *P. myriotylum*, *P. palmivora* and *R. solani* is clearly demonstrated in the present investigations.

A. sydowii has caused the dieback and disintegration of the test organism R. solani by overgrowing and parasitising it (Table 36). There was no considerable inhibition of the test organism in the dual culture which was evident also from the data of cell free culture filtrate studies, where A. sydowii showed no inhibitory action on P. myriotylum or P. palmivora while it recorded 20 per cent inhibition of test organism R. solani (Table 20). The present investigation indicates that A. sydowii is not able to produce any strong inhibitory substances against the fungi tested and the antagonistic property of A. sydowii against R. solani is mainly by parasitic activity.

In the case of Streptomyces sp. (with straight sporophores) there was no clear indication of the antagonistic character of dieback and disintegration, but flattening of hyphae of the test organism P. myriotylum on the sides nearest to the antagonist was observed in dual culture. The antagonistic activity of Streptomyces spp. against Pythium was reported by earlier workers (Chi, 1967; Domsch et al., 1980). But in the present investigation, the species of Streptomyces used have not shown any antagonistic property against the three test organisms but showed adverse effect on growth of

P. myriotylum by way of flattening of hyphae nearest to antagonist.

In the present study, four species of bacteria have been isolated and studied. Of these, one is identified as *B. subtilis* and the other three isolates are found to be identical to *B. subtilis* but having different cell size of *Bacillus* sp. All these bacteria have shown good antibiotic property to all the organisms tested. In dual culture with *P. myriotylum* all of them have shown dieback and disintegration property, but with the other two test organisms they showed the character of mutual inhibition at a distance (Table 36).

The cell free culture filtrates of *Bacillus subtilis* inhibited 100 per cent growth of *P. myriotylum* whereas it was 78 and 70 per cent in respect of *P. palmivora* and *R. solani* respectively.

The antagonistic and antibiotic properties of *B. subtilis* and other spp. of *Bacillus*, are well established as evident from the reports of earlier workers (Henis and Inbar, 1968; Olsen and Baker, 1968; Agarwal *et al*., 1977;

Ashour *et al.*, 1980; Utkhade and Rahe, 1980; Odigie and Ikotun, 1982; Tschen and Kuo, 1985; Mukherjee *et al.*, 1987 and Podile and Dube, 1987).

The importance of this group of bacteria for the management of soil borne diseases by means of biological control is getting great attention of plant pathologists all over the world.

Antibiotic bioassay studies reveal that some of the isolates especially *P. citrinum*, *P. simpliciasimum* and *Streptomyces* spp. are producing very powerful biotic substances which are equivalent in their antibiotic property with 325, 150 and 250 ppm respectively of tetracycline hydrochloride against *Escherichia coli*. The different spp. of *Trichoderma* and *Aspergillus* have shown some antibiotic property against *E. coli* which was equivalent to 150 ppm of tetracycline hydrochloride.

The antibiotic properties of the organisms especially *Penicillium* and *Streptomyces* are well known and abundant literature is available on this subject.

The present investigation has shown that the forest soils of Kerala are very rich in microorganisms especially

fungi. Of these, many of them have shown good antagonistic property against the notorious soil borne pathogens viz. P. myriotylum, P. palmivora and R. solani. Due to the continuous cultivation, the ecological equilibrium of the population dynamics of the microbes in the soil has been disrupted, and cultivated soils are having generally more plant pathogenic organisms than the antagonistic organisms. The following organisms isolated and studied during the present investigations viz. T. harzianum, T. koningii, T. longibracheatum, A. niger, P. citrinum, P. simplicissimum, B. subtilis and allied species of bacteria are found to be very powerful antagonistic and antibiotic producing organisms which can be very successfully utilized for the biological control of the major soil borne plant pathogens of Kerala like P. myriotylum, P. palmivora and R. solani. The proper development of food bases and method of application of these antagonistic microbes in the field will help to get better result for management of the soil borne plant diseases by means of biological control.

Summary

SUMMARY

1. The antibiotic producing and antagonistic micro-organisms in the forest soils of Kerala were studied and the results are presented in this thesis. Soil samples were collected from the moist evergreen forest areas of Wynad and Idukki districts. In both localities the soils were lateritic in origin and they were typically forest soils with acidic reaction. The average rain fall for the last ten years was 1297.27 mm in Wynad and 1763.86 mm in Idukki.

2. A total of 64 species of higher plants, distributed among 40 phanerogamic families was identified from the areas designated for the collection of soil samples.

3. Total microbial population was estimated and it was found that Idukki soils were very high in microbial population with 39.422 - 57.98 x 10^6 per g of soil while that in Wynad it ranged from 34.24 - 38.74 x 10^6 per g of soil.

4. The depth of soil has direct relationship with the microbial population. When the depth increased, microbial population significantly reduced in both the forest soils.

5. The mean fungal population in different depths of Wynad soil ranged from $2.32 - 4.41 \times 10^4$ per g of dry soil and that of Idukki, it ranged from $2.25 - 5.03 \times 10^4$ per g of dry soil. Twenty different species of fungi were isolated. Of these, 18 were identified upto species level. The predominant genera of fungi were *Mucor*, *Syncephalastrum*, *Trichoderma*, *Microascus*, *Cunninghamella*, *Absidia*, *Aspergillus*, *Penicillium*, *Talaromyces*, *Paecelomyces* and *Fusarium*. In Wynad, seventeen species of fungi were isolated whereas it was only twelve species in Idukki district. Of these, nine were common in both the districts.

6. The actinomycetes population in different depths of soils was generally high when compared to fungi and ranged from $0.047 - 0.210 \times 10^6$ per g of dry soil in Wynad and $0.087 - 0.451 \times 10^6$ per g of dry soil in Idukki. Three species of actinomycetes were identified upto generic level. Two species were common in both the soils and one was restricted to Idukki alone.

7. Among the micro-organisms, bacterial population was maximum in all the three layers of soils in both the districts. It ranged from $34.2 - 38.5 \times 10^6$ per g of dry

soil in Wynad. It was much higher in Idukki and ranged from 38.88 - 57.62 x 10^6 per g of dry soil. Four types of *Bacillus* spp. were identified. Of these, two were common in Wynad and Idukki, while the other two spp. were restricted to Idukki district alone.

8. The antagonistic properties of all the isolates were studied by using three soil borne plant pathogens *viz*. *Pythium myriotylum*, *Phytophthora palmivora* and *Rhizoctonia solani* as test organisms by dual culture method.

9. The dual culture characters of the soil microorganisms isolated from the forest soils were studied along with the three test organisms and classified into following five groups.

   (a) Intermingling and overgrowth

   (b) Mutual inhibition on contact

   (c) Mutual inhibition at a distance

   (d) Inhibition at a distance and disintegration of test organism.

   (e) Die-back and disintegration of test organism.

   Of the above reactions, intermingling and overgrowth did not show any antagonistic or antibiotic property. The reaction of mutual inhibition on contact showed only slight antagonistic property.

10. Mucor sp. and Cunninghamella elegans showed the reaction of intermingling and overgrowth with all the three test organisms. Absidia corymbefera, Syncephalastrum recemosum, Aspergillus meleus, A. sydowii, A. terreus, Microascus cinereus and Fusarium oxysporum showed the reaction of intermingling and overgrowth with Pythium myriotylum and Phytophthora palmivora. Paecelomyces lilacinus has shown intermingling and overgrowth character in dual culture with P. palmivora and R. solani.

11. Talaromyces wortmanni has shown the reaction of mutual inhibition on contact with all the three test organisms in dual culture. A. versicolor, Streptomyces sp. (with flexuous sporophores) and Streptomyces sp. (with faciled sporophores) showed the reaction of mutual inhibition on contact with P. myriotylum and R. solani in dual culture. On the other hand, Absidia corymbefera, Syncephalastrum racemosum, Aspergillus meleus, Microascus cinereus, Fusarium oxysporum and Streptomyces sp. (with straight sporophores) showed this reaction only with R. solani. But Paecelomyces lilacinus showed this reaction only with P. myriotylum.

12. *Penicillium citrinum*, *P. simplicissimum*, *Bacillus subtilis* and three unidentified species of *Bacillus* have the dual culture reaction of mutual inhibition at a distance against *P. palmivora* and *R. solani*. None of the above species has shown this reaction against *P. myriotylum*.

13. *A. niger* showed the reaction of inhibition at a distance and disintegration of test organisms in dual culture with *P. myriotylum* and *R. solani*, while *P. citrinum* and *P. simplicissimum* have shown this reaction only with *P. myriotylum*. None of these organisms showed this reaction with *P. palmivora*.

14. Three species of *Trichoderma viz*. *T. harzianum*, *T. koningii*, *T. longibracheatum* have shown the dual culture reaction of dieback and disintegration of test organisms, with all the three plant pathogens tested. *A. niger* has shown this property only against *R. solani*. *Streptomyces* sp. (with straight sporophores), *Bacillus subtilis* and three other species of *Bacillus* have shown this character against *P. myriotylum* alone.

15. The micro-organisms which showed conspicuous antagonistic characters in dual culture against the three test

organisms were selected and further studied for production of antibiotic substances by means of assay of cell free culture filtrates by employing poisoned food technique with the three soil borne pathogens. The three species of *Trichoderma* inhibited the growth of all the test organisms to some extent which ranged from 13-26 per cent, while *A. sydowii* has inhibited 20 per cent growth of *R. solani* and against the other two test organisms no inhibition was showed. *A. terreus* has no inhibitory action against *Pythium myriotylum* and *Phytophthora palmivora*, but it inhibited 78 per cent growth of *R. solani*.

16. *A. niger* produced very powerful toxic metabolite and inhibited 100 per cent growth of all the three test organisms while *P. simplicissimum* produced powerful toxic metabolites which inhibited 100 per cent growth of *P. myriotylum* and 70 and 68 per cent growth of *P. palmivora* and *R. solani* respectively. *P. citrinum* was found to produce more or less the same inhibitory metabolites which inhibited 100 per cent growth of *P. myriotylum*, 87 per cent of *P. palmivora* and 67 per cent of *R. solani*.

17. The cell free culture filtrates of *Bacillus subtilis* inhibited 100 per cent growth of *P. myriotylum* whereas it was 78 and 70 per cent in respect of *P. palmivora* and *R. solani* respectively.

18. The antibiotic properties of the antagonists were studied and showed that *P. citrinum* produced maximum antibiotic property which is equivalent to 325 ppm of tetracycline hydrochloride, followed by *Streptomyces* sp. (with straight sporophores) which gave an equivalence of 250 ppm of tetracycline hydrochloride. *T. longibracheatum*, *P. simplicissimum* and *A. versicolor* produced their antibiotic property equivalent to 150 ppm of tetracycline hydrochloride. Of the 14 organisms studied for their antibiotic property, all the other 11 organisms produced only less than 100 ppm equivalence of tetracycline hydrochloride.

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ANTIBIOTIC PRODUCING AND ANTAGONISTIC MICROORGANISMS IN THE FOREST SOILS OF KERALA

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

Department of Plant Pathology

COLLEGE OF HORTICULTURE

Vellanikkara, Trichur

1988

ABSTRACT

The antibiotic producing and antagonistic fungi, actinomycetes and bacteria in the evergreen forest soils of Ladysmith forest of Thariyode in Wynad, and Cheriyakanom forest of Thekkadi in Idukki districts of Kerala State were studied.

The phanerogamic flora around the sites of soil sample collection in both localities were identified.

The total microbial population was studied in relation to the depth of soil. The microbial population was maximum in the top layer and decreased with increase in depth of soil. The total microbial population was higher in Idukki and in both districts, population of bacteria was maximum followed by actinomycetes and fungi. A diversified group of fungi consisting, *Mucor*, *Syncephalastrum*, *Trichoderma*, *Microascus*, *Cunninghamella*, *Absidia*, *Aspergillus*, *Penicillium*, *Talaromyces*, *Paecelomyces* and *Fusarium* was present. Three types of actinomycetes *viz*. *Streptomyces* sp. with straight sporophores, flexuous sporophores and fascicled sporophores were present while four types of bacteria *viz*., *B. subtilis*, *Bacillus* sp. identical to *B. subtilis*, *Bacillus* sp. with small cell and fast growth in NA and *Bacillus* sp. with small cell and slow growth in NA were present.

Antagonistic properties of the isolates were studied with the test organisms *Pythium myriotylum*, *Phytophthora palmivora* and *Rhizoctonia solani*.

Mucor sp. and *Cunninghamella elegans* showed intermingling and overgrowth with all the test organisms while *Absidia corymbefera*, *Syncephalastrum racemosum*, *Aspergillus meleus*, *A. terreus*, *Microascus cinereus* and *Fusarium oxysporum* showed this character with *P. myriotylum* and *P. palmivora*. Intermingling and overgrowth character was observed in *Paecelomyces lilacinus* with *P. palmivora* and *R. solani* whereas *A. versicolor* and three species of *Streptomyces* showed this character only with *P. palmivora*.

Mutual inhibition on contact was exhibited by *Talaromyces wortmannii* with all the three test organisms, while *A. versicolor* and *Streptomyces* spp. with flexuous sporophores and fascicled sporophores showed this character with *P. myriotylum* and *R. solani*. This character was observed in case of *A. corymbefera*, *S. racemosum*, *A. meleus*, *M. cinereus*, *F. oxysporum* and *Streptomyces* sp. with straight sporophores, with *R. solani* while *P. lilacinus* showed this with *P. myriotylum*.

Mutual inhibition at a distance was shown by *Penicillium citrinum*, *P. simplicissimum*, *B. subtilis* and the other three *Bacillus* spp. when tested with *P. palmivora* and *R. solani*, but *A. terreus* showed this reaction only with *R. solani*.

Inhibition at a distance and disintegration of test organism was shown by A. niger with P. myriotylum and R. solani while P. citrinum and P. simplicissimum showed this character only with P. myriotylum.

All the three spp. of Trichoderma showed die-back and disintegration of all the three test organisms, while A. niger showed this character only with P. palmivora and A. sydowii showed this character with R. solani only. Streptomyces sp. with straight sporophores, B. subtilis and the other three Bacillus spp. showed this character with P. myriotylum alone.

Inhibitory properties of antagonists using cell free culture filtrates were estimated and found that A. niger inhibited 100 per cent growth of all the three test organisms while P. citrinum, P. simplicissimum and B. subtilis showed 100 per cent inhibition of P. myriotylum and a range of 67-87 per cent in case of P. palmivora and R. solani. A. terreus did not inhibit P. myriotylum and P. palmivora, but inhibites 78 per cent of R. solani. All the three Trichoderma spp. moderately inhibited all the three test organisms (13-26 per cent) while A. sydowii showed 20 per cent inhibition of R. solani only.

Antibiotic property of the antagonists was determined and P. citrinum exhibited maximum equivalent to 325 ppm tetracycline followed by Streptomyces with straight sporophores having

250 ppm. *T. longibracheatum*, *P. simplicissimum* and *A. versicolor* recorded antibiotic property equivalent to 150 ppm tetracycline hydrochloride while the other isolates had <100 ppm equivalence of tetracycline hydrochloride.