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IDENTIFICATION OF SOIL MICROORGANISMS (BACTERIA ANDFUNGI) IN DIFFERENT COCONUT GROWING SOILS IN SRI LANKA

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ABSTRACT

Bacteria and fungi present in the manure circles (rhizosphere) of twenty selected soil series in coconut growing areas were studied. *Bacilli spp* were the dominant bacteria in all soil series. In addition, *Micrococcus, Staphylococcus, Enterobacter, Serratia, Corynebacterium, Pseudomonas* and *Actinomycetes* were also identified. Among the fungi *Penicillium, Aspergillus* and *Curvularia* were common in most of the soil series.

INTRODUCTION

In Sri Lanka, coconut is grown in different agro-ecological regions of the main agro-climatic zones, namely the Dry zone, the Intermediate zone and the Wet zone. Coconut plantations in the Puttalam, Kurunegala, Colombo, Gampaha and Kalutara Districts comprise the coconut triangle (Somasiri, *et al.,* 1993). The performance of coconut depends mainly on physical, chemical and biological properties of soils such as, moisture availability, rooting depth, aeration, fertility, organic matter content, microbial activity etc. (Somasiri, *et al.,* 1993). The various biochemical processes associated with nutrient cycling are mediated by soil enzymes which are derived from microorganisms and plant roots (Tabatabai, 1982). Bacteria, actinomycetes and fungi are the organisms most actively involved in the decay of organic materials. The bacteria are the most numerous and most diverse with each species having specific functions such as cellulose users, pectin decomposers, protein decomposers, ammonifiers, nitrifiers etc. There are also bacteria with different adaptation to various environmental conditions. Therefore identification of microorganisms is very important in determining the soil quality. The objective of this study was to identify bacteria and fungi present in the different coconut growing soils.

METODS

Site selection

Twenty different coconut growing soils within the coconut triangle were selected for this study. Three locations were selected in each soil series, where fertilizer or manure has not been applied at least for the last three years.

The selected soil series, locations, major soil types, agro-ecological regions and mean annual rainfall (mm) are given in Table 1.

Table 1 Selected soil series, major soil types agro-ecological regions and rainfall of the studied locations (IL - Intermediate Low Country, WL - Wet Low Country, DL - Dry Low Country)

Soil series	Major soil type	Locations	Agro-ecological region	75% of the expected rainfall
Rathupasa	Latosols and regosols on old red and yellow sands	Dummaladeniya Mahawewa Koswadiya	IL_1 IL_1 IL_1	1000 1000 1000
Madampe	Latosols and regosols on old red and yellow sands	Tummodara Kirimetiyana Madampe	IL_1 IL_1 IL_1	1000 1000 1000
Sudu	Latosols and regosols on old red and yellow sands	Suduwella Karukkuwa Maradawella	IL_1 IL_1 IL_1	1000 1000 1000
Wilattawa	Colluvial members of red yellow podzolics with soft or hard laterites	Wannirasnayakapura Mahagama Mudalakkuliya	IL_3 IL_1 IL_3	750-875 1000 750-875
Andigama	Red yellow podzolics	Mudalakkuliya Weerakodiyana Wadumunnegedara	IL_3 IL_1 IL_1	750-875 1000 1000
Boralu	Red yellow podzolic with soft or hard laterites	Divulapitiya Alabodagama Bogamuwa	WL_3 WL_3 WL_3	<1875 >1500 1500 >1500
Ambakelle	Alluvial soils	Sedawatta Arachchikattuwa Pottukulama	DL_1 IL_1 IL_3	750-875 1000-1125 875-1000
Wariyapola	Non calcic brown soils	Kobeigane Awulegama Wariyapola	IL_3 IL_3 IL_3	<875 <875 <875
Maho	Non calcic brown soils	Alahena Wannigama Wariyapola	IL_3 IL_3 IL_3	>875 <875 >875
Melsiripura	Reddish brown latosolic soils	Galwarama Adirillawatta Panliyadda	IL_1 WL_2 DL_1	>1000 >1875 >750
Warakapola	Red yellow podzolic soils	Arangalakanda Wewaldeniya Neligama	WL_2 WL_2 WL_2-WL_3	1500-1875

Soil series	Major soil type	Locations	Agro-ecological region	75% of the expected rainfall
Kurunegala	Red yellow podzolic soils	Metiyagane Yaggapitiya Ambanpola	IL_1 IL_1 DL_1	750-875 750-875 <875
Kuliyapitiya	Red yellow podzolic soils	Amangalla Metiyagane Katupotha	IL_1 IL_1 IL_1	>1000 750-875 1125>1000
Pallama	Colluvial members of red yellow podzolic with soft or hard laterites	Alabodagama Divulapitiya Bogamuwa	WL_3 WL_3 WL_3	>1750 <1875>1500 >1500
Weliketiya	Sandy regosols	Karambe Kadiyamutte Marawila	DL_3 DL_3 IL_1	500-750 500-750 1000
Tambarawa	Non calcic brown soils	Koorikulama Kadigawa Welipennagahamula	IL_3 IL_3 IL_1	875-1000 875-1000 1000
Kalpitiya	Sandy regosols	Kurunchipitiya Palakudawa Nawakkaduwa	DL_3 DL_3 DL_3	500-750 500-750 500-750
Negombo	Sandy regosols	Kandatoduwawa Karukapana Modarawella	DL_1 IL_1 IL_1	500-875 875-1000 1000
Gambura	Red latosols	Vijayapura Bodhirajapura Sirambiadiya	DL_3 DL_3 DL_3	575-750 575-750 575-750
Katunayaka	Latosols and regosols	Welihena Ekala Kadirana	WL_4 WL_4 WL_4	>1500 >1500 >1500

Sampling procedure :

Soil samples were taken from the manure circles (within 1.75 m radius from the base of the palm). Four samples of soil from 0 - 15 cm were drawn from four positions of each manure circle and mixed and prepare a composite sample. Sampled soils were passed through 2 mm sieve and mixed well to get a composite sample and were stored at 0-4 °C in polythene bags until isolation of microorganisms .

Isolation of Microorganisms

For the isolation of both fungi and bacteria, first, the Dilution Plate technique was carried out under aseptic conditions using Potato Dextrose Agar and Nutrient Agar, respectively (Parkinson, *et al.,* 1971). Colonies of different external appearance, from mixed population, were streaked on separate Nutrient Agar media. Pure cultures were then obtained by re-streaking those

colonies several times in same media. Bacterial identification was carried out using pure cultures obtained from morphologically different bacterial colonies grown in nutrient agar. Later they were subjected to basic morphological tests (colony morphology, pigmentation, staining characteristics, shape and arrangement of cells, motility, presence or absence of spores) and thereafter bio-chemical characteristics were tested (Cowan and Steel, 1974, Sneath, 1986). Identification of fungi was carried out following the keys and by the microscopic examinations of the structures (Onions, *et al.,* 1981).

RESULTS AND DISCUSSION

Identification of bacteria

The bacteria identified in different soil series are presented in Table 2. Different types of bacteria i.e. *Bacillus spp, Micrococcus spp, Staphylococcus sp, Enterobacter sp, Serratia sp, Corynebacterium sp, Pseudomonas sp* and *Actinomycetes sp* were found. Most of these bacteria are commonly found in tropical soils. *Bacillus spp* were the dominant bacteria in all the soil series. In addition to *Bacillus species, Micrococcus luteus, Enterobacter cloacae* and *Pseudomonas aeruginosa* were also prominent in most of the soil series. Some of bacteria were specific to some soil series, i.e. *Bacillus thuringiensis* was found only in Katunayake soil series; *Aerococcus viridans* was found in only Weliketiya soil series; *Bacillus pasteurii* was found in only Pallama soil series. A greater number of bacterial species was found in soil series such as Rathupasa, Kalpitiya, Ambakelle, Boralu, Katunayaka and Melsiripura which can be considered as fertile or soil series with high nutrient availability.

Identification of fungi

Fungal species identified in different soil series under investigation are shown in Table 3 and some common fungi species are shown in Plates 1 to 8. Fungal species such as *Penicillium, Aspergillus, Mucor, Rhizopus, Absedia, Syncephalastrum, Mortierella, Curvularia, Humicola, Ulocladium, Fusarium* and *Trichoderma* were present in most of the soil series and *Penicillium spp, Aspergillus spp* and *Curvularia spp* were the most abundant species in the coconut growing soils. *Aspergillus japonicus* and *Aspergillus niger* were found in all soil series while *Penicillium frequentans* was the next most common.

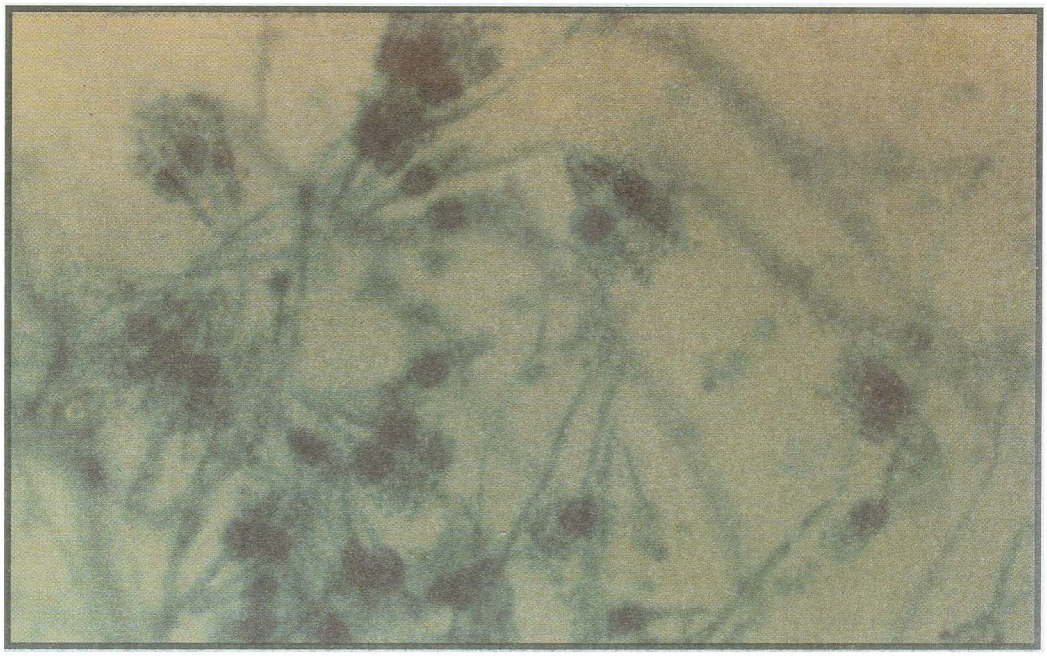
Table 2 **Bacteria identified in different soil series**

Bacterial species	Soil series																			
	Wilattawa	Andigama	Weliketiya	Gambura	Kalpitiya	Negombo	Kuliyapitiya	Rathupasa	Madampe	Sudu	Wariyapola	Ambakelle	Tambarawa	Maho	Warakapola	Pallama	Boralu	Katunayaka	Kurunegala	Melsiripura
Bacillus cereus				x	x			x					x							
Bacillus thuringiensis																		x		
Bacuillus sphaericus				x	x						x	x		x			x	x		
Bacillus megaterium		x			x			x	x		x		x					x		
Micrococcus varians					x	x					x		x							x
Bacillus circulans				x			x			x		x							x	
Micrococcus luteus			x		x								x	x						x
Staphylococcus epidermidis							x					x								x
Aerococcus viridans					x															
Alcaligenes odorans						x														x
Chromobacterium vialaceum								x									x			x
Bacillus alvei								x				x	x				x			
Actinomycetes sp	x							x	x	x		x						x		x
Bacillus insolitus												x				x	x	x		
Enterobacter cloacae		x						x	x		x			x						
Serratia marcescens									x											
Corynebacterium hofmanii			x												x	x				
Pseudomonas aeruginosa							x		x			x				x	x	x	x	
Bacillus licheniformis	x	x						x									x	x	x	
Bacillus subtilis	x		x					x		x		x			x					
Bacillus firmus	x	x						x							x	x				
Bacillus pasteurii																x				
Bacillus mycoides				x	x	x				x							x			
Bacillus polymyxa					x	x							x							
Bacillus brevis				x				x	x		x			x			x	x	x	x

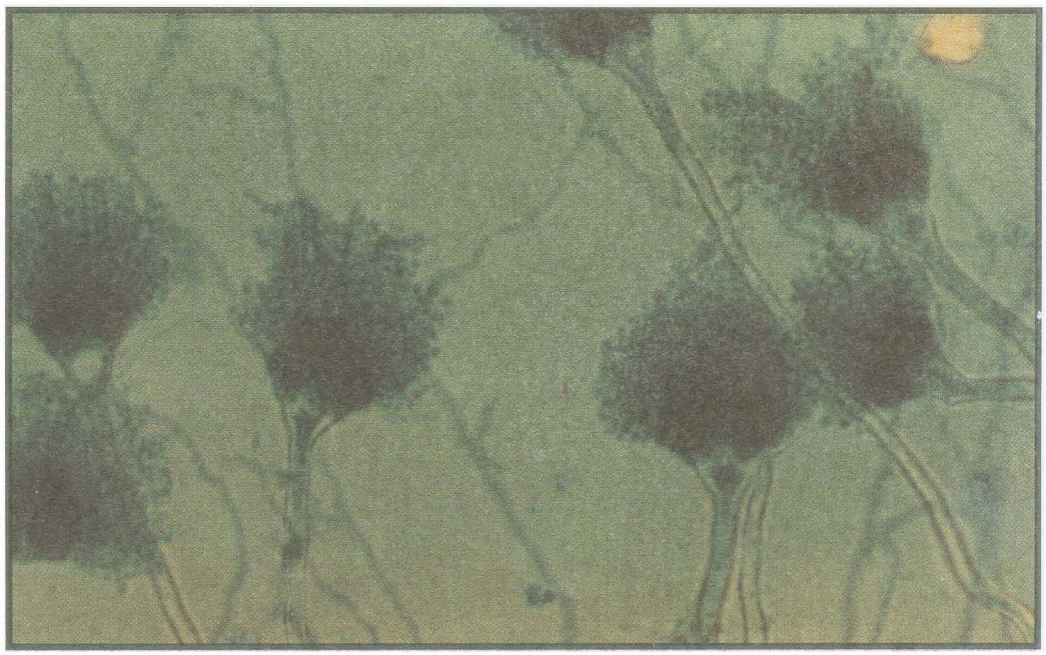
Table 3 **Fungi species identified from different soil series**

Fungi species	Wilattawa	Andigama	Weliketiya	Gambura	Kalpitiya	Negombo	Kuliyapitiya	Rathupasa	Madampe	Sudu	Wariyapola	Ambakelle	Tambarawa	Maho	Warakapola	Pallama	Boralu	Katunayaka	Kurunegala	Melsiripura
Syncephalastrum			x	x				x	x			x						x	x	x
Fusarium oxysporum					x			x	x			x								
Penicillium citrinum	x				x	x										x				
Penicillium oxalicum						x									x	x				
Aspergillus terreus			x								x									
Fusarium moniliforme											x		x							
Penicillium sp	x		x	x			x	x	x	x				x	x		x			
Aspergillus sp	x				x															
Penicillium citreonigrum								x										x		
Aspergillus niger	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Aspergillus japonicus	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Aspergillus flavipes							x	x	x				x							
Aspergillus ochraceus			x	x																
Absedia sp								x	x			x					x	x		
Penicillium restrictum						x												x		
Mortierella sp		x						x	x			x				x				
Curvularia sp	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Humicola sp				x	x															
Mucor sp							x	x				x				x		x	x	x
Rhizopus sp								x											x	x
Ulocladium sp									x											
Trichoderma sp																	x	x		x
Penicillium frequentans	x	x			x				x	x	x			x				x		x

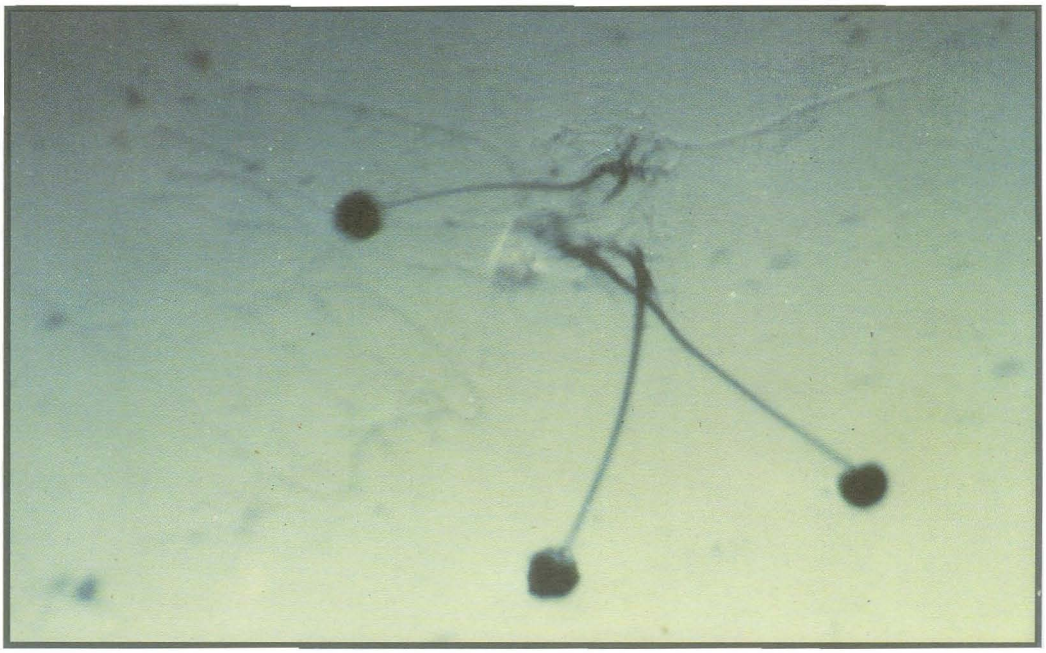
Some fungi species present in the soil



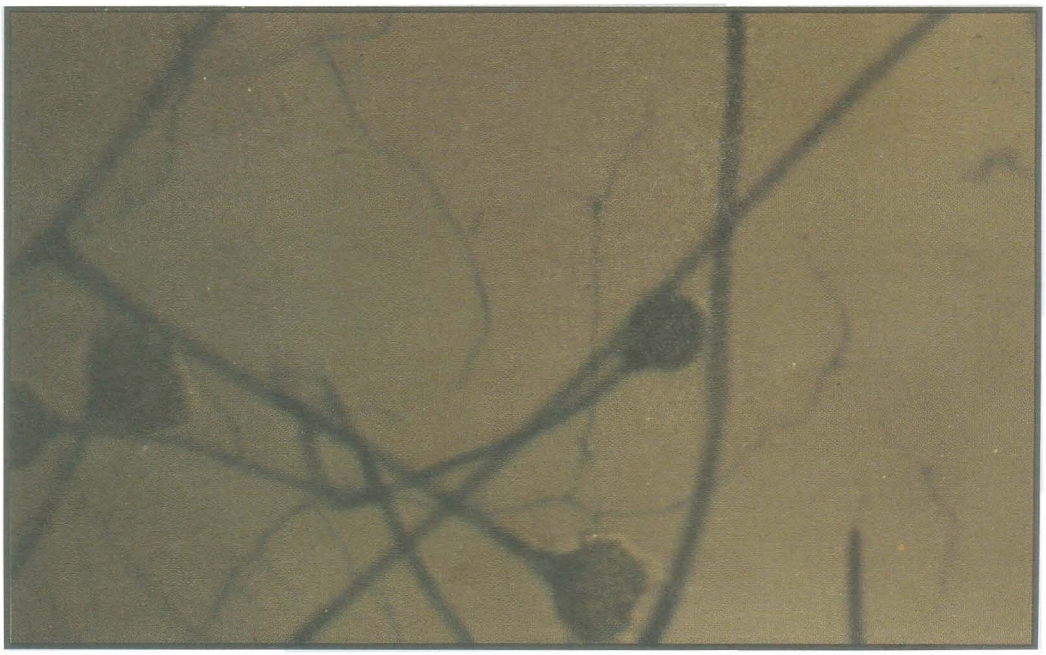
Penicillium species



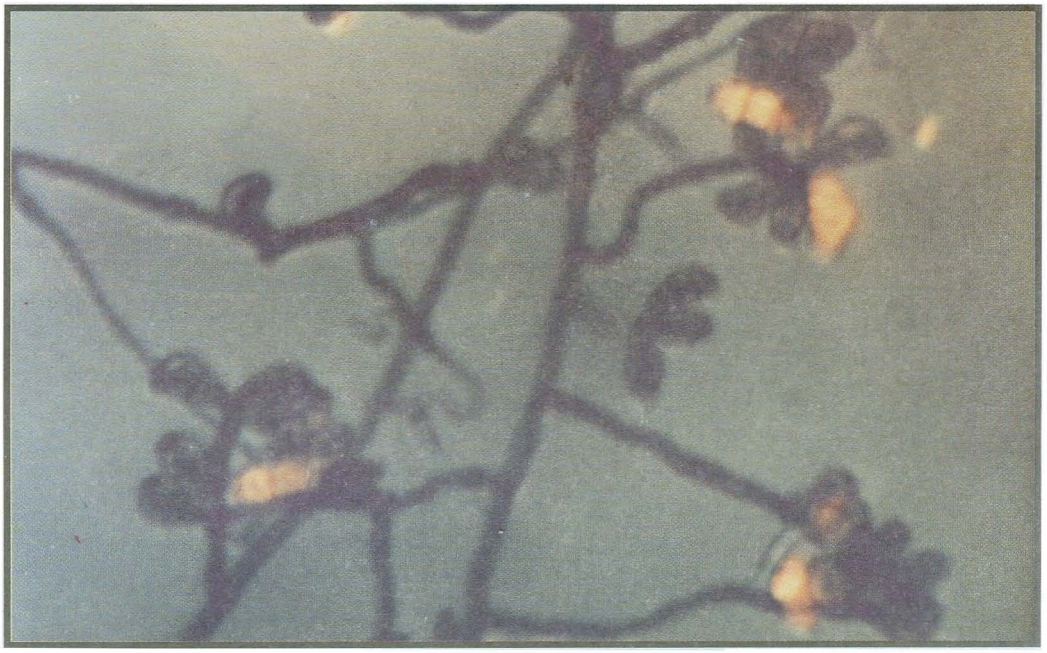
Aspergillus species



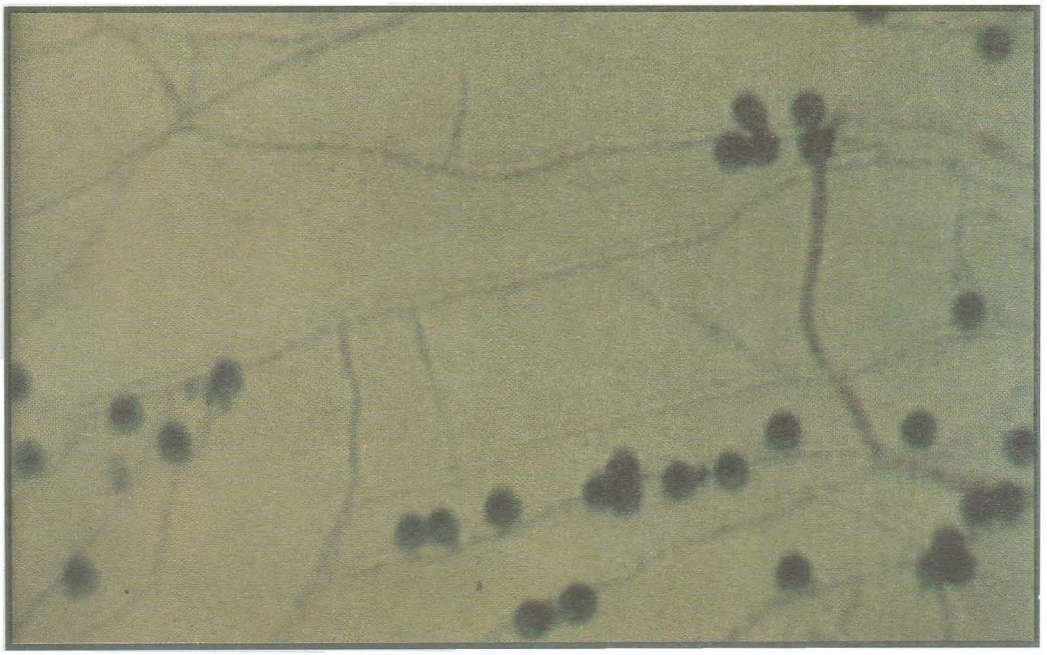
Rhizopus species



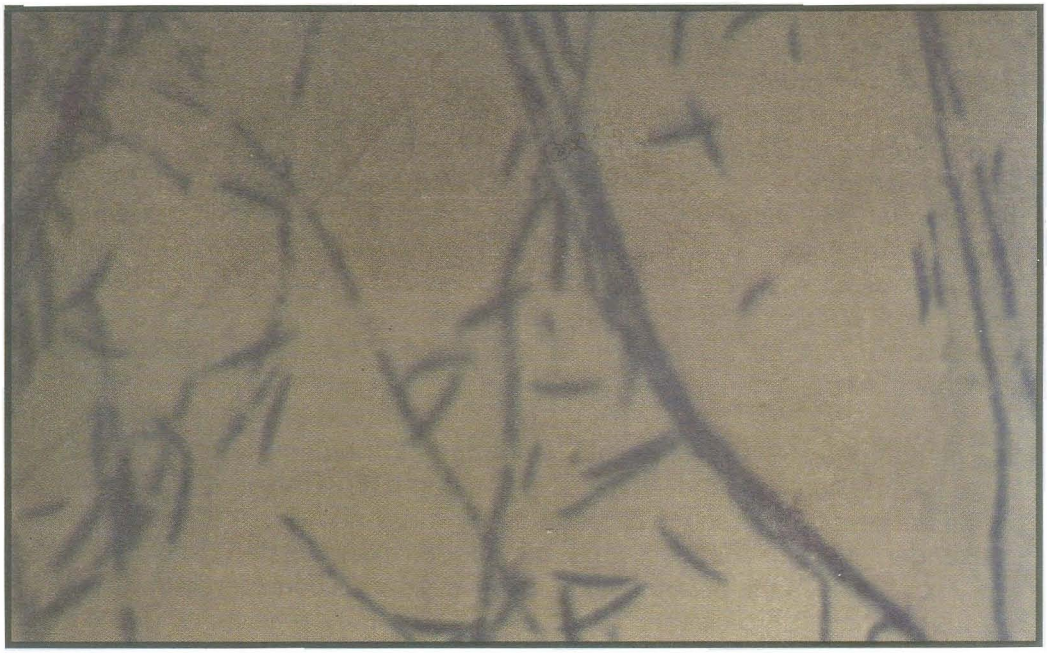
Mortierella species



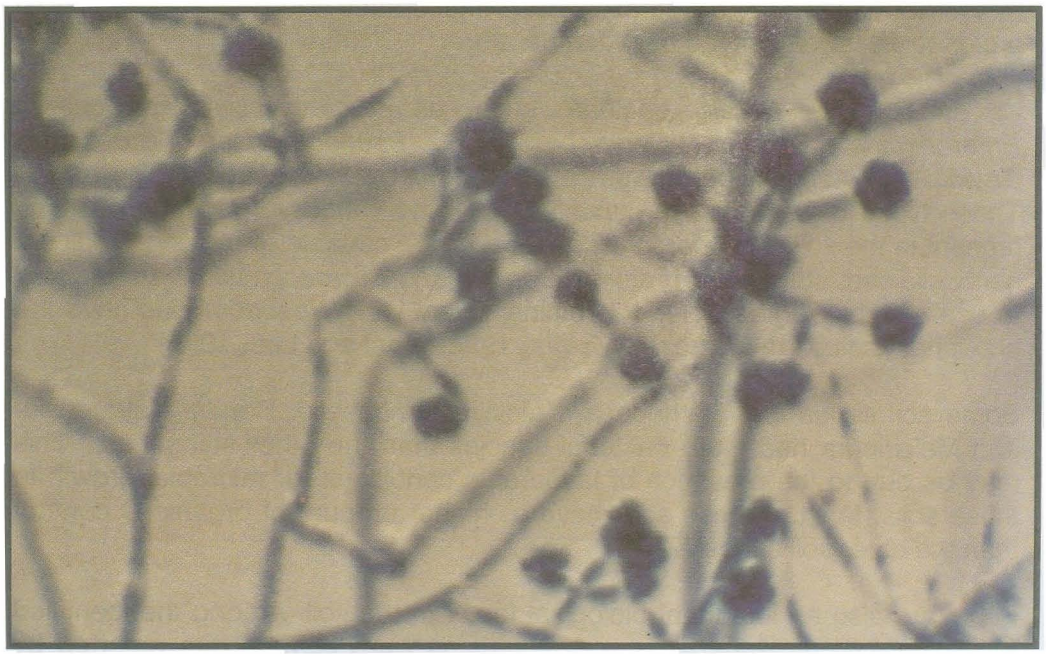
Curvularia species



Humicola species



Fusarium species



Trichoderma species

Some fungi species were specific to some soil series i.e. *Trichoderma sp* in Pallama, Boralu and Melsiripura soil series. *Ulocladium sp* in Rathupasa and Madampe soil series; *Rhizopus sp* in Rathupasa, Kurunegala and Melsiripura soil series; *Penicillium citreonigrum* in Rathupasa and Katunayaka soil series; *Penicillium oxalicum* in Negombo, Warakapola and Pallama soil series; *Aspergillus terreus* in Weliketiya and Wariyapola soil series; *Fusarium moniliforme* in Wariyapola and Thambarawa soil series; *Aspergillus ochraceus* in Weliketiya and Gambura soil series.

More fungal species were found in Rathupasa, Madampe and Ambakelle soil series which can be considered as fertile soils or having more available nutrients than the other soil series.

Soil microorganisms are of critical importance in nutrient cycling processes, and also as sources and sinks of plant nutrients. Bacteria are the most prominent among the soil microbes usually more numerous than the others (Alexander, 1977). *Bacillus* were the predominant bacteria isolated from every soil series in the present study. The soil series considered in this study are from various agro-climatic zones and vary in their physical and chemical properties. Some of the soil series had favourable properties for microbial growth while others did not. But *Bacilli* were found in both categories. This is probably because *Bacilli* has the ability to persist in unfavourable conditions by the formation of endospores. These endospores show resistance to both prolonged desiccation and high temperature.

In addition to *Bacilli*, bacteria such as *Micrococci, Staphylococci, Enterobacter, Serratia, Corynebacterium, Pseudomonas, Aerococcus, Erwinia* and *Actinomycetes* were also commonly in coconut growing soils. These types of bacteria are capable of utilizing various organic compounds present in the soil. *Pseudomonas* and *Bacilli* are capable of utilizing cellulose, hemicellulose, starch and proteins; *Serratia* and *Micrococcus* have the ability to use starch; *Corynebacteria* proteins; *Bacilli* and *Pseudomonas* have the phosphate solubilization ability (Verma, 1998; Wani and Lee, 1995).

Some soil series e.g. Rathupasa, Kalpitiya, Ambakelle, Boralu, Katunayake and Melsiripura had more bacterial species than the other soil series. This may be due to the presence of favourable conditions for microbial growth in these soils. Most of these soils have a higher amount of organic C content than the other soil series.

Fungi are also an important biological factor in the soil. Among the identified fungi, *Penicillium*, *Aspergillus* and *Curvularia* were common as reported by Cosico (1994). Fungi such as *Mucor, Rhizopus, Absedia, Syncephalastrum, Mortierella, Humicola, Ulocladium* and *Trichoderma* were also found in this study. Anderson and Anderson (1986) reported that fungi are the main

cellulose decomposing organisms and these include *Trichoderma, Fusarium, Penicillium, Aspergillus* and *Curvularia.*

Some soil series have shown more fungi species. This may be due to the favourable environmental conditions of these soils which affect the growth of fungi.

CONCLUSION

Some bacteria and fungi were specific to certain coconut growing soils i.e. *Bacillus thuringiensis* only in Katunayake series, *Staphylococcus epidermidis* in Kuliyapitiya and Ambakelle and Melsiripura series, *Alcaligenes odorans* only in Negombo and Melsiripura series; *Serratia marcescens* only in Madampe soil series. Fungal species *Trichoderma* species in Pallama, Boralu and Melsiripura soil series; *Ulocladium* sp only in Rathupasa and Madampe series; *Rhizopus* species in Rathupasa, Kurunegala and Melsiripura soil series. Growth of bacteria and fungi are mediated by environmental condition. Therefore certain type of microorganisms grow in environments where they have favourable conditions. Soil series differ from each other in chemical and physical properties. Therefore according to their physical and chemical properties specific microorganisms were found in specific soil series.

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