STUDIES ON RHIZOSPHERE MICROFLORA OF *ACANTHUS ILICIFOLIUS*

Dissertation submitted by Kum. MINI RAMAN in partial fulfilment for the Degree of Master of Science (Mariculture) of the Cochin University of Science and Technology

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CERTIFICATE

This is to certify that this dissertation is a bonafide record of work carried out by Kumari. MINI RAMAN under my supervision and that no part there of has been presented before for any other degree.

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C O N T E N T S

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PREFACE

Growing general interest in marine mangrove and estuarine habitats in recent years has also led to an increase in studies pertaining to marine microflora which were often overlooked as 'participants' in the ecological processes. The biological agents of decomposition in the mangroves are indigenous heterotropic bacteria, fungi and actinomycetes.

The microflora are influenced by the structure and texture of mangrove swamps, the humic fraction and the amount of mineralizable organic matter. Microflora from water and sediments of mangrove area has been studied by many authors. However, information on rhizosphere microflora of a tropical mangrove plant Acanthus illicifolious are not available in the Cochin area

The characteristic soil community is distinctly different from the rhizosphere microflora which are constantly affected by the natural exudation of allelo-chemicals from the roots. Naturally exuded allelochemicals also serve to reduce tissue damage in roots and also check pathogens and other grazing organisms. The plant in turn is markedly affected by the population it has stimulated since the root zone is the active site through which inorganic nutrients are obtained.

By quantitative estimation of total microbial population it appears to be possible to determine the dynamic state of the rhizosphere microflora and to measure the biological activity of microorganism in their natural habitat. Determinations of

the activities of the hetrotropic microflora and the study of the physical and chemical factors influencing such activity may be of value as they help to obtain a better understanding of the relations between the productivity and the biological fertility of the mangrove soil which in turn will have an effect in the nearshore marine environment, as inshore environment is always enriched by nutrients from the mangrove sediments by the incoming and outgoing tidal action.

The present study was made to know whether there is any difference in the seasonal distritution of microflora from rhizosphere and the non-rhizosphere mangrove environment. Investigations were also made on the seasonal variation of environmental parameters such as temperature, pH, Eh organic carbon content, available nitrate and available phosphate in the rhizosphere and to study the significant relationship if any between these phyico-chemical factors and the distribution of microflora in these environment.

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INTRODUCTION

The mangrove ecosystem comprises a group of floristically diverse trees and shrubs which characterize the intertidal vegetation of many tropical and sub-tropical areas. Mangroves are one among the several specialized marine ecosystems in which the productivity at different trophic levels and energy flow assume unusual importance as it has direct influence in enriching the inshore environment. Although the occurrence of mangroves is limited, the ecological role of this ecosystem has been recognized (Heald & Odum, 1962). Mangrove swamps comprising of foliage as a major organic material support a detrital type of food chain in the tropical marine environment. (Heald and Odum, 1962).

Concern about mangroves has grown steadily in recent years by the increased awareness about its ecological significance and benefits to mankind. Many aspects of this ecosystem are still unknown. Limited investigations are being made in India during the past on ecology, phyto-geography, microbiology, forestry etc. of mangroves. Some observations on the distribution, ecology and the environmental features of the mangroves from two major

estuarine systems of Goa have been reported by Untawale et. al. (1973). Dwivedi et.al. (1973) studied the ecology of mangrove swamps of the Mandovi estuary, Goa. Untawale et. al. (1977) studied the structure and production in a detrital rich estuarine mangrove swamp in Kollur estuary near Coondapoor (Karnataka) along the central west coast of India. The distribution of trace elements in the Pichavaram mangroves was done by Ramdhas et. al. (1975). The distribution of heterotrophic bacteria of mangrove ecosystem in the area Cochin was studied by Surendran (1985).

Acanthus illicifolius is a gregarious ever green under shrub which grows about a metre high. They are characterized by their dense, coriaceous spinous, holly like leaves and big blue flowers which are abundant from June to August. It may be found as pure isolated strands around pools rather deeply inland apparently isolated from any possible marine invasion. Muralidharan (1984) studied the colonisation of Acanthus illicifolius in relation to various physico-chemical parameters, in Cochin.

Published reports and studies on the microflora from mangrove environment are very few. Matonodkar et. al. (1981) studied seasonal variation of microflora from mangrove swamps of Goa situated along the Mandovi-Zuari estuary. Studies were conducted on heterotrophic bacterial flora by the same authors in 1981. Venkatesan and Ramamurthy (1971)

conducted marine microbiological studies of Mangrove swamps of Killai backwaters and reported the presence of physiologically active groups of bacteria. Matonodkar (1981) observed similar physiologically active groups from mangrove swamps of Goa. Hummadkar and Agate (1985) isolated 21 bacterial species from mud and water collected from mangroves of Sindhudurg and Malvan area in Konkan, Maharashtra. Chandrika et. al. (1985) encountered green sulphur bacteria responsible for detritus decomposition from mangrove mud of Karuthedum near Cochin. Rublee (1981) studied the seasonal distribution of bacteria in salt marsh sediments in North Carolina.

Quite a lot of investigations has been carried out on the fungi in tidal salt marshes. Recent mycological investigations indicate that fungi may be as important decomposers in salt marshes as are the bacteria. Gessner, (1977) examined the seasonal occurrence and distribution of fungi on aerial, parts of the salt-marsh plant Spartina alterniflora in Rhode Island estuary. Other ecological surveys in Rhode Island (Gessner, 1977) also concerned fungi active in the decomposition of Spartina alterniflora. Apinis and Chesters (1964) studied the Ascomycetes colonizing various halophytes of salt marshes and sand dunes in England. (Pugh, 1962) studied the mycorrhizal associations in salt-marsh plants.

Mason (1928) found mycorrhiza in species of Agrostis, Armeria, Aster and Plantago. Gessner and Kohlmeyer (1976) studied the influence of salinity and latitude on the occurrence of certain common fungi on Spartina alterniflora from Canada to Argentina. The same observations were made with fungi on Salicornia spp. in Europe, North America and South America, and Bermuda (Kohlmeyer and Kohlmeyer, 1977).

Similar investigations have been carried out on the fungi inhabiting the mangrove vegetation or 'mangal' which is the tropical counterpart of tidal salt marshes of temperate regions. Cribb and Cribb (1956) in Australia were the first mycologists to collect marine fungi from mangroves. Several others reported on the higher and lower species from different tropical areas (Kohlmeyer, 1969). Knowledge on marine manglicolous fungi is limited in spite of wide distribution and importance of mangrove trees in tropics. Newell (1976) extensively studied the microbial colonisation of mangrove seedlings and investigated the succession of fungi on submerged seedlings of the mangrove species Rhizophora mangle. Heald and Odum (1962) determined the detritus products as being over 3 metric tons (dry weight) per acre per year from mangrove leaf-fall alone in a South Florida estuary. According to these authors, mangrove twigs, bark and leaf scales are less important contributors than leaves to the food web. The role of micro organisms

in the breakdown of the leaves has been studied by Fell and Master (1973) and Fell et. al. (1975) examined the the activities of higher and lower fungi in the degradation of R. Mangle leaves. Swart (1958) did the first comprehensive studies on fungi of soil under and east African mangrove vegation. Ulken (1972) isolated phycomycetes from mangrove sediments in Brazil and Hawaii, and Lee and Baker (1972) isolated soil microfungi from a Hawaiian Mangrove swamp investigated. Other reports deal with the description of single species isolated from mangrove soils, for example that by Swart (1970) on a pencillium from Australia. Kohlmeyer (1969) observed the vertical and horizontal zonation of manglicolous fungi and reported that no distinct pattern of vertical distribution of Ascomycetes was observed.

Only very few reports on the manglicolous fungi are available in India. Investigations on Indian mangalvsoils were conducted by Pawar and Thirumalachar (1967). Other reports deal with the descriptions of a single species isolated from mangrove soils, for example, that by Rai and Tewari (1963) on Preussia isolates, and by Pawar et. al. (1967) on Phoma spp.

Published works on marine actinomycetes are few and still fewer are the studies on actinomycetes in mangroves.

The term Rhizosphere was introduced in 1904 by the German Scientist Hiltner to denote that region of the soil which is subjected to the influence of plant roots. Rhizosphere effect indicates the overall influence of plants roots on soil microorganisms. The rhizosphere is not a uniform part of the soil but a zone with physical, chemical and microbial gradients.

There are few reports on the rhizosphere in the mangrove environment eventhough the studies and investigations are mainly limited to agriculture as the rhizosphere have considerable significance for crop production and fertility. Zuberer studied the ultrastructure of the rhizoplance of naturally occuring plants of three Florida Mangrove species (Rhizophora mangle, red mangrove; Avincennia germinans stern; black mangrove and Laguncaria racemosa white mangrove) by transmission electron microscopy. These investigations were undertake to obtain information as to the nature of the association between plant roots and epiphytic bacteria in an effort to explain the nitrogen fixation activity which has been observed with washed roots of these plants.

Considerable work has been done on the rhizosphere of crop plants. Rovira and Davey (1974), demonstrated the validity of rhizosphere in terms of microbiological and chemical gradients. Their studies showed that the roots of

plants are frequently surrounded by a muicilaginous layer varying in composition from a relatively simple oligosaccharide to a complex pectic acid polymer permeated by loose cellulose microfibrils. Fine structure studies on the epithelial layer of plant roots after inoculation with specific bacteria have shown that the microorganisms get embeddedd in the surface of the root with the help of the mucilaginous external layer or mucigel.

Among the rhizosphere microflora bacteria are the predominant ones. Although most bacteria are stimulated by roots some are most reponsive and outstrip their competitors in the race for the nutrients provided. Several genera of bacteria - Pseudomonas, Flavobacterium, Arthrobacter, Agrobacterium and others have been reported to be either abundant or sparse in rhizosphere. Direct light microscopy of the microorganisms on roots has shown that only 5-10% of the root surface is covered by microorganisms (Bowen, 1976). It was observed that rhizosphere effect, which is the ratio of the number of microbes/gm. of rhizospheresoil is greater for bacteria.

Rouatt and Katznelson (1961) showed that Pseudomonas sp. were dominant in rhizosphere while Arthobacter sp. were characteristic of root free soil. Furthermore they showed that relative incidence of Pseudomonas species also increased

on the root surface where they could out grow and inhibit Arthrobacter in the presence of extracts from soyabean roots. Brown et. al. (1973) studied the microbial populations and nitrogen in soil growing consecutive cereal crops. It was noticed that bacteria are strongly influenced by the nitrogen status of their host. This fits with the evidence that plants supplied with extra mineral nitrogen exude more amino acids (Bowen, 1969) and that many rhizosphere bacteria are amino acid users (Gray and Williams, 1971). Loutit and Brooks (1970) studied the rhizosphere organisms and molybdenum concentrations in plants. Zagatto and Katznelson (1957) observed the metabolic activity of bacterial isolates from rhizosphere and control soil. Vancura and Macura (1962) studied the effect of foliar application of some readily metabolised substances, growth regulators and antibiotics on rhizosphere microflora.

Several workers have studied the colonization of roots by the bacteria. To understand the dynamics of colonization of roots several workers adopted the use of model systems in which the growth rates once around the roots are measured. Bowen and Rovira (1976) stressed the need of 'generation time' of bacteria on roots as the basis for studying the dynamics of colonization. Bowen and Foster (1973) eliminated the problem of different colonization times on parts of roots of different ages by planting axenically grown roots into soil and counting

the bacteria on selective media at intervals. The application of pattern analysis to microbial microflora on roots has shown irregular distribution with the bacteria aggregating on two scales viz., small clumps and also larger aggregations. Several works has shown that aggregation is associated with cell junctions (Rovira, 1956).

Less attention has been paid to actinomycetes in the rhizosphere although they are quite numerous on active roots. Most of the studies has been concentrated on detecting antagonistic actinomycetes producing antibiotics which inhabit root pathogens. Studies on the types of actinomycetes found in the root region revealed that they are similar to those from the root free soil, usually Streptomyces and Nocardia species predominate. The physiological activities of actinomycetes from rhizosphere and non-rhizosphere soil of several plants were compared by Abraham and Herr (1964).

Considerable attention has been paid to the root-surface inhabiting fungi during the past and many workers have been able to define a distinct flora of Fusarium species, Cylindrocarpon radicicola and non sporing isolates being among the dominant forms. Taylor and Parkinson (1965) studied the colonisation of the root surface cortex and stele of dwarf bear roots and reported that some fungi confined to the surfaces of young roots were able to penetrate tissues of older roots.

Root exudates play an important role in the development of microflora. Literature indicates that plant stress increases root exudations which would itself increase the associated bacterial flora (Rovira, 1969). (Rovira 1965) pointed out that fungi and actinomycetes do not respond to plant root exudates as dramatically as bacteria.

The qualitative and quantative nature of microbial populations around roots are influenced by many factors. Starkey (1918) in a series of important papers showed that rhizosphere effect depended upon the type of plant, age of plant, health of plant, the position and type of root and the soil type and environment.

The effect of environmental factors like high light intensity and temperature on the microflora was studied by Rovira (1956) who reported that high light intensity and high temperature increases the stimulation of bacteria while a decrease has been shown to decrease the rhizosphere stimulation of bacteria. Peterson (1961) was unable to detect any effect of different light intensities on fungi but reported that High temperatures led to a stimulation of certain fungi (especially the nonsporing hyaline forms) and decreased bacterial numbers. The effect of soil type on the rhizosphere microflora was studied by Abraham and Herr (1964).

Gibberllin and gibberllin like substances are known to be produced by bacterial genera commonly occuring in the rhizosphere such as Pseudomonas, Azotobacter, Arthobacter. Broadbent et.al. (1971) studied the bacteria and actinomycetes antagonistic to fungal roots pathogens in Australlian soils. Cook (1986) studied the fungal-bacterial interaction in the root region.

The existence of assured energy source makes the rhizosphere a habitat more or less independent of fluctuation in substrate availability, which is the major limiting factor for the heterotrophic soil microflora. The loss of photosynthate as root exudate enhances the growth of chelate producing microbes and facilitates the solubilisation of primary minerals which act through a positive feed back mechanism to increase the productivity of the ecosystem.

MATERIALS AND METHODS

Study areas

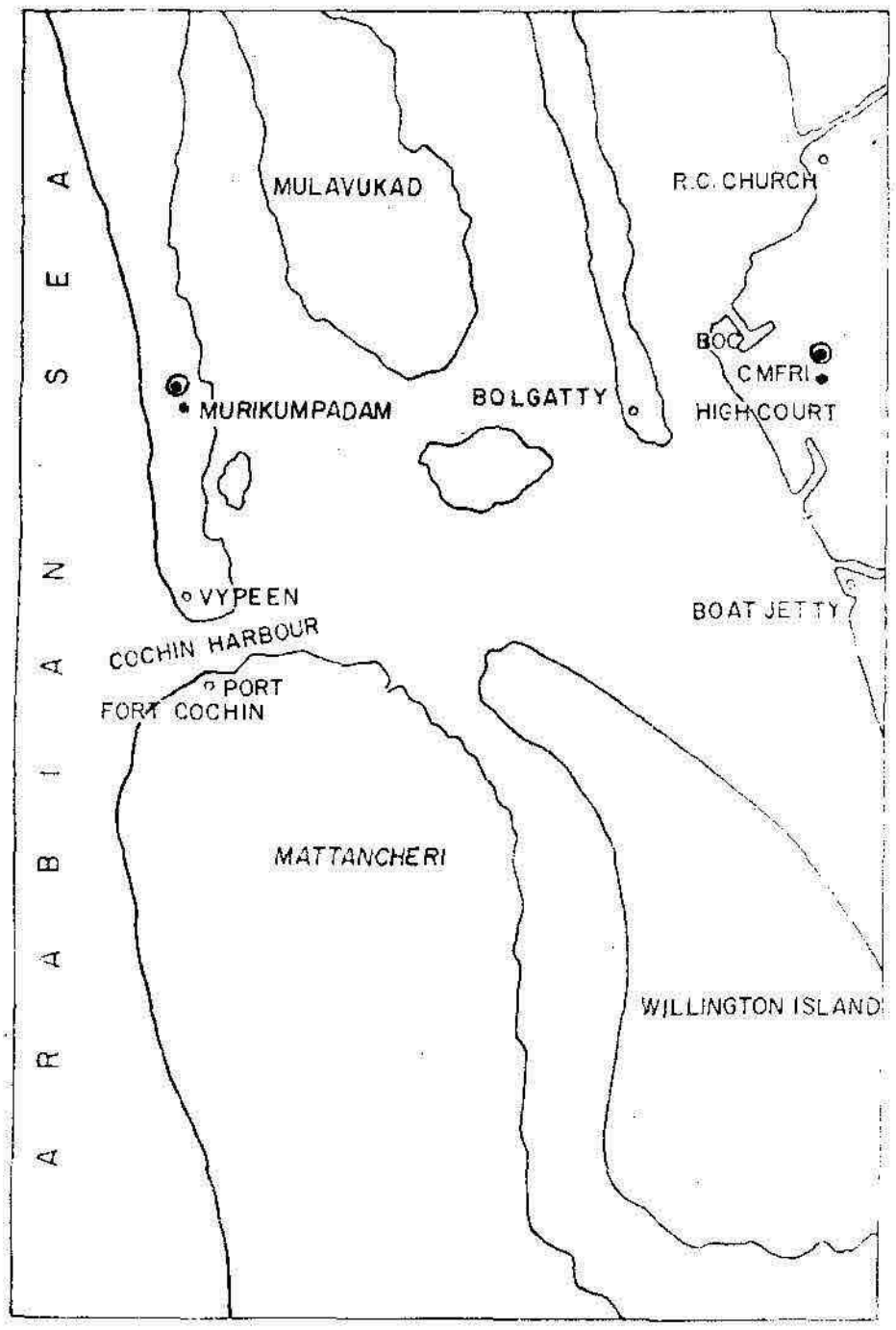
The studies were conducted for a period of 5 months, during June to October 1986. Two sites were selected for this purpose, one around Murikkumpadam area in Vypeen Island near Cochin and another near the Cochin backwaters in front of the Central Marine Fisheries Research Institute. The site selected in the Murikkumpadam area is a typical tropical mangrove ecosystem with an intricate network of channels, creeks and canals and is influenced by the tide. This typical mangrove ecosystem harbours an abundant and varied flora of mangroves. The other site near Cochin backwaters represents a mangrove habitat comprising mainly of Acanthus illicifolius and Avicenia officianalis This site was always influenced with tidal inundation (Fig-1)

Parameters of study

Quantitative and qualitative studies on the three predominant microflora namely bacteria, fungi and actinomycetes in the rhizosphere of the mangrove species Acanthus illicifolius were carried out for a period of 5 months (June-October, 1986) using different selective media.

The effect of variations of some important environmental parameters such as soil temperature, pH, Eh, salinity, organic carbon, available nitrate and available phosphorous on the microfloral population were also studied.

Fig.1 - Map of Vypeen Island and Cochin showing the study areas (Station I and II)



Collection of samples:

Fortnightly samples were collected from the 2 sites throughout the period of study. Sampling were done between 0700 and 0900 hours invariably in all sampling dates. The plant was carefully removed from the field and the superflous soil was dislodged by gentle agitation. Under aseptic conditions the plant with its root system intact was carefully and quickly transferred to sterile polyethene bags. Samples were taken randomly from each site. Care was taken not to contaminate the soil samples.

Soon after collection the samples were transported immediately to the Bacteriological Laboratory. They were then subjected to bacteriological investigations within three hours of sampling.

Bacteriological investigations:

Both quantitative and qualitative analysis of microflora were done in the rhizosphere samples of the above mentioned sites during the period of study (June to October, 1986).

Quantative Analysis

1. Enumeration of total viable bacteria, fungi and actinomycetes:

The viable count of total bacteria, fungi and actinomycetes present in the rhizosphere was determined by pour plating method (Rodina, 1972) using selective media.

a) Enumeration of total viable microorganisms in the rhizosphere

(i) From the surface of the root - Under aseptic conditions each plant was taken out from the polythene bag and held by the stem with the help of a sterilised scalpel. The adhering soil was carefully removed and placed in a sterilised container with a lid. The roots were given a few washings with sterile water (Harley and Waid, 1955). The washed roots were cut and placed in a petridish containing sterile water. Small pieces approximately 1cm. in length were cut up with a sterilised scalpel. The pieces were transferred to a sterile mortar and covered by a petridish lid to prevent contamination. The pieces were slightly curshed with a sterile pestle (Stover & Saite 1953; Clarke & Parkinson, 1960). It was transferred to a container containing 99 ml of sterile aged water which was collected from the same ecosystem. After shaking for ten minutes serial dilutions were made by adopting standard procedures given by Rodina (1972). One ml of the inoculum was transferred to 10mm diameter sterile glass petridishes and pourplated. The sea water agar medium was used for pour plating.

(ii) From the rhizosphere soil - Approximately 1 gm of the soil sample was aseptically transferred to a sterilised glass mortar, ground well with pestle and mixed well with 99ml of sterile aged water collected from the same ecosystem. After through mixing and shaking serial dilutions were made as mentioned above and 1ml of the inoculum was transferred to petridishes and pourplated with seawater agar.

The plates were incubated at room temperature ($28 \pm 2c^0$) for 48 hours in a bell jar. The colonies developed in the petridis were counted after 3-4 days and represented on dry weight basis. All the innoculation proceduresweres carried out in an innoculation chamber sterilised with U-V radiation.

For the enumeration of fungi and actinomycetes selective media like mycological agar and starch casein agar were used adopting the above mentioned procedure. The actinomycetes developed were counted after 7-10 days, the fungal colonies after 5-6 days.

(II) Quantitative Enumeration of chitinolytic, ureolytic, proteolytic caseinolytic and lipolytic populations:

The various zymogenous populations were quantitatively estimated by pour plate method (Rodina, 1972) using selective media.

(a) Enumeration of chitinolytic population using mineral medium supplemented with Chitin precipitate (Aaronson, 1970). The composition of the media is as follows:

Mineral		Media
K_2HPO_4	-	1.0 gm
$Mg\ SO_4\ 7H_2O$	-	5 gm
Nacl	-	5 gm
$Fe(NH_4)_2\ SO_4\ 6H_2O$		.005 gm
Agar	-	15.0 gm
Water	-	1l

About 20gm of Chitin precipitate was supplemented to the medium till it became turbid and pH adjusted to 7.0. The inoculated media were incubated for 2 weeks in darkness at 20 C^{O}. Colonies of chitinolytic bacteria were recognized by the development of transparent halos surrounding the colonies.

6. Enumeration of ureolytic population using Christensen's Urea Agar. The composition of the media is as follows.

Peptone	- 1.0 g
KH_2 PO_4	- 2.0 g
D.Glucose	- 1.0 g
Agar	-20.0 g
Phenolred (.2% solution)	- 6.0 ml.
Water	- 1 L.
pH - 6.8	- 7.0.

The medium was sterilised by intermittent heating for 3 days and cooked to 50C°. 20% Urea solution previously sterilised by filtration through a membrane filter was then added to give a final concentration of 2%. The plates were incubated at room temperature (28 ± 2C°) for 7 days. Ureolytic activity was detected by the change in the colour of the medium from light yellow to pink.

c) Enumeration of proteolytic population using modified Franzier's gelatin agar (Harrigen & McCance, 1972).

Peptone	- 10.0 g.
Meat Extract	- 10.0 g.
Gelatin	- 4.0 g.
Agar	- 15.0 g.
Water	- 1 L.
pH	- 7.2

The medium was sterilized by autoclaving for 20 minutes at 15 C°. The inoculated plates were incubated at room temperature (28 ± 2C°) for 8 days and tested using mercuric chloride solution of the following composition.

Mercuric Chloride	- 15.0 g.
Sterilised water	- 100 ml.

The plates were flooded with 8-10 ml of the reagent. Unhydrolysed gelatin formed a white precipitate with the reagent. Gelatin hydrolysers were identified by the clear halos around the colonies.

d) Enumeration of caseinolytic population by using casein media (Harrigon&McCance 1972).

Peptone	- 10.0 g.
Meat Extract	- 10.0 g.
Casein	- 30.0 g.
Agar	- 15.0 g.
Water	- 1l.

The medium was sterilized at 15 lbs pressure for 15 minutes and inoculated medium was incubated at room temperature (28 ± 2C°) for at least 7 days. Caseinolytic colonies were detected by the appearance of clear zones around the colonies.

d) Enumeration of lipolytic population using Tween Agar medium (Harrigan and McCance, 1972). The composition of the media is given as

Peptone	- 10.0 g.
$Cacl_2$	- 0.1. g.
Tween 80 (Sarbitol monocleate)	- 10.0 ml.
Agar	- 15.0 g.
Water	- 1l
PH - 7.0	- 7.4

Medium was sterilised at 15 lbs pressure for 15 minutes. The inoculated plates were inoculated at room temperature($28 \pm 2C^{o}$) for 7 days. Lipolytic colonies were detected by the appearance of opaque zones surrounding them. Appearance of waxy material around the colonies was the identification of the liberation of insoluble oleic acid as a result of the lipase action.

Qualitative analysis

Since it was impossible to examine in detail all the colonies that grew on the count plate, a limited no was isolated for qualitative analysis.

The colonies selected from the primary plates were isolated and sub-cultured both in peptone broth and agar slants. Further purification was done by repeated streaking on the sea water agar medium of the same composition. All the purified isolates were subsequently examined for colony characteristics, cell morphology and gram stain. They were subjected to a series of biochemical tests for identification purpose. The various tests employed in the identification procedure are as follows:

Gram Staining

Hucker's modified technique was employed to stain the isolated strains. Broth cultures were used for staining the heat fixed smear.

Motility test: Hanging drop preparations were made and motility was observed directly under oil immersion objective.

Test for detecting nitrate reduction

This test was conducted to detect the reduction of nitrate by bacteria. The test was done by adding two drops of sulphanilic

acid and two drops of α- napthyl amine solution to a 24 hours peptone broth culture of bacteria. The presence of nitrite was indicated by a pink red colour and the presence of ammonia was confirmed by the orange brown precipitate when treated with nesseler's reagent.

Test for amylotytic activity of bacteria

The test was employed for the detection of the enzyme amylase in bacteria (enzyme involved in the hydrolysis of starch). The bacterial strain was streaked on a beef extract agar plate containing 2% of soluble starch and incubated at room temperature for 2 days. To make the test 2:3 iodine crystals were placed on the petridish cover and the petriplate was slightly warmed to trigger the vaporisation of iodine, The clear zone developed was taken as an indication of starch hydrolysis.

Test for detecting H_2S formation:

The enzymatic decomposition of proteins or peptones composed of sulphur containing amino acids by bacteria, results in the formation of hydrogen sulphide. The test was done by inoculating the bacterial culture in L-cysteine broth above which strips of filter paper, soaked with a saturated solution of lead - acetate were dried and suspended. The presence of hydrogen sulphide was indicated by black colour on the lead acetate paper. The colour was due to the formation of lead sulphide by the action of escaping hydrogen sulphide on lead acetate paper.

Hugh and Leifson's test or Oxferm test:

The test was employed to distinguish between oxidative and fermentative utilisation of carbohydrates. Carbohydrate media

was prepared with phenol red as an indicator in duplicate tubes and were inoculated with bacterial test strains. They were then incubated for 24 hours - one aerobically while the other one anaerobically by sealing the surface of the medium with 2cm of liquid paraffin. The results were observed as

Oxidative metabolism - acid in aerobic tubes only (Yellow Colour)

Fermentative metabolism - acid in both tubes.

The Catalase Test: Catalase is an enzyme capable of decomposing hydrogen peroxide into water and molecular oxygen and the enzyme is produced by all aerobic bacteria. The presence of the enzyme was detected by adding hydrogen peroxide to the bacterial culture and noting the evolution of oxygen.

Test of Sensitivity towards Pencillin

Antibiotic sensitivity of isolated bacterial culture towards pencillin was tested with pencillin discs (2.5 I.U/disc)

The Oxidase Test

The test was conducted to detect the presence of certain oxidases in the bacteria that will catalyse the transport of electrons between the electron donors in the bacteria and redox-dye-N' N' N' Tetramethyl paraphenylene diamine dihydrochloride. The dye was reduced to a deep purple colour within ten seconds in a positive reaction.

Gelatin Liquifaction Test:

Ability to liquify gelatin is a diagonistic criterian of same value because the proteolytic activity of a bacterial species may be measured in a gelatin medium (Rodina, 1972). The test was carried out by inoculating gelatin media with 24 hours broth culture incubating

the tubes at 37C° for 48 hours, refrigerating the tubes for about one hour and observing the solid nature of the gelatin.

Identification of bacterial genera :

A total of 30 strains of bacteria were isolated and identified upto genera level by following the scheme given by Usio Simidu and Kayuyoshi Aiso(1962). The results were compared and verified with Bergey's Manual of determinative bacteriology (1975).

Physico - Chemical Investigations:

An attempt has been made to study some important physico-chemical parameters and find out their possible relationship if any with the microfloral population of the rhizosphere.

The following parameters have been studied:

(1) soil temperature.
(2) soil pH
(3) salinity of soil
(4) organic carbon
(5) available Nitrogen.
(7) Available Phosphorous.

Soil temperature was estimated from the study area by using a Jenson Delux alcoholic thermometer of range 0-110°C each division being 1°C. Soil Eh was also determined from the study area itself by using an Eh meter. After transportation to the laboratory, the sample were immediately subjected to pH estimation by using an Elico pH meter (Model LI - 10T).

The soil samples obtained from rhizosphere were air dried by spreading on polythene sheets in trays, ground well using mortar and pestle, and sieved through .425 mm sieve for chemical analysis (Devis and Freithus, 1970). These samples were stored in plastic jars with screw lid.

Salinity was estimated by making soil extract in the ratio 1:2 with distilled water and subsequent filteration with silver nitrate adopting the standard technique of salinity determination.

Determination of organic carbon in the soil was done by adopting Walkley and Black's rapid titration method.

Available nitrate present in the soil was estimated by colorimetric method (Mackereth 1957).

Available phosphorus was estimated by Olsen's method.

PLATE (a): Sampling station

PLATE-1 - Pure culture of bacteria in agar slants.

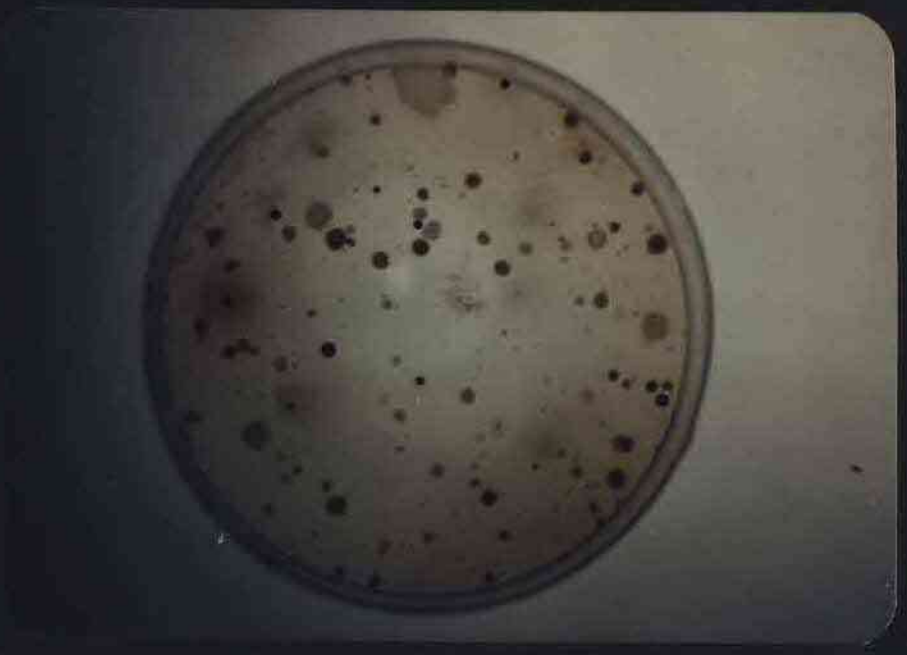


PLATE 2: Heterotrophic bacterial colonies developed on casein agar media

PLATE-4 - Penicillin resistant and sensitive forms.

PLATE-6 - Antagonistic forms from rhizosphere.

PLATE-5 - Purple sulphur and green sulphur bacteria.

RESULTS

Distribution and composition of heterotrophic microbial flora in the Rhizosphere of A.ilicifolius.

Station I

The total bacterial population in the root of A.ilicifolius ranged from 71.42 x 10^4/g to 262.62 x 10^6/g while in the soil it ranged from 72.5 x 10^4/g to 226.53 x 10^6/g. A reduction in the bacterial population in the root or rhizoplane was observed during August followed by a steep increase during the later half of September and throughout October whereas the distribution of rhizosphere microflora showed only minor decline during August and an increase in the following months (Fig.1a).

The seasonal cycle of fungi revealed highest count of 25.71 x 10^4/g in August to 4 x 10^6/g during September in the rhizoplane. The population of fungi was generally recorded low when compared to bacterial distribution. In the rhizosphere soil the fungal counts varied from 15 x 10^4/g during July to 10 x 10^6/g during October. A seasonal cycle was clearly evident in the typical mangrove area. The fungal population in the rhizoplane and rhizosphere samples steeply increased in station 1 during August but the counts declined considerably during September and fluctuated during October (Fig.3)

The total actinomycetes population ranged from 0.98 x 10^4 to 21.42 x 10^5/g in the rhizoplane while in rhizosphere soil maximum was 7.05 x 10^5/g and minimum being 0.46 x 10^4/g. Seasonal fluctuations in the actinomycete population were similar to that of fungi in the rhizosphere. (Fig.5)

Station II

In the typical mangrove habitat the bacterial flora ranged from 90.9 x 10^4/g to 333.33 x 10^6/g in the rhizoplane whereas in the rhizosphere soil it varied from 93.75 x 10^4/g to 322.5 x 10^6/g. Seasonal cycle in the bacterial population was prominent and the counts showed drastic decline in the rhizoplane and rhizosphere soil during August. This was followed by an increase in September and October in both rhizoplane and rhizosphere (Fig .2)

The fungal population had a minimum of 2.8 x 10^4/g in July and the maximum was encountered during August in the rhizoplane whereas in the rhizosphere the counts ranged from 32.25 x 10^4/g in June to a maximum of 13.15 x 10^5/g in August. A similar seasonal cycle in the distribution of fungi was recorded in station 2 like station 1 (fig.4)

The actinomycetes population ranged from 0.62 x 10^4/g to 11.11 x 10^5/g in the rhizoplane and 32 x 10^4/g to 12.5 x 10^5/g in rhizosphere soil. Station 2 and station 1 exhibited same seasonal trend in the distribution of actinomycetes both in rhizoplane and rhizosphere (Fig.6)

BACTERIAL TAXONOMY

For the identification of bacteria the application of physiological criteria is necessary but in the case of fungi macroscopic and microscopic observations were sufficient for identification. The physiological criteria of the bacterological isolates were considered for generic classification of the isolates using a modified scheme of Usio Simidu and Kayuyoshi Aiso (1962). (Table 10).

A total of 30 strains were isolated and identified upto genera level with the help of the scheme and the results were compared with Bergey's manual of Determinative Bacteriology (1974)

a) Morphological and Biochemical observations:-

Morphological and Biochemical characteristics of all the isolates were investigated and the results were summarised in the table (5).

Among the 30 isolates gram positive strains were rare (3.34%) when compared to gram negative strains (96.66%). 96.6% of the total isolates were found to be motile. Pigmented forms were recorded as 26.4%. Hugh and Leifson's test employed to distinguish between oxidative and fermentative utilisation of carbohydrates revealed that fermentative metabolism was predominant among the isolates (98,2%). Only 1.8% of the isolates were found to be oxidative. None of the isolates were found to be alkaline. All the isolates were catalase positive. 30% pencillin resistant forms (plate-4) and 90% oxidase positive forms were also recorded.

6. Identification and relative abundance of genera

The following six genera were identified out of 30 bacterial strains isolated from the rhizosphere of Acanthus ilicifolius (Table.6).

Alcaligenes, Flavobacterium, Cytophaga, Vibrio, Enterobacteriaceae Aeromonas.

Among the 30 isolates Alcaligenes were predominant (50%) followed by Vibrio (16.6%), Aeromonas (10%), Cytophaga (10%), Flavobacterium (3.3%) and Enterobacteriaceae (3.33%) (Fig.9)

(C) Physiologically active isolates from rhizosphere

Among the 30 isolates 70% reduced nitrate to nitrite, 80% hydrolysed starch, 60% liquified gelatin and 90% produced H_2S from sulphur containing amino acids like L-cysteine (Table.7) Purple Sulphur and green sulphur bacteria were also encountered from the rhizosphere using selective media after an incubation period of 3 months. (Plate.6).

Identification and Generic distribution of fungi

Based on the morphological features the dominant fungal population isolated from the rhizosphere belonged to the following genera. Fusarium, Pencillium, Aspergillus and Rhizopus. The frequency distribution of rhizosphere fungi is given in the table.8. The relative abundance of the rhizosphere fungi during various months has also been recorded in both the stations (Table.9)

Fusarium was found to occur in maximum numbers in the rhizosphere in both the stations. Pencillium came next followed by Aspergillus. Rhizopus was found to occur only in minimum numbers. In station I maximum percentage of Fusarium was recorded during October, (80%) and minimum in August (52.17%). Pencillium and Aspergillus also showed maximum counts during July and August, respectively. A minimum value was recorded during October for both the species. Rhizopus was encountered maximum in August and minimum in October.

In station 2, the maximum counts for Fusarium was recorded during June while a minimum in July. Similarly for Pencillium Aspergillus and Rhizopus the maximum counts were observed in, July, a minimum in June.

Interrelationship of microflora in the rhizosphere.

STATION-I

Maximum B/F ratio & B/A ratio was recorded during the month of October. (629:142, & 842:94) respectively indicating the dominance of bacteria over fungi and actinomycetes. Maximum F/A ratio (1.34 and 1.39) was obtained during the month of October indicating the predominance of fungi over actinomycetes. A minimum F/A ratio (0.56) was observed in September indicating the dominance of actinomycetes over fungi.

STATION-II

Maximum B/F and B/A ratios were recorded during October, (517:17 and 716:33) respectively. The minimum F/A ratio (0.335) was recorded during July.

Distribution of Zymogenous population:-

Total zymogenous bacteria like proteolytic, chitinoclastic caseinolytic & lipolytic were isolated from the rhizosphere and rhizoplane using selective media. (Fig.26-29)

STATION-I

All the zymogenous population showed seasonal variation during the study period (June-October). A decrease in the 4 bacterial populations were observed during August in both rhizoplane and in rhizosphere. The decrease was followed by a gradual increase during the month of September and October.

Caseinolytic population: A drastic reduction in the number of caseinolytic population was observed during August in both rhizoplane and rhizosphere.

Ureolytic population:

Ureolytic bacteria showed an exceptional rise in the counts in rhizoplane as well as in the rhizosphere soil during August. The count was maximum during August and minimum was obtained during June - July.

Proteolytic population:-

Maximum counts of proteolytic bacteria was observed during September and minimum in August. The distribution was found to be similar in the rhizoplane as well as in the rhizosphere soil.

Lipolytic population:- A gradual decrease in the lipolytic population was observed which reached a minimum during August and increased slightly during September and October.

Chitinoclastic population:- Like the distribution of lipolytic bacteria, the chitinoclastic bacteria also showed a decrease in August followed by a slight increase in September and October.

STATION-II

A seasonal variation was observed in the zymogenous populations similar to that of station I.

Caseinolytic population:- Caseinolytic population showed a drastic reduction in the month of August in the rhizoplane and rhizosphere. An increase in the count was observed during September and October.

Ureolytic population:- The bacterial population exhibited a distribution pattern similar to that of station I. An exceptional increase was noticed in the month of August while minimum counts were observed in June and July.

Proteolytic population:- The distribution of proteolytic population was maximum during September and minimum was recorded is August in the rhizosphere soil. However, a uniform pattern in the distribution of proteolytic population was observed only in the rhizoplane.

Lipolytic population:- Maximum count of lipolytic bacteria was observed during September and October. A decrease in the population was observed during August, during the peak monsoon period.

Chitinoclastic population:- The chitinoclastic population declined in number in August followed by a slight increase in September and October.

Parallel relationship was found among certain zymogenous populations. In station II, in the rhizoplane the distribution of lipolytic and caseinolytic populations were proportional.

ENVIRONMENTAL PARAMETERS

Temperature:

Soil temperature did not vary much among the 2 stations. Initially in June, the temperature was high in both the stations. With the onset of monsoon, the temperature declined and reached a minimum in August. During post monsoon the temperature increased and reached a maximum in October. (Fig. 10-13)

pH

The pH did not show any drastic seasonal variation in station I, but slightly higher values were obtained during post monsoon months. A minimum pH of 7.3 was recorded during August and a maximum of 7.8 during October. The pH showed some fluctuations during the post-monsoon months in station II. A minimum pH of 6.46 was recorded during July and a maximum pH of 7.21 in September. The pH range was different for the 2 Stations observed, station I being mostly acidic and station 2 being alkaline. (Fig. 14-17)

Eh

No drastic variation in Eh could be recorded from station I during the study period. Moreover it showed positive values throughout the study period. Soil Eh varied from +62mv to +150mv. The Eh of +62 was recorded during August and +150mv during October. Fluctuations in the Eh was observed in station 2 during the study period. Eh varied from +85 mv in June to -170mv in October, (Fig 18 - 21)

SALINITY:- Salinity showed an obvious seasonal variation in the 2 stations. Salinity was maximum during the post-monsoon months and the peaks were reported as 8.8‰ and 7.5‰ in (October) in station I and 2 respectively. During monsoon the salinity was found to be decreasing and a sudden decrease was observed during August in both the stations, and the values became as low as 1.003‰ and 0.76‰ in stations I and 2 respectively (Fig. 22-25)

Organic Carbon in Soil:- Organic Carbon neither showed considerable seasonal variation nor it varied much in the 2 stations. However, slightly higher organic carbon content was observed during monsoon period in station I as well as in station 2. In station I the organic carbon content varied from 7.8% to 9.5% and was recorded during October and during August. In station 2 the values varied from 7.9% to 9.6% and was recorded during October and July.

Available nitrate in the soil:-

Not much variation in the available nitrate could be recorded from both the stations during the study period.

However minor fluctuations were observed in the two stations. In station I the available nitrate varied from 0.1150ppm to 0.388ppm and was recorded during the months of July and October. In station 2 the available nitrate showed values ranging from 0.187 ppm to 0.4464 ppm. The lowest value was observed during the month of July and highest during October.

Available phosphorous content was high during post monsoon months in both the stations. During the monsoon a decline in the phosphorous content was observed. A maximum of 63 and 65 ppm was recorded during October and a minimum of 28 ppm and 26 ppm was recorded during August in Station 1 and 2 respectively.

Statistical Analysis:-

The correlation between environmental parameters and total microflora, bacteria, fungi and actinomycetes was worked out separately for each station, because it is possible that the interrelationship between the variables might change from station to station.

Correlation between the total microbial population and bacteria, fungi and actinomycetes populations were analysed during the study period (from June - October) in both the stations.

In stations I and 2 significant positive correlation at 5% level was observed between microbial and bacterial populations of rhizoplane and rhizosphere. A significant negative correlation was observed between the actinomycetes population of root and total microflora. A significant negative correlation was observed between bacteria and actinomycetes population. A similar correlation was observed in station 2 also.

Similarly correlation between the total rhizosphere bacteria and various zymogenous population was analysed during a period of 6 months.

In station I, a significant negative correlation between ureolytic and total bacterial population of rhizoplane was observed. A positive correlation between proteolytic bacteria and total bacteria of rhizoplane and rhizosphere was obtained, Total bacteria and chitinoclastic population of soil showed a positive correlation.

Ureolytic population of rhizoplane showed a negative correlation with caseinolytic population. Similarly a negative correlation was observed between proteolytic and ureolytic populations. Lipolytic and chitinolytic populations showed positive correlation.

In station 2 a positive correlation between protelytic and total bacterial population of rhizoplane and rhizosphere was observed. A negative correlation between ureolytic and total bacterial population of root was recorded. Significant positive correlation between Caseinolytic and lipolytic populations of rhizoplane and chitinoclastic and lipolytic populations of rhizosphere was also recorded.

ANALYSIS OF VARIANCE TEST:- To judge whether numerical differences between various microbial groups and between stations were significant, analysis of variance was carried out through $\log_{10}$ transformation of raw numbers. There was no significant difference in the total plate counts between the stations. (Fig. 30-31)

The Z-test was carried out to study the homogenity of relationship between the environmental parameters and the microflora in the 2 stations which gave values 0.92 and 0.96 in station 1 and station 2 respectively. The values obtained were below 1.96 in the two stations which indicated that stations 1 and 2 are more or less homogenous.

The distribution of microflora showed some clustering in September and October. It was found that the distribution of the microflora was characterised by overdispersion.

TABLE - 1

STATION - 1.

Months	Total Microbial population			Total microbial population		
	Bacteria No/g x 10^6	Fungi No/g x 10^4	Actinomycetes No/g x 10^5	Bacteria No/g x 10^6	Fungi No/g x 10^4	Actinomycetes No/g x 10^5
JUNE	66.66	56.66	71.66	43.5	47.5	5
	133.3	50	3.75	93.75	42.5	3.75
JULY	180	33.33	5	78.75	23.75	2.5
	175	23.33	3.33	101.33	15	2.5
AUGUST	51.42	40	21.42	64	52	6
	57.14	25.71	15.71	72	42	6
SEPTEMBER	126	66	16	64.70	35.29	7.05
	150	42	10	81.76	30.58	4.70
OCTOBER	262.62	45.45	5.05	226.53	38.77	4.08
	237.37	40.40	1.01	203.06	30.61	1.02

TABLE -2

STATION - 2.

MONTHS	TOTAL MICROBIAL POPULATION ON ROOT			TOTAL MICROBIAL POPULATION IN SOIL		
	BACTERIA	FUNGI	ACTINOMYCETES	BACTERIA	FUNGI	ACTINOMYCETES
	No/g x 10^6	No/g x 10^5	No/g x 10^5	No/g x 10^5	No/g x 10^4	No/g x 10^5
JUNE	91.25	66.66	6.66	50	57.5	7.5
	216.66	60	3.33	112.5	55	3.75
JULY	333.33	33.33	2.66	225	37.5	12.5
	253.33	28	2.66	140	33.75	8.75
AUGUST	116.02	59.25	11.11	64.81	50	11.25
	34.09	44.44	9.25	55.75	32.25	7.5
SEPTEMBER	194	72	4	67.74	45.16	3.75
	198	62	2	103.22	33.33	3.75
OCTOBER	239.77	62.5	2.27	261.29	63.44	5
	244.31	52.27	2.27	322.58	49.46	3.22

TABLE - 3

DISTRIBUTION OF MICROFLORA AND THEIR INTER-RELATIONSHIP IN THE RHIZOSPHERE

STATION - 1.

Months	Total Microbial population	% to total root Microflora in Bacteria	Fungi	Actinomycets	Ratio between different Microflora in root B:F	B:A	F:A	Total Microflora population X $10^6/g$		% to total Microflora in soil B.	F.	A.	Ratio between Microflora in soil B:F	B:A	F:A
JUN	202.56	98.71	.5265	.7607	187.48	129.76	.692	132.02		98.72	.6473	.629	152.51	156.87	1.03
JUL	356.39	99.60	.1589	.2337	626.18	426.18	.679	180.96		99.00	.2141	.276	464.73	360.24	.775
AUG	112.93	96.130	.5818	3.287	165.22	29.24	.177	138.14		98.45	.6804	.861	144.69	114.39	.791
SEPT.	279.66	98.64	.3861	.9296	255.58	106.15	.415	148.29		98.76	.4441	.792	222.38	124.64	.560
OCT.	501.44	99.71	.1712	.1208	582.41	825.07	1.42	430.78		99.72	.1585	.118	629.14	842.94	1.34

TABLE -4

Occurrence of Microflora and Their Inter relationship in the Rhizosphere

STATION - 2.

Months	Total Microbial population 10^6/g	% of Total Microflora in root			Ratio between the microflora in root			Total microbial population X 10^6/gm.	%of Total microflora in soil			Ratio between Microflora in the soil		
		B	F	A	B:F	B:A	A:F		B	F	A	B:F	B:A	A:F
JUNE	310.17	99.27	.4083	.3220	243.13	308.29	1.268	164.74	98.64	.683	.683	144.46	144.46	1
JULY	487.79	99.80	.1043	.0905	956.64	1102.83	1.153	367.87	99.21	.1936	.577	512.44	171.76	.335
AUGUST	153.17	98.00	.6769	.329	144.78	73.74	.509	123.25	97.81	.617	1.52	146.57	64.30	.438
SEPT.	393.94	99.50	.3401	.1523	292.54	653.31	2.233	172.48	99.11	.455	.435	217.82	227.84	1.046
OCT.	485.67	99.67	.2363	.0934	421.79	187.13	2.536	585.81	99.66	.193	.140	517.17	710.33	1.39

TABLE-5

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF 30 BACTERIAL STRAINS ISOLATED FROM THE RHIZOSPHERE OF Acanthus ilicifolius.

S.NO.	CHARACTERISTIC	FREQUENCY OF OCCURRENCE (%)
1.	Gram	
	Positive	3.34
	Negative	96.66
2.	Motility	96.66
3.	O/F Media	
a.	Oxidative	---
b.	Alkaline	
c.	Fermentative	98.2
4.	Gelatin Hydrolysers	60
5.	Starch Hydrolysers	80
6.	Nitrate Reducers	70
7.	H_2S Producers	73.33
8.	Oxidase	90
9.	Catalase	100
10.	Pencillin resistance Test	30
11.	Pigmentation	26.4

TABLE - 6

RELATIVE ABUNDANCE OF 30 DIFFERENT BACTERIAL GROUPS ISOLATED AND IDENTIFIED FROM THE RHIZOSPHERE OF *Acanthus ilicifolius*

NUMBER OF ISOLATES - 30

S.NO.	BACTERIAL GROUPS	NUMBER OF BACTERIA	% BACTERIAL GROUPS
1.	Alcaligenes	15	50
2.	Flavobacterium	1	3.33
3.	Cytophaga	3	10
4.	Vibrio	7	23.33
5.	Enterobacteriaceae	1	3.33
6.	Aeromonas	3	10

TABLE - 7

DISTRIBUTION OF PHYSIOLOGICALLY ACTIVE ISOLATES FROM THE RHIZOSPHERE OF *Acanthus ilicifolius*

NUMBER OF	% BACTERIAL GROUPS			
ISOLATES	NITRATE REDUCERS	STARCH HYDROLYSERS	GELATIN LIQUIFIERS	H_2S PRODUCERS
30	70	80	60	90

TABLE - 8

QUALITATIVE NATURE OF PREDOMINANT FUNGI ISOLATED FROM RHIZOSPHERE AND THEIR FREQUENCY

RHIZOSPHERE FUNGI	FREQUENCY %
FUSARIUM	33.33
PENICILLIUM	16.66
ASPERGILLUS	25
RHIZOPUS	8.33

TABLE - 9

RELATIVE ABUNDANCE OF DIFFERENT FUNGI DURING THE STUDY PERIOD

STATION-1	RHIZOSPHERE FUNGI (%)				STATION-2	RHIZOSPHERE FUNGI (%)		
MONTHS	FUSARIUM	PENICILLUM	ASPERGILLUS	RHIZOPUS	FUSARIUM	PENICILLUM	ASPERGILLUS	RHIZOPUS
JUNE	73.52	11.76	14.70	-	83	5.19	6.49	6
JULY	68.75	18.75	12.5	6.25	44	20	24	12
AUGUST	52.17	15.21	15.21	17.39	66	12.6	11.11	9.52
SEPTEMBER	63.63	10.90	14.5	10.90	50	20	22.8	7.1
OCTOBER	80	5.33	10	3.33	72.8	8.7	9.7	8.7

TABLE - 10

THE OUTLINE OF PROCEDURE FOR SCREENING OF CULTURES (MARINE BACTERIA) SCHEME OF USIO SIMIDU AND KAYUYOSHI AISO (1962)

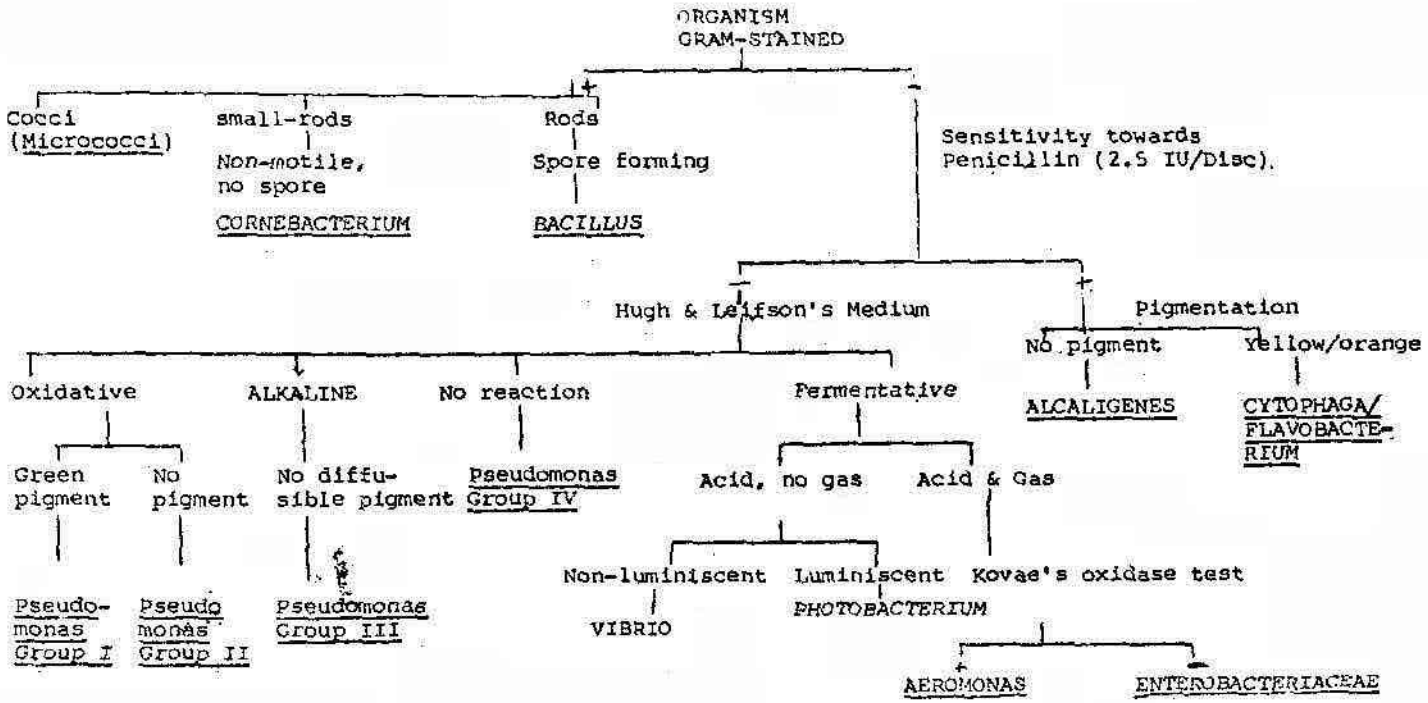


Fig.1a - Occurrence of total heterotrophic bacteria in rhizoplane and rhizosphere soil from station I.

Fig.2 - Occurence of total heterotrophic bacteria in rhizoplane and rhizosphere soil from station II.

Fig. 1a

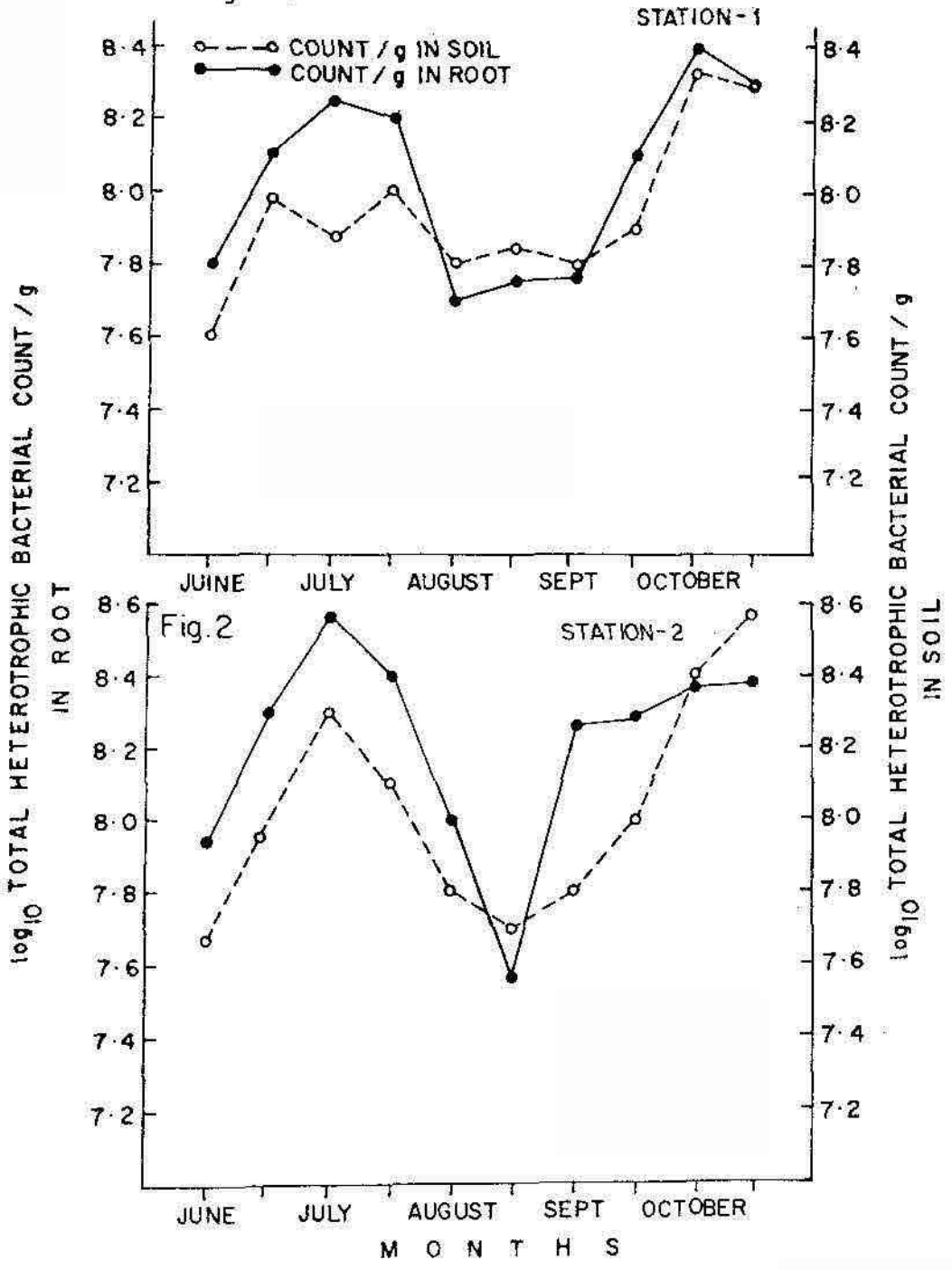

STATION-1
COUNT / g IN SOIL
COUNT / g IN ROOT
8·4
8·2
8·0
7·8
7·6
7·4
7·2
JUINE
JULY
AUGUST
SEPT
OCTOBER
Fig. 2
STATION-2
8·6
8·4
8·2
8·0
7·8
7·6
7·4
7·2
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S
log10 TOTAL HETEROTROPHIC BACTERIAL COUNT / g IN ROOT
log10 TOTAL HETEROTROPHIC BACTERIAL COUNT / g IN SOIL

Fig.3 - Occurrence of fungi in rhizoplane and rhizosphere soil from station I.

Fig.4 - Occurence of fungi in rhizoplane and rhizosphere soil from station II.

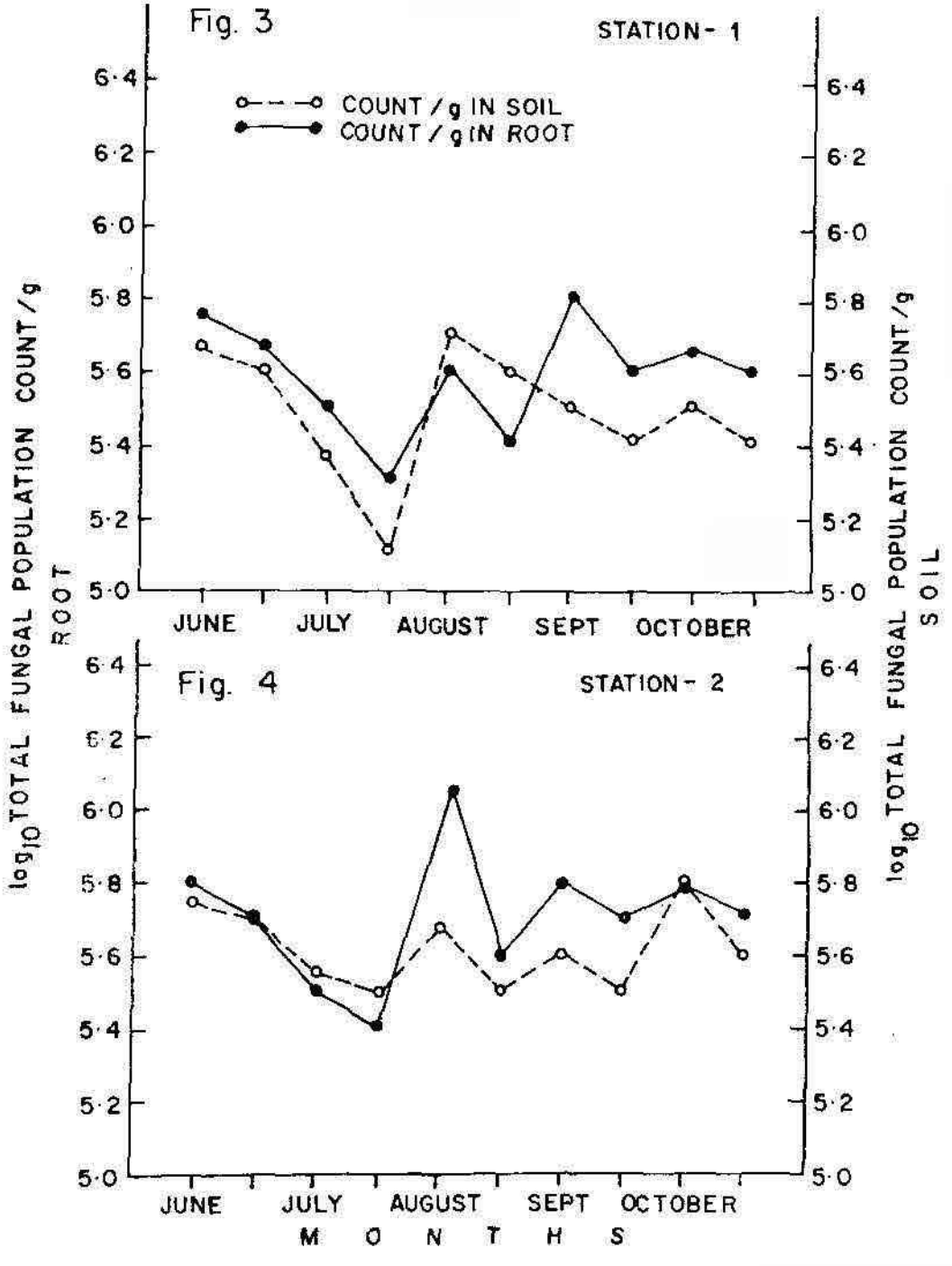


Fig. 3
STATION - 1
COUNT / g IN SOIL
COUNT / g IN ROOT
log10 TOTAL FUNGAL POPULATION COUNT / g ROOT
log10 TOTAL FUNGAL POPULATION COUNT / g SOIL
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
JUNE
JULY
AUGUST
SEPT
OCTOBER
Fig. 4
STATION - 2
MONTHS

Fig.5 - Occurrence of actinomycetes in rhizoplane and rhizosphere soil from station I.

Fig.6 - Occurrence of actinomycetes in rhizoplane and rhizosphere soil Station II.

Fig. 5

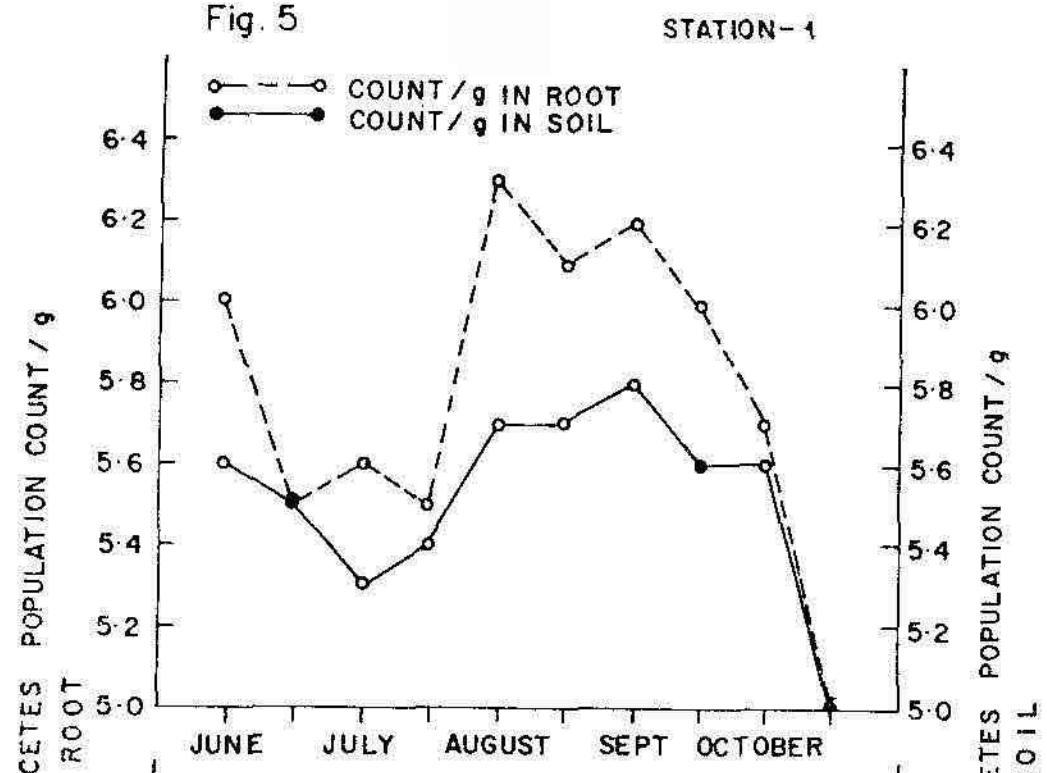

STATION-1
COUNT/g IN ROOT
COUNT/g IN SOIL
log10 TOTAL ACTINOMYCETES POPULATION COUNT/g ROOT
log10 TOTAL ACTINOMYCETES POPULATION COUNT/g SOIL
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
JUNE
JULY
AUGUST
SEPT
OCTOBER

Fig. 6

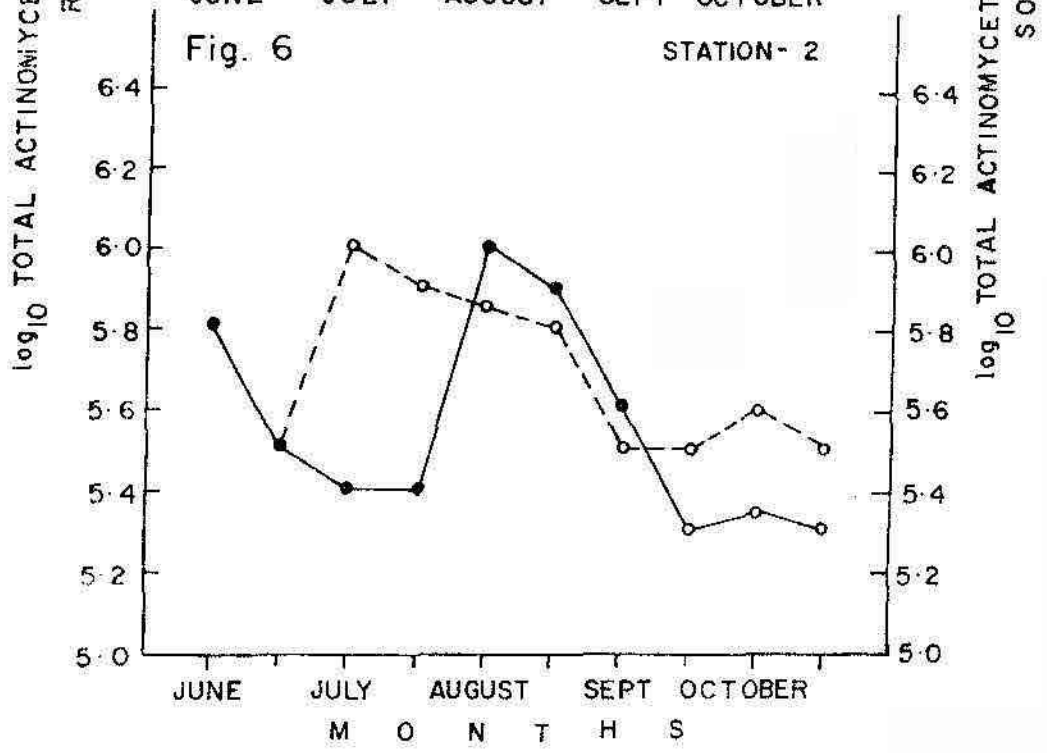

STATION-2
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig.7 - Seasonal variation in the bacteria, fungi and actinomycetes populations in the rhizoplane.

Fig.8 - Seasonal variation in the bacteria, fungi and actinomycetes populations in the rhizosphere soil.

Fig. 7

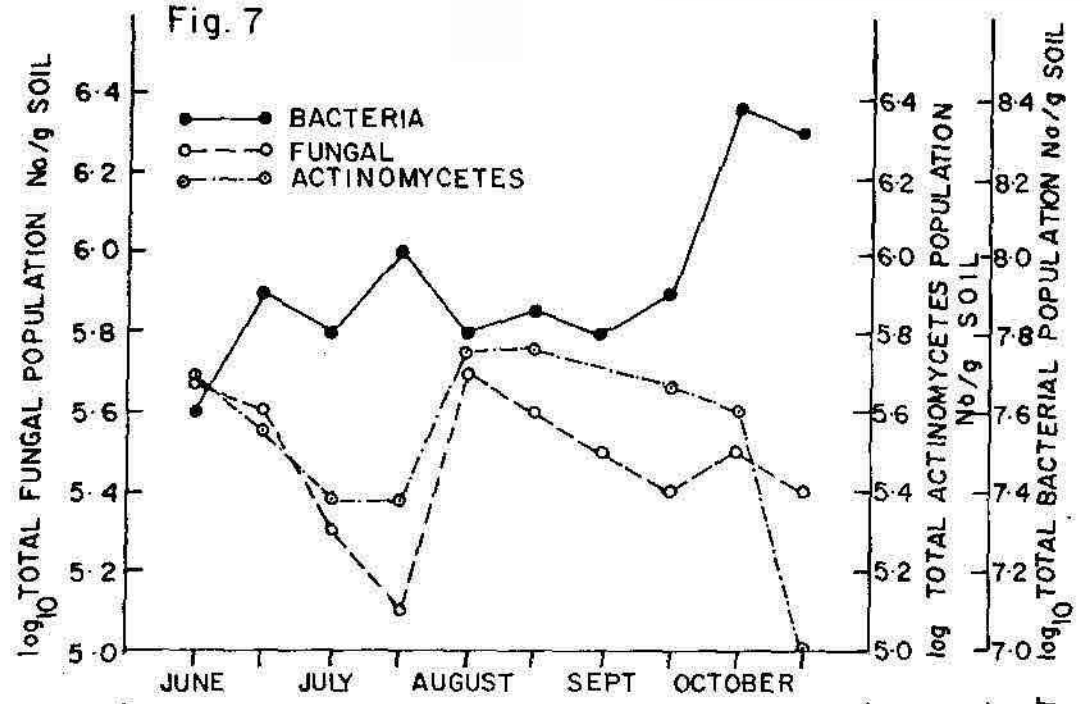

BACTERIA
FUNGAL
ACTINOMYCETES
log10 TOTAL FUNGAL POPULATION No/g SOIL
log TOTAL ACTINOMYCETES POPULATION No/g SOIL
log10 TOTAL BACTERIAL POPULATION No/g SOIL
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
8·4
8·2
8·0
7·8
7·6
7·4
7·2
7·0
JUNE
JULY
AUGUST
SEPT
OCTOBER

Fig. 8

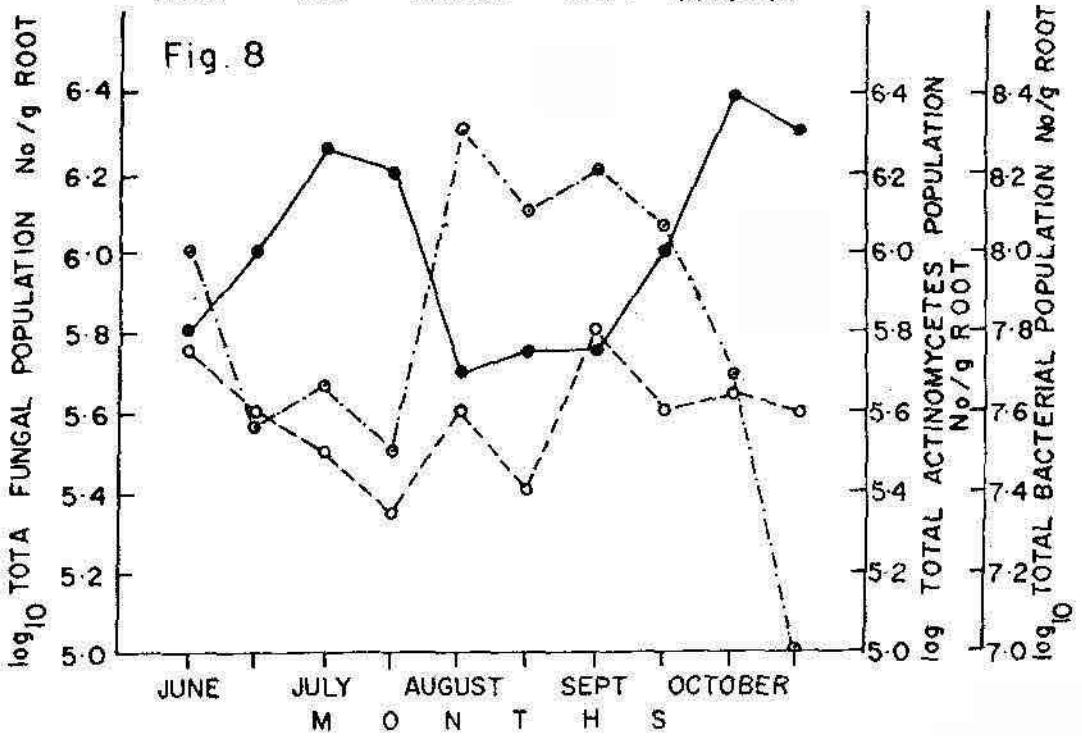

log10 TOTA FUNGAL POPULATION No/g ROOT
log TOTAL ACTINOMYCETES POPULATION No/g ROOT
log10 TOTAL BACTERIAL POPULATION No/g ROOT
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
8·4
8·2
8·0
7·8
7·6
7·4
7·2
7·0
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig.9 - Percentage occurrence of the bacterial genera isolated from the rhizosphere of *Acanthus illicifolius*.

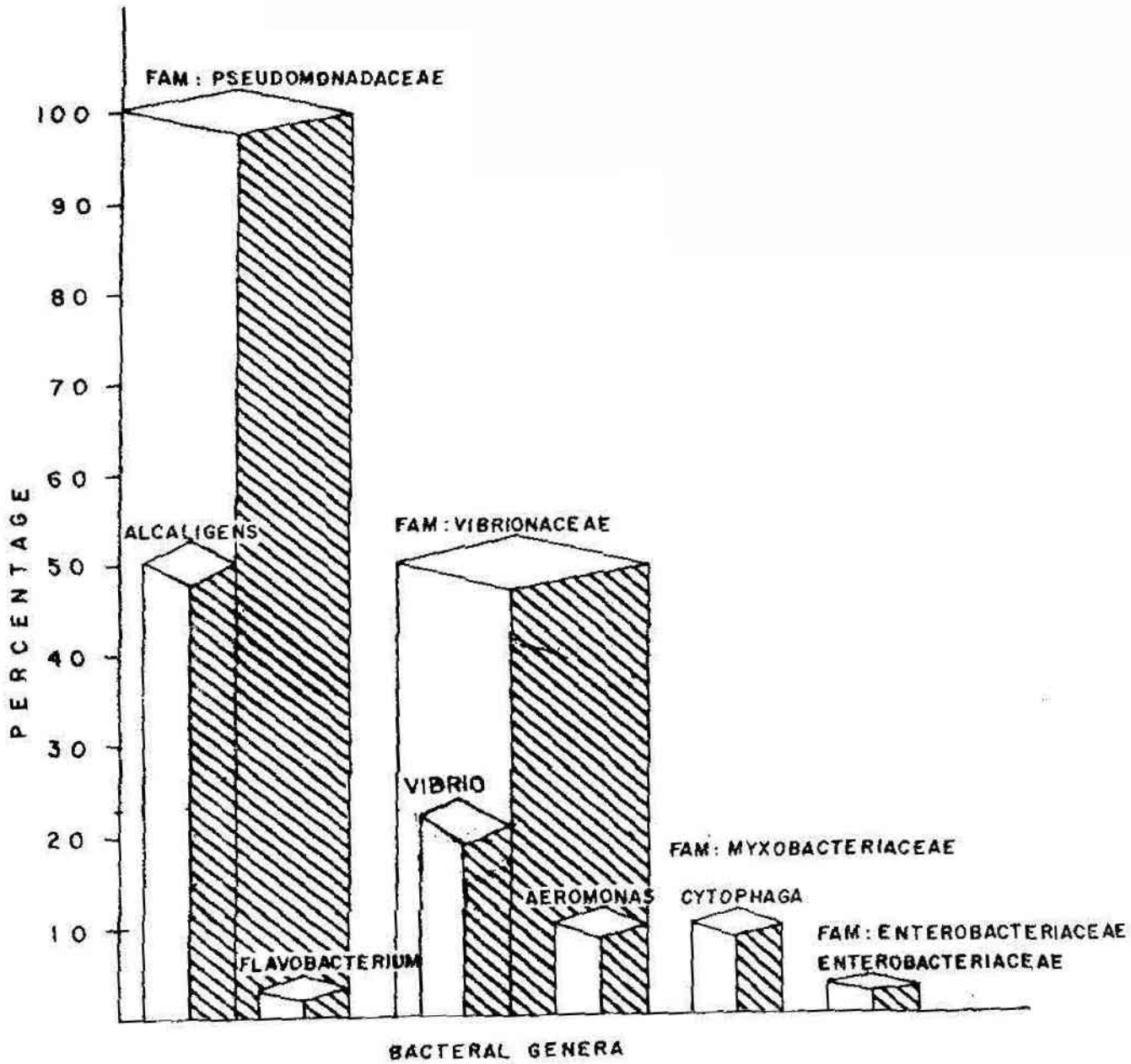
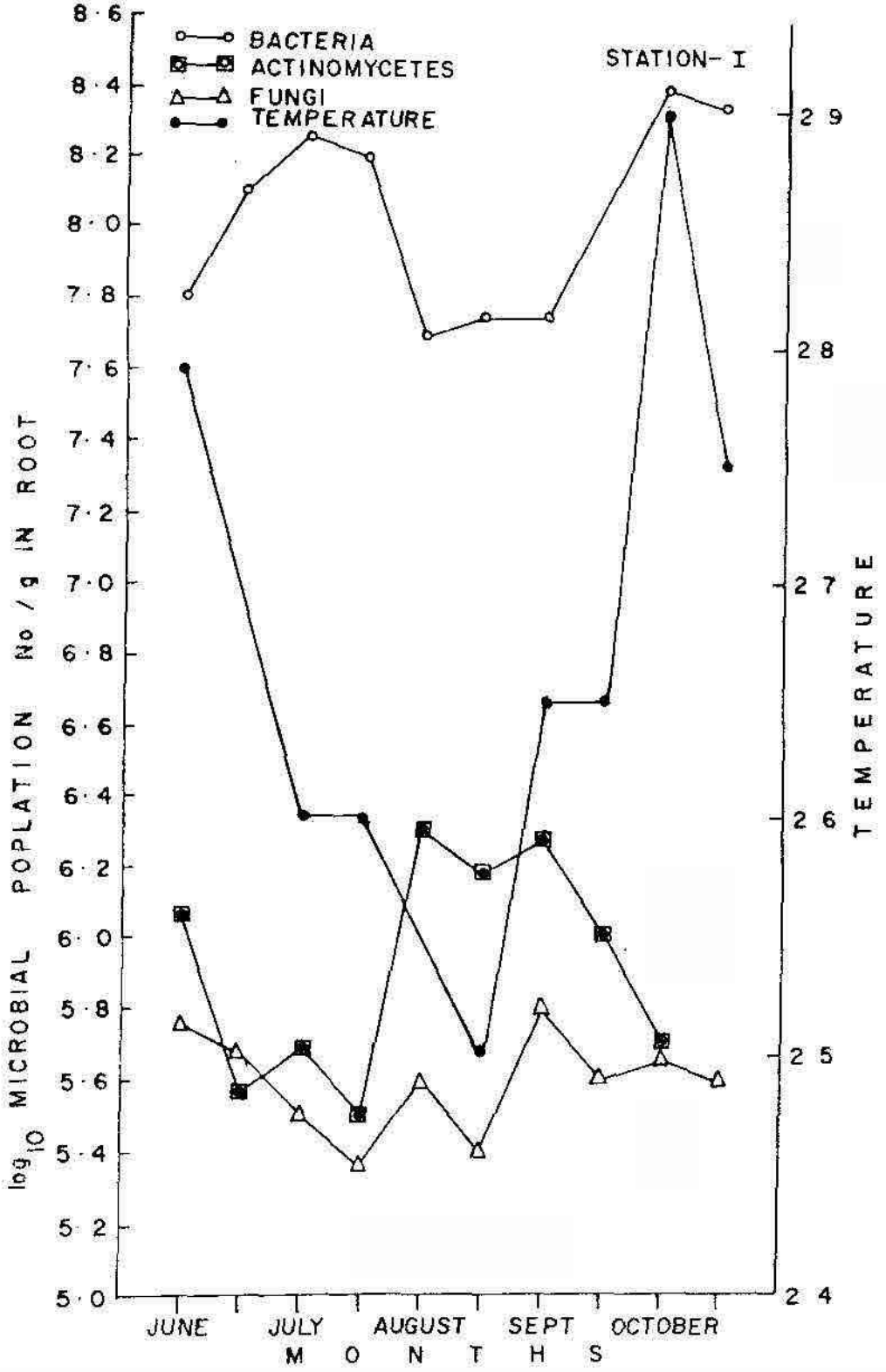

FAM : PSEUDOMONADACEAE
100
90
80
70
60
50
40
30
20
10
PERCENTAGE
ALCALIGENS
FLAVOBACTERIUM
FAM : VIBRIONACEAE
VIBRIO
AEROMONAS
FAM : MYXOBACTERIACEAE
CYTOPHAGA
FAM : ENTEROBACTERIACEAE
ENTEROBACTERIACEAE
BACTERAL GENERA

Fig. 9

Fig. 10-11 Variation in the temperature and microbial population in the rhizoplance and rhizosphere from Station I.

Fig. 10


BACTERIA
ACTINOMYCETES
FUNGI
TEMPERATURE
STATION- I
log10 MICROBIAL POPLATION No / g IN ROOT
TEMPERATURE
8·6
8·4
8·2
8·0
7·8
7·6
7·4
7·2
7·0
6·8
6·6
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
2 9
2 8
2 7
2 6
2 5
2 4
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig·11

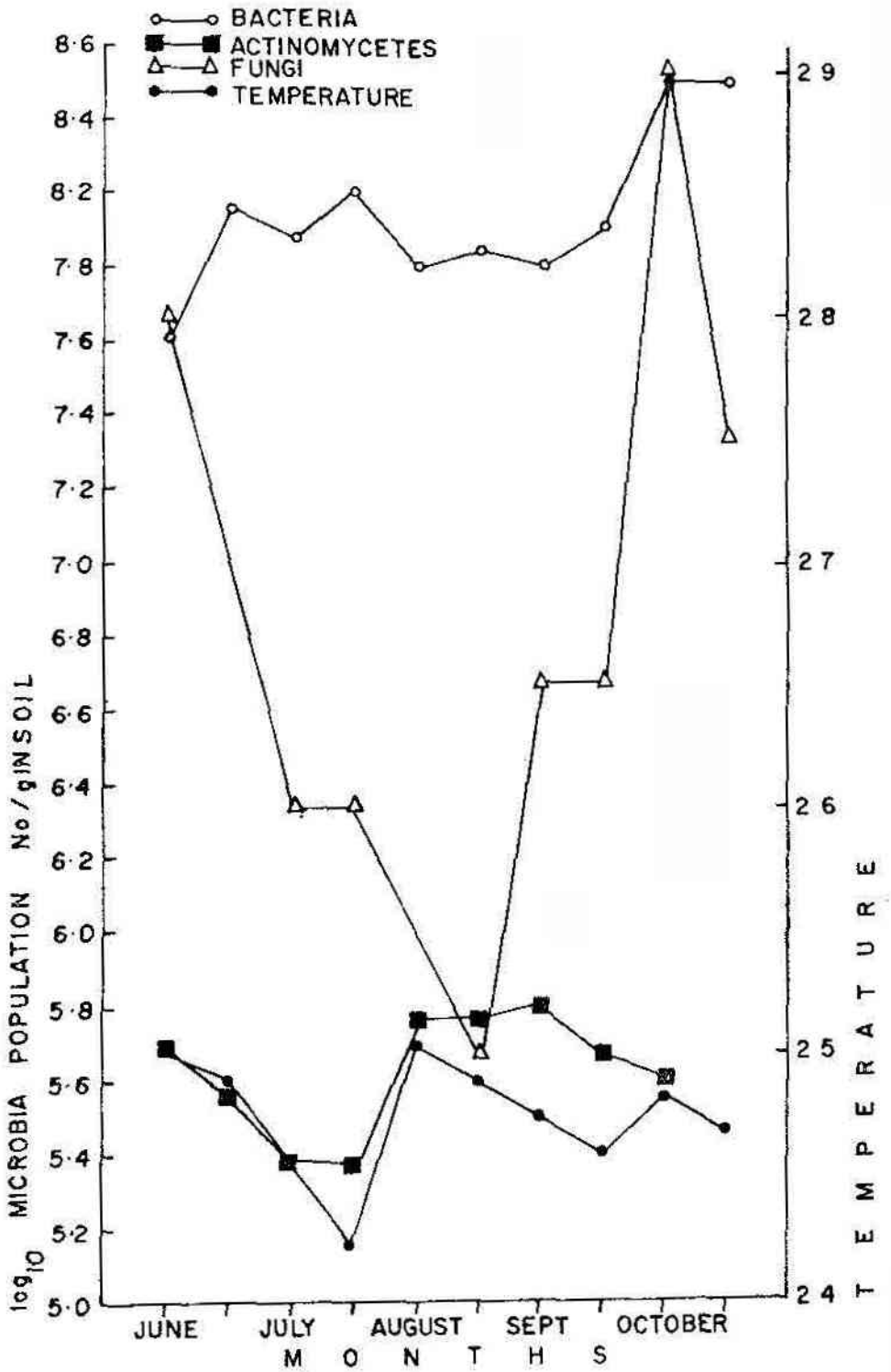

BACTERIA
ACTINOMYCETES
FUNGI
TEMPERATURE
8·6
8·4
8·2
7·8
7·6
7·4
7·2
7·0
6·8
6·6
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
log10 MICROBIA POPULATION No/gINSOIL
29
28
27
26
25
24
TEMPERATURE
JUNE
JULY
AUGUST
SEPT
OCTOBER
MONTHS

Fig. 12-13 Variation in the temperature and microbial population in the rhizoplane and rhizosphere from station II.

Fig. 12

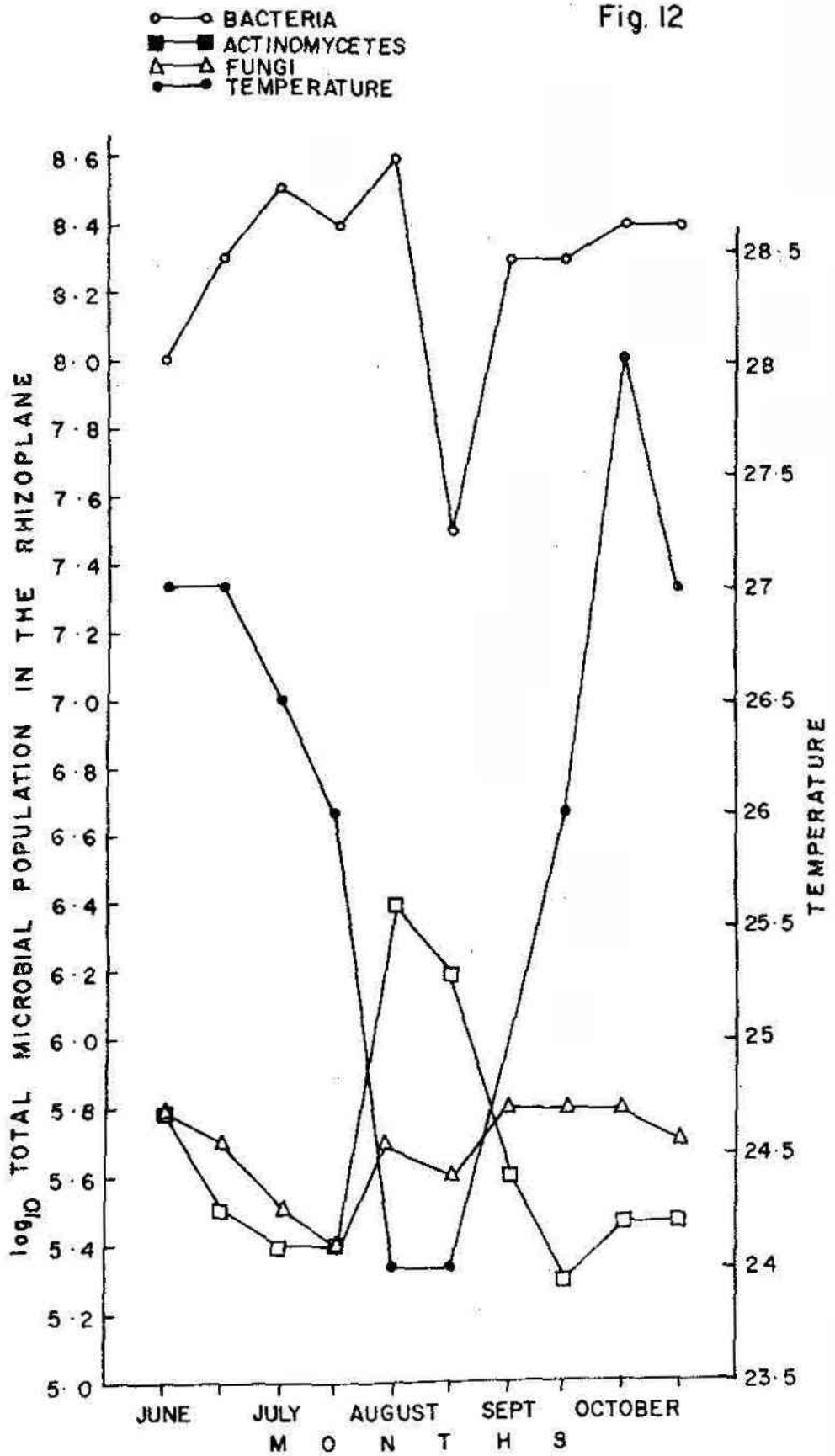

BACTERIA
ACTINOMYCETES
FUNGI
TEMPERATURE
log10 TOTAL MICROBIAL POPULATION IN THE RHIZOPLANE
TEMPERATURE
8·6
8·4
8·2
8·0
7·8
7·6
7·4
7·2
7·0
6·8
6·6
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
28·5
28
27·5
27
26·5
26
25·5
25
24·5
24
23·5
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig. 13

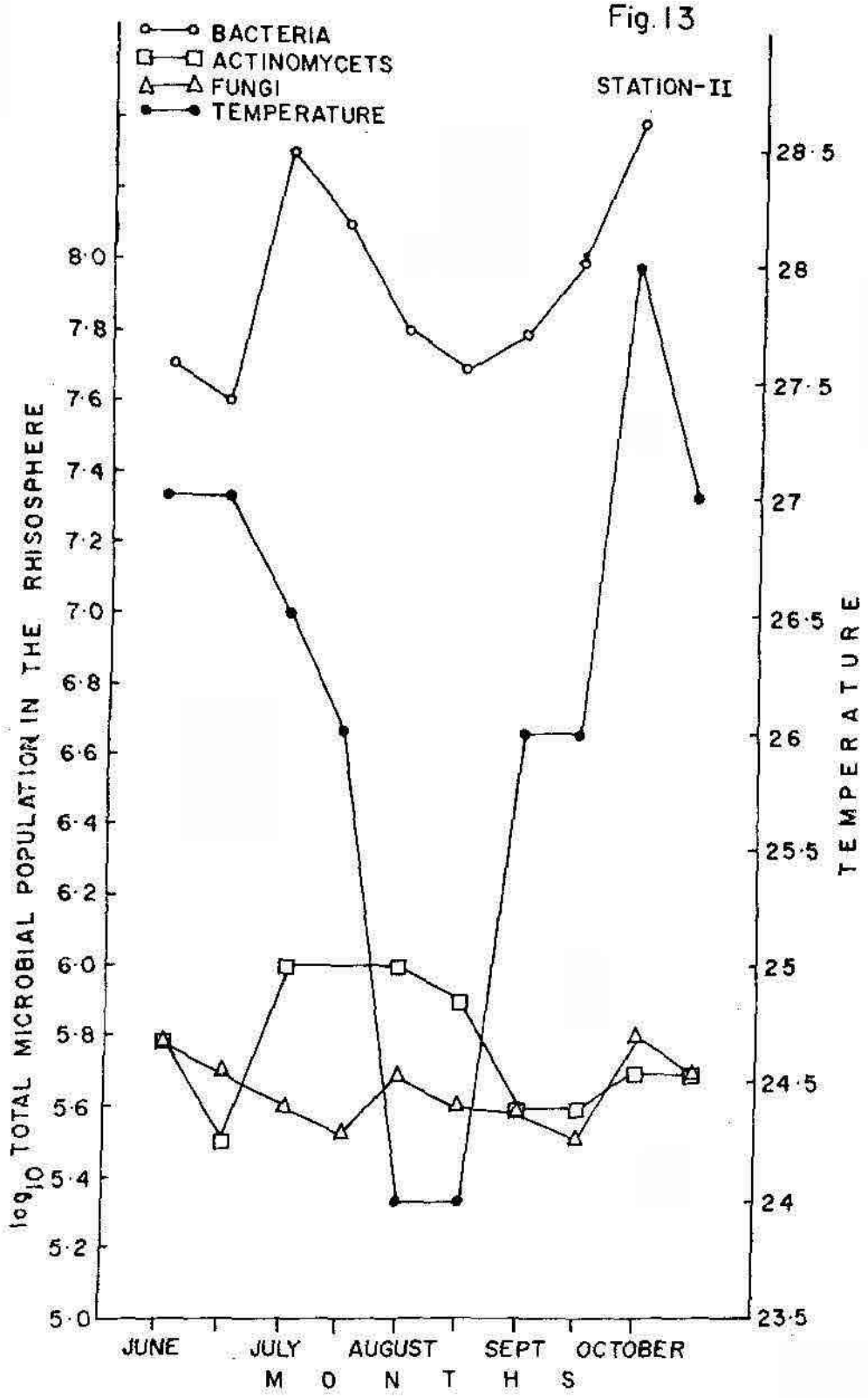

BACTERIA
ACTINOMYCETS
FUNGI
TEMPERATURE
STATION-II
log10 TOTAL MICROBIAL POPULATION IN THE RHISOSPHERE
TEMPERATURE
8·0
7·8
7·6
7·4
7·2
7·0
6·8
6·6
6·4
6·2
6·0
5·8
5·6
5·4
5·2
5·0
28·5
28
27·5
27
26·5
26
25·5
25
24·5
24
23·5
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig. 14-15 Variation in the pH and microbial population in the rhizoplane & rhizosphere in station I.

Fig. 15

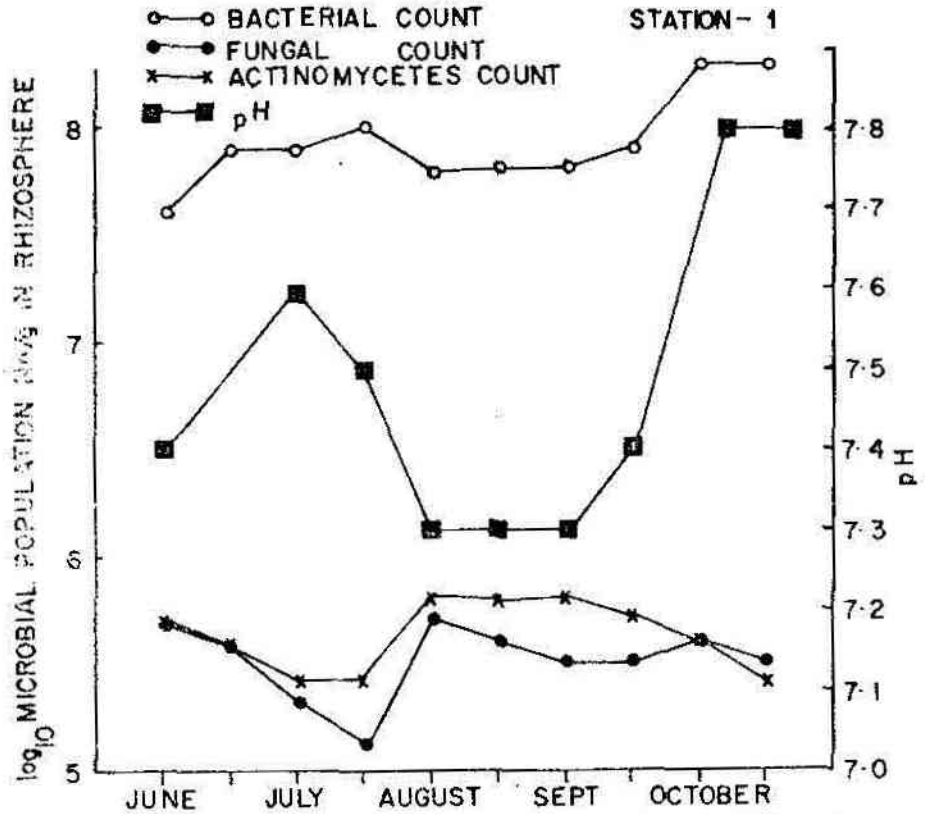

BACTERIAL COUNT
FUNGAL COUNT
ACTINOMYCETES COUNT
pH
STATION - 1
log10 MICROBIAL POPULATION
8
7
6
5
7·8
7·7
7·6
7·5
7·4
7·3
7·2
7·1
7·0
pH
JUNE
JULY
AUGUST
SEPT
OCTOBER

Fig. 14

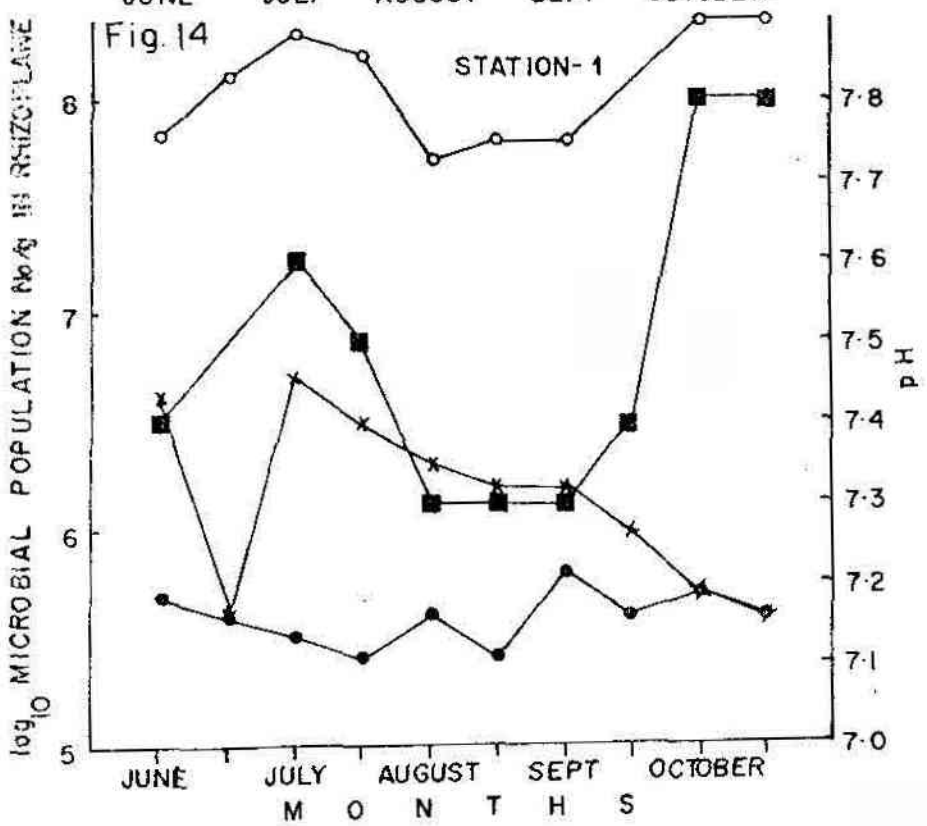

STATION - 1
log10 MICROBIAL POPULATION
8
7
6
5
7·8
7·7
7·6
7·5
7·4
7·3
7·2
7·1
7·0
pH
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig. 16-17 Variation in the pH and microbial population in the rhizoplane and rhizosphere soil from station II.

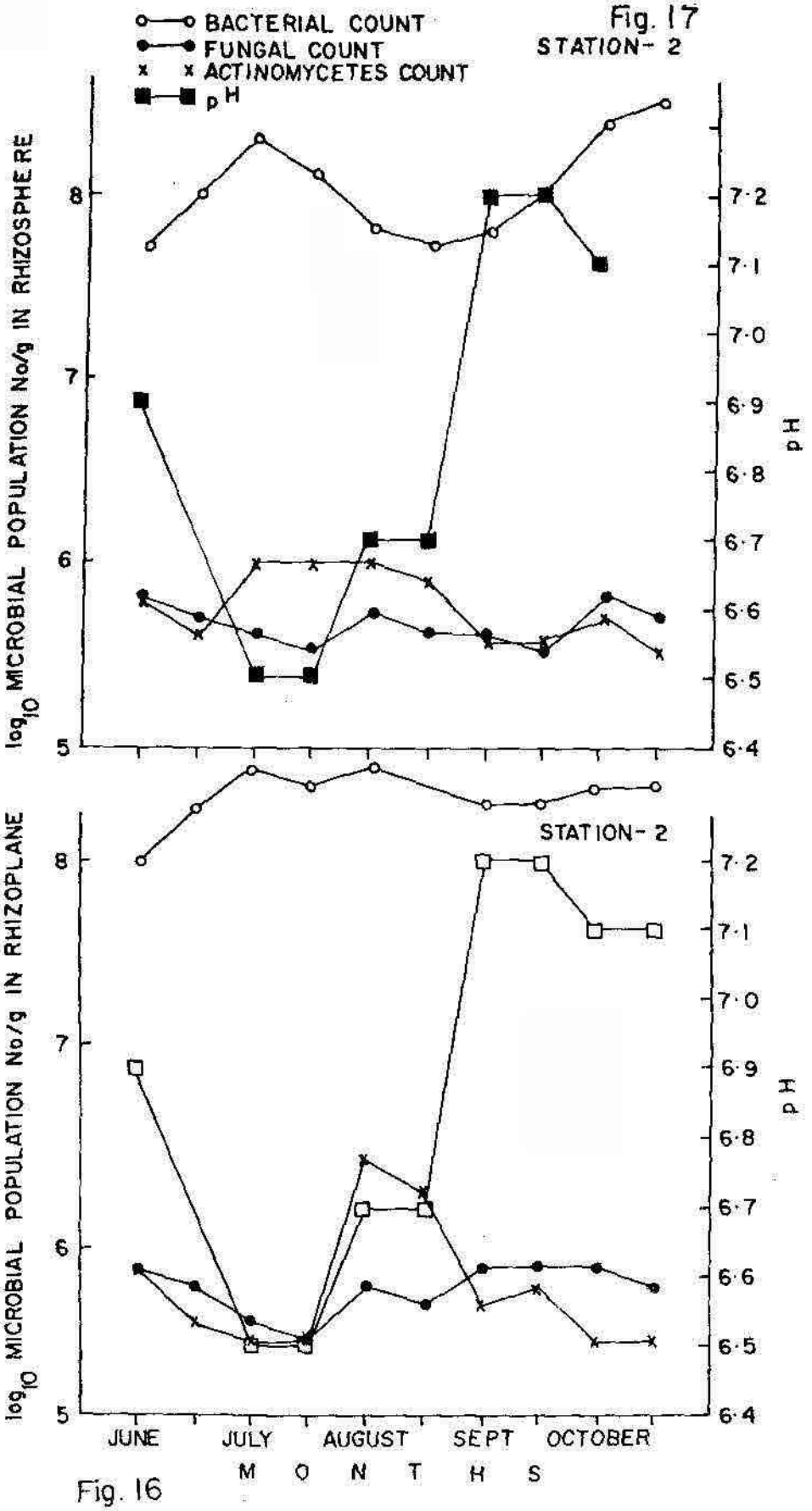

BACTERIAL COUNT
FUNGAL COUNT
ACTINOMYCETES COUNT
pH
STATION- 2
log10 MICROBIAL POPULATION No/g IN RHIZOSPHERE
log10 MICROBIAL POPULATION No/g IN RHIZOPLANE
pH
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig. 17

Fig. 16

Fig. 18-19 Variation in the Eh and microbial population in the rhizoplane and rhizosphere soil from station I

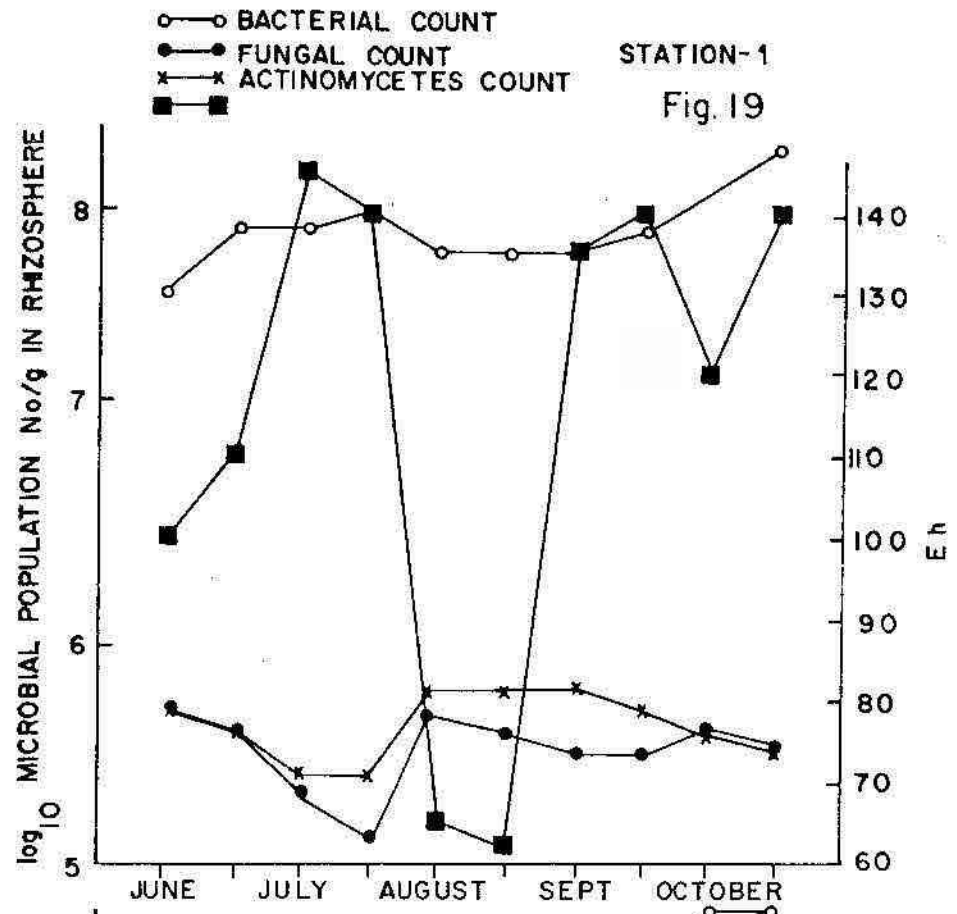

BACTERIAL COUNT
FUNGAL COUNT
ACTINOMYCETES COUNT
STATION-1
log10 MICROBIAL POPULATION No/g IN RHIZOSPHERE
8
7
6
5
140
130
120
110
100
90
80
70
60
Eh
JUNE
JULY
AUGUST
SEPT
OCTOBER

Fig. 19

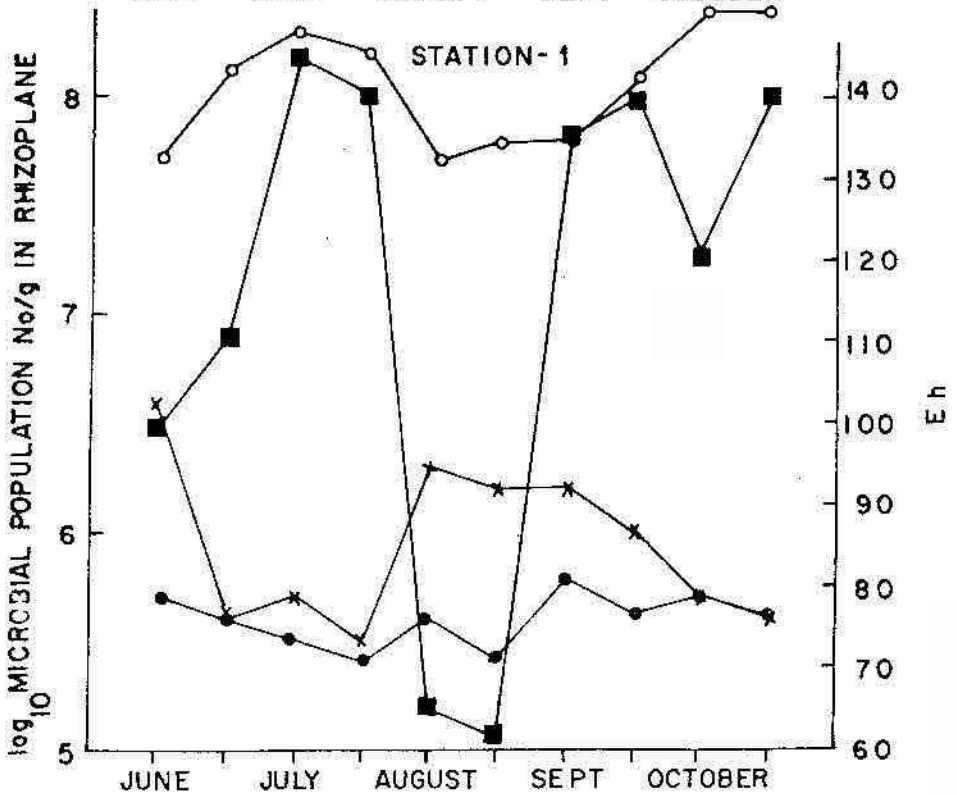
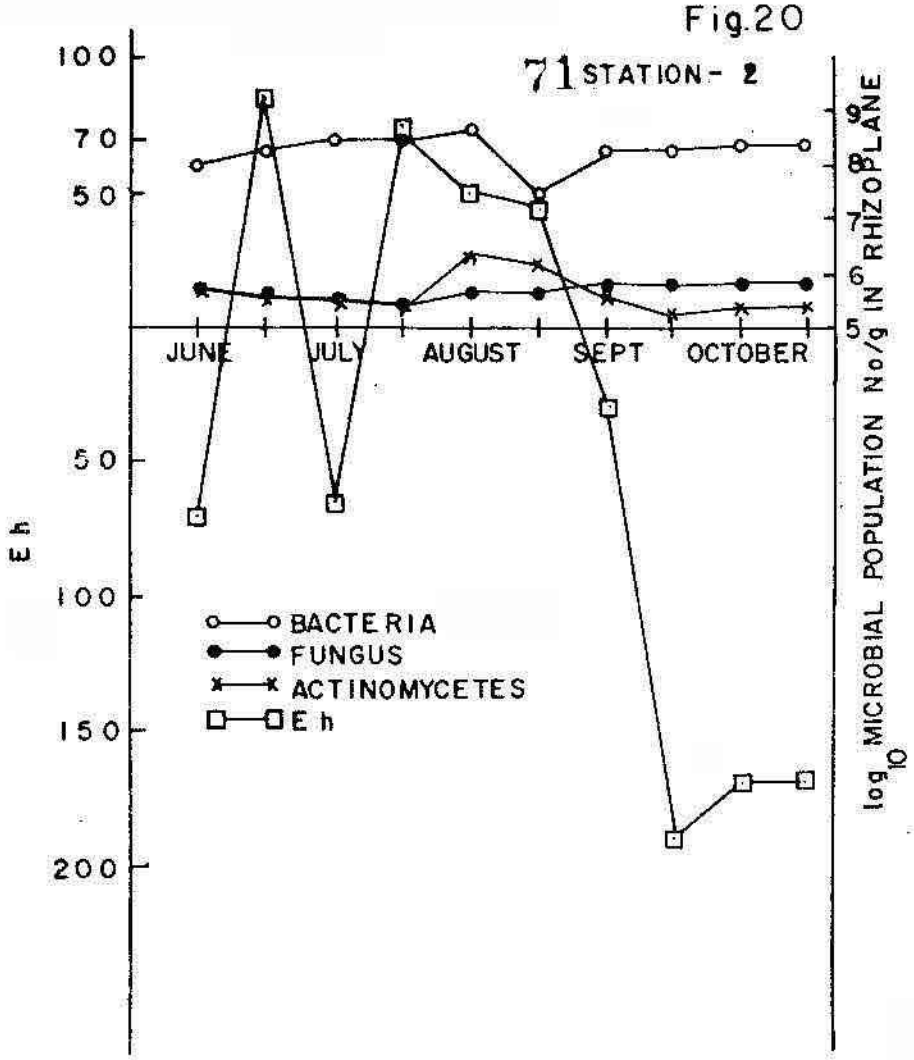

STATION-1
log10 MICROBIAL POPULATION No/g IN RHIZOPLANE
8
7
6
5
140
130
120
110
100
90
80
70
60
Eh
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig. 18

Fig. 20-21 Variation in the Eh and microbial population in the rhizoplane and rhizosphere soil from station II

Fig.20


71 STATION - 2
100
70
50
50
100
150
200
Eh
JUNE
JULY
AUGUST
SEPT
OCTOBER
BACTERIA
FUNGUS
ACTINOMYCETES
Eh
9
8
7
6
5
log10 MICROBIAL POPULATION No/g IN RHIZOPLANE

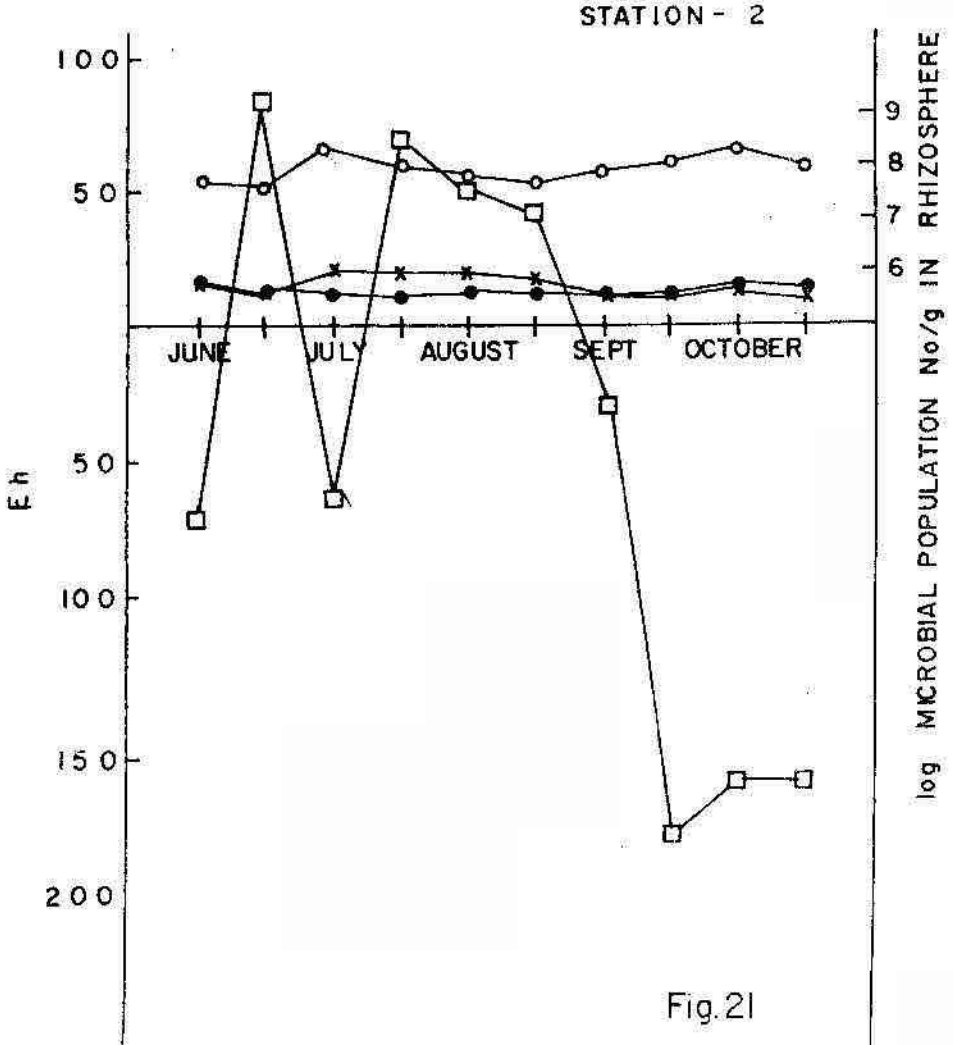

STATION - 2
100
50
50
100
150
200
Eh
JUNE
JULY
AUGUST
SEPT
OCTOBER
9
8
7
6
log MICROBIAL POPULATION No/g IN RHIZOSPHERE

Fig.21

Fig. 22-23 Variation in the salinity and microbial population in the rhizoplane and rhizosphere soil from station I.

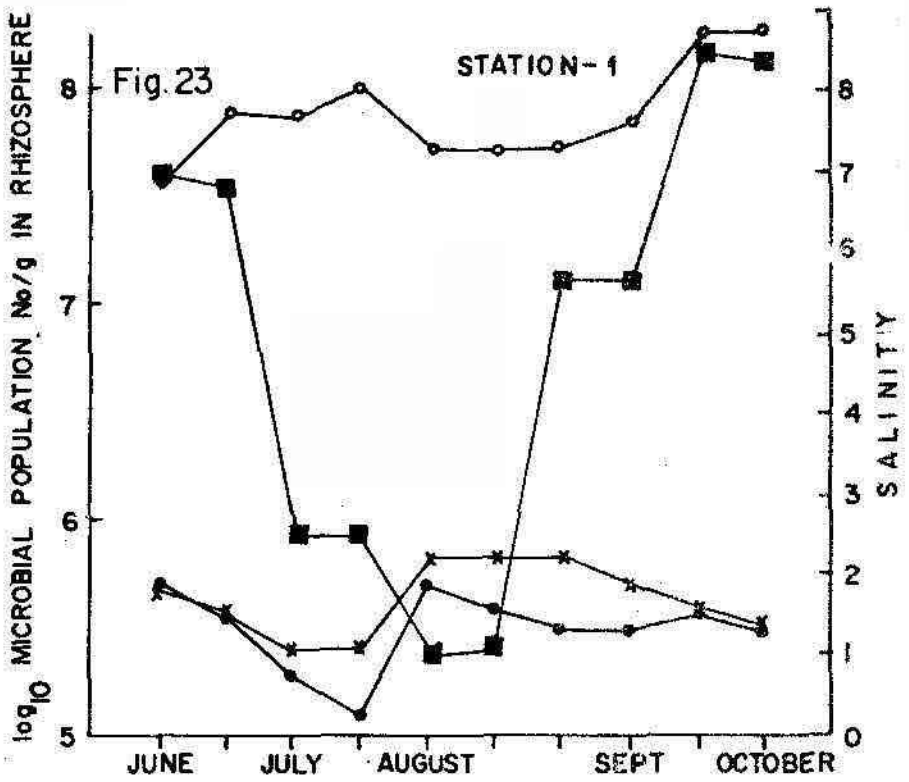


Fig.23
STATION-1
log10 MICROBIAL POPULATION No/g IN RHIZOSPHERE
SALINITY
JUNE
JULY
AUGUST
SEPT
OCTOBER

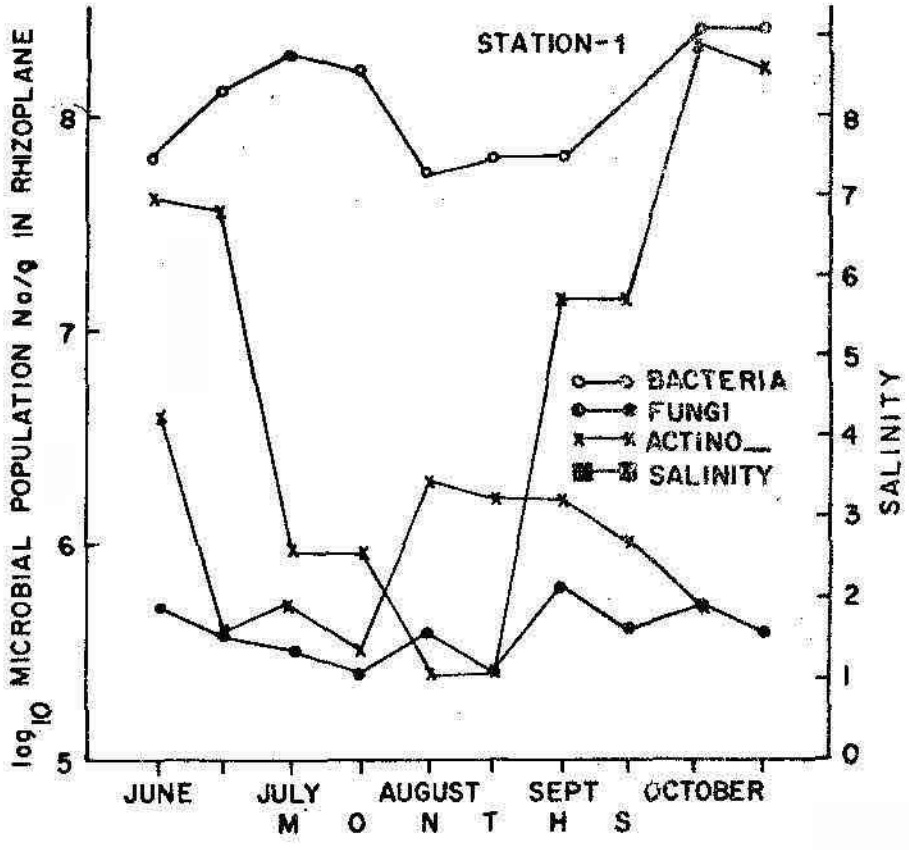

STATION-1
log10 MICROBIAL POPULATION No/g IN RHIZOPLANE
SALINITY
BACTERIA
FUNGI
ACTINO_
SALINITY
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig.22

Fig. 24-25 Variation in the salinity and microbial population in the rhizoplane andrhizosphere soil from station II

Fig.25

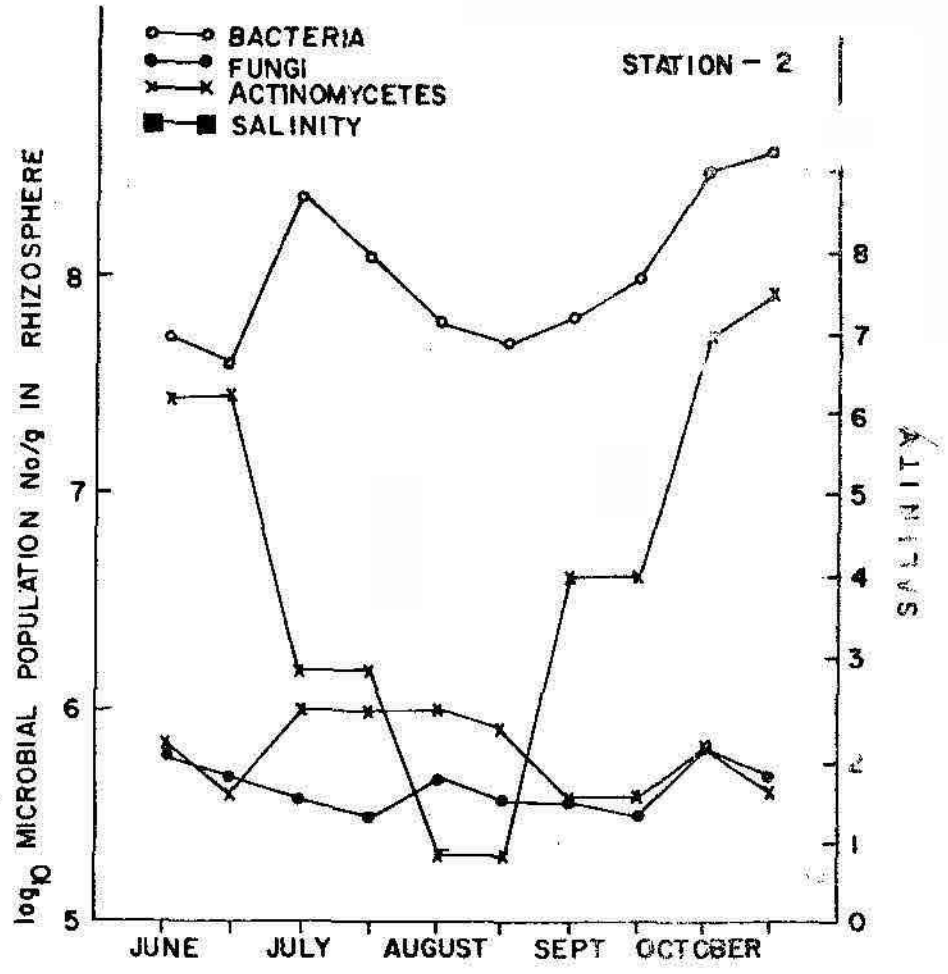

BACTERIA
FUNGI
ACTINOMYCETES
SALINITY
STATION – 2
log10 MICROBIAL POPULATION No/g IN RHIZOSPHERE
SALINITY
JUNE
JULY
AUGUST
SEPT
OCTOBER

Fig.24

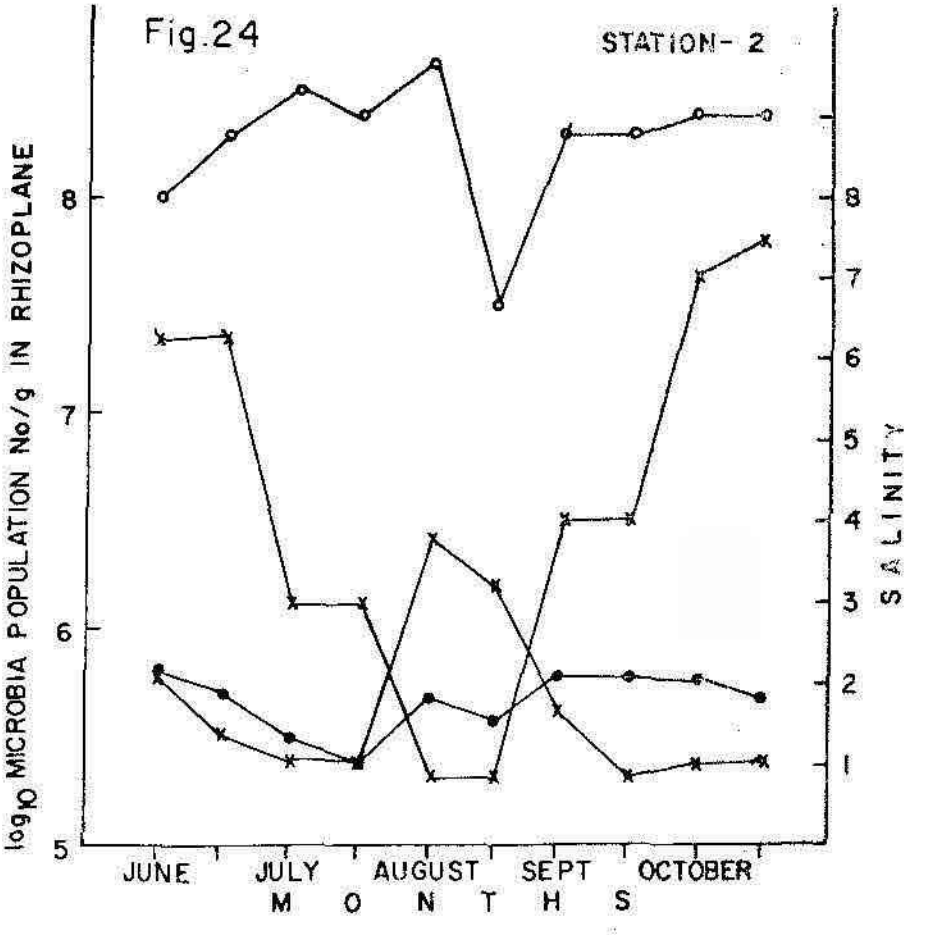

STATION- 2
log10 MICROBIA POPULATION No/g IN RHIZOPLANE
SALINITY
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig. 26-27 Seasonal variation of various zymogenous population in rhizoplane and rhizosphere soil from station I

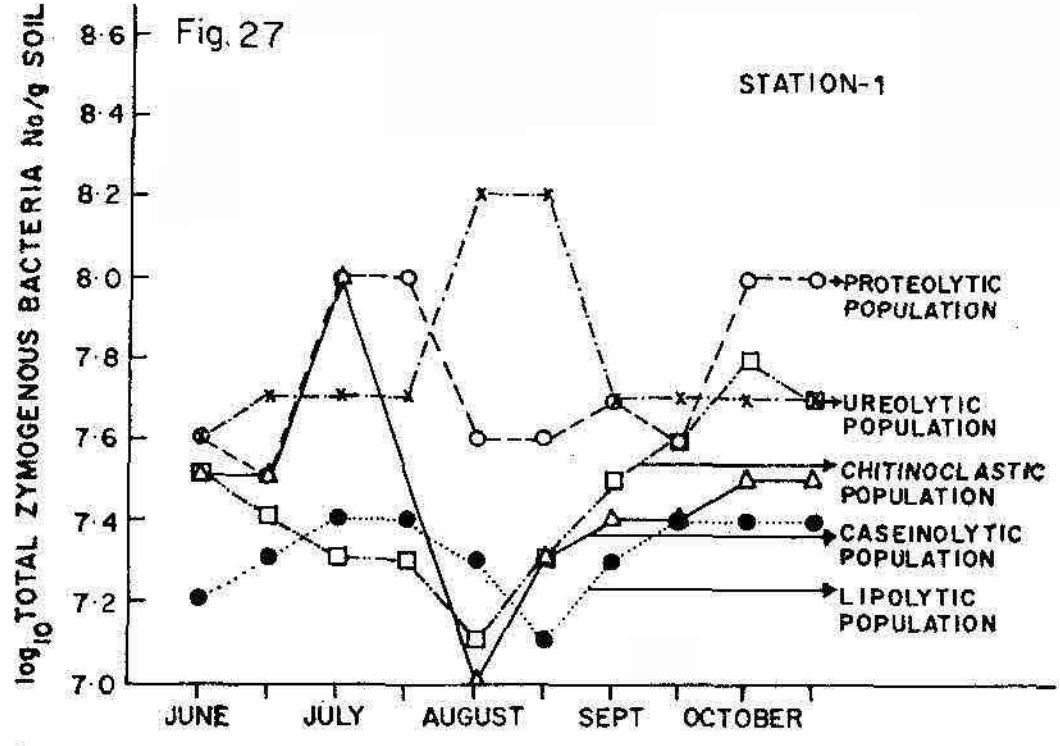


Fig. 27
STATION-1
log10 TOTAL ZYMOGENOUS BACTERIA No/g SOIL
8·6
8·4
8·2
8·0
7·8
7·6
7·4
7·2
7·0
PROTEOLYTIC POPULATION
UREOLYTIC POPULATION
CHITINOCLASTIC POPULATION
CASEINOLYTIC POPULATION
LIPOLYTIC POPULATION
JUNE
JULY
AUGUST
SEPT
OCTOBER

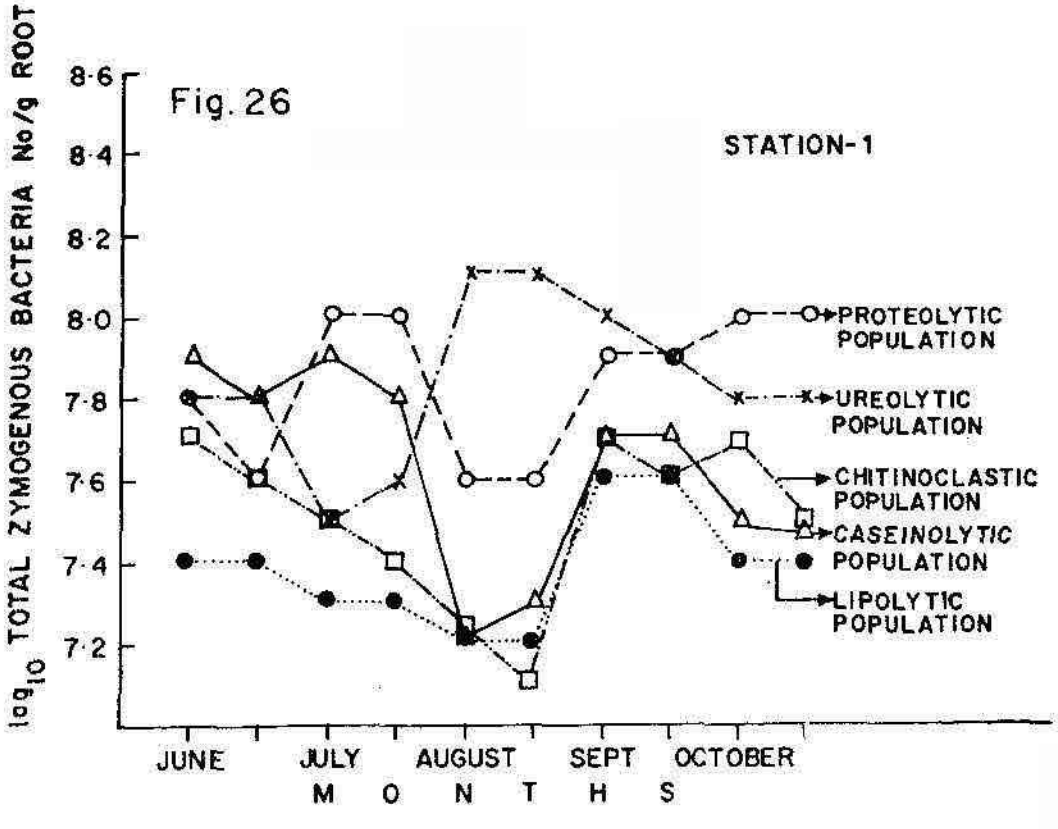


Fig. 26
STATION-1
log10 TOTAL ZYMOGENOUS BACTERIA No/g ROOT
8·6
8·4
8·2
8·0
7·8
7·6
7·4
7·2
PROTEOLYTIC POPULATION
UREOLYTIC POPULATION
CHITINOCLASTIC POPULATION
CASEINOLYTIC POPULATION
LIPOLYTIC POPULATION
JUNE
JULY
AUGUST
SEPT
OCTOBER
M O N T H S

Fig.28-29 Seasonal variation of various zymogenous population in the rhizoplane and rhizosphere soil from station II

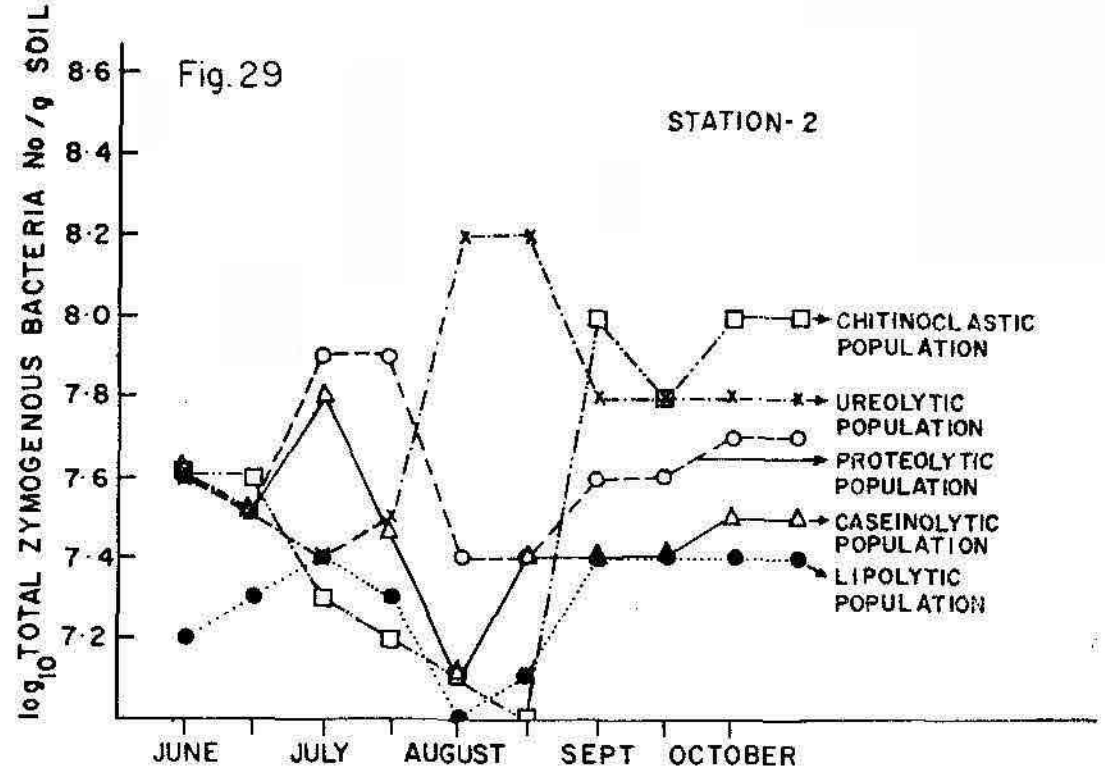


Fig. 29
STATION-2
log10 TOTAL ZYMOGENOUS BACTERIA No/g SOIL
8·6
8·4
8·2
8·0
7·8
7·6
7·4
7·2
CHITINOCLASTIC POPULATION
UREOLYTIC POPULATION
PROTEOLYTIC POPULATION
CASEINOLYTIC POPULATION
LIPOLYTIC POPULATION
JUNE
JULY
AUGUST
SEPT
OCTOBER

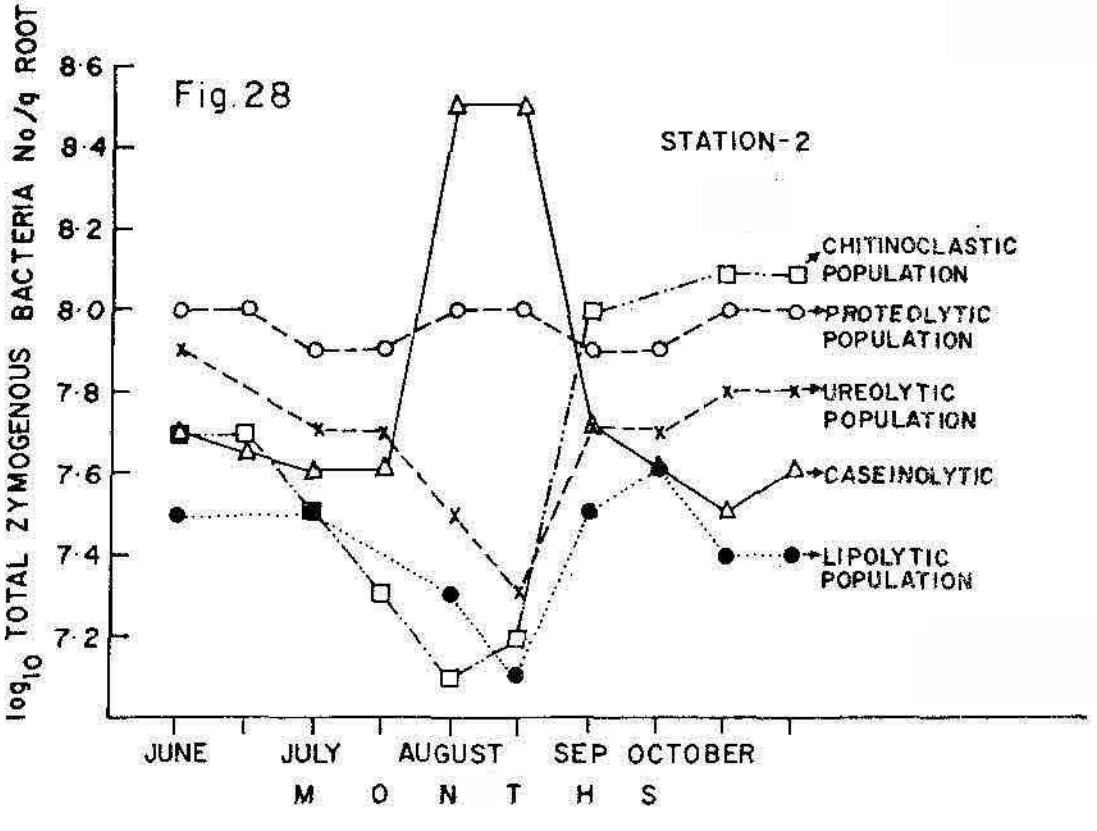


Fig. 28
STATION-2
log10 TOTAL ZYMOGENOUS BACTERIA No/g ROOT
8·6
8·4
8·2
8·0
7·8
7·6
7·4
7·2
CHITINOCLASTIC POPULATION
PROTEOLYTIC POPULATION
UREOLYTIC POPULATION
CASEINOLYTIC
LIPOLYTIC POPULATION
JUNE
JULY
AUGUST
SEP
OCTOBER
M O N T H S

Fig.30-31 Sampling variance between the stations I and II.

Fig. 31

STATION - 2

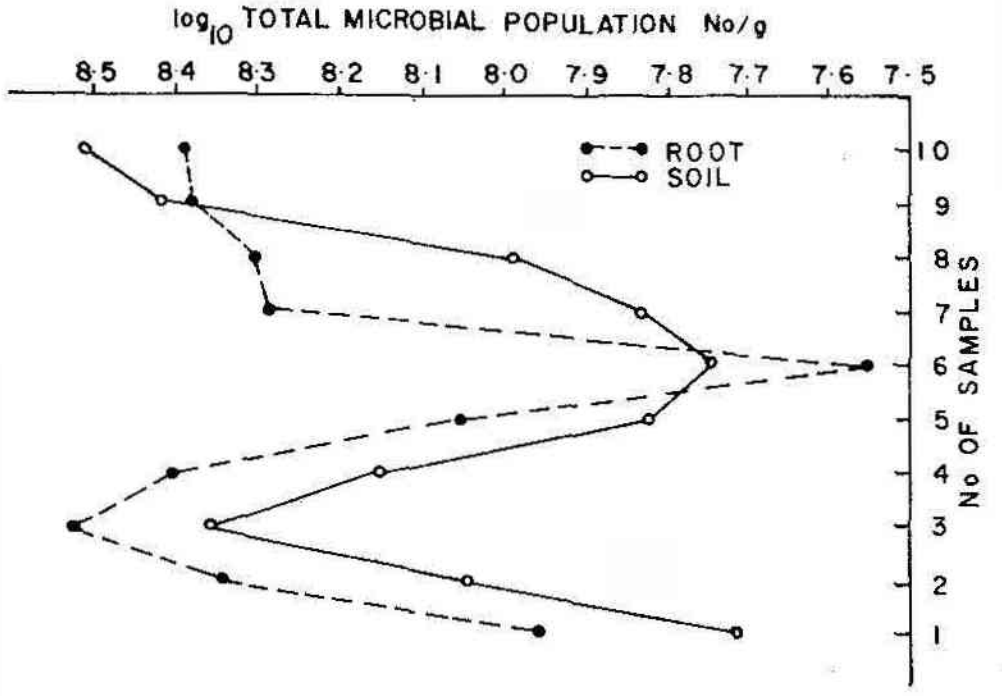
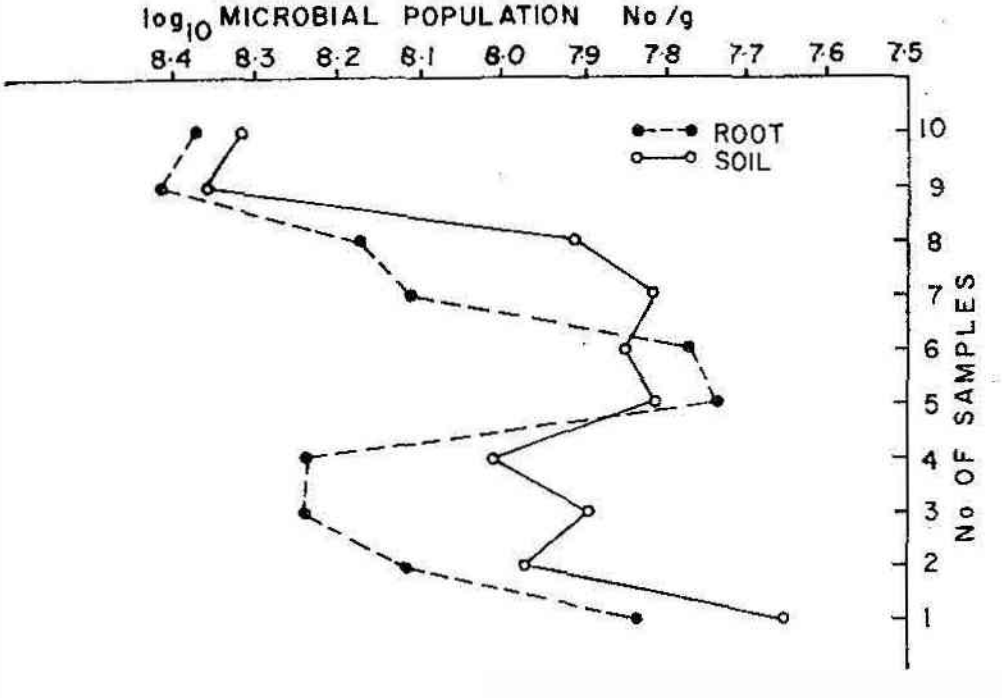


Fig. 30

STATION - 1



DISCUSSION

In the present study the distribution pattern of rhizosphere microflora of Acanthus illicifolious was analysed both quantitatively and qualitatively. The viable heterotrophic bacterial counts in the rhizosphere showed seasonal variation giving the maximum in post monsoon months in both the stations.

Seasonal cycle of fungi revealed the maximum in monsoon months and the minimum in post monsoon months in both the stations. The population of fungi was generally low when compared to bacterial distribution. Total actionomycetes population also showed a similar trend as fungi in the seasonal distribution.

In the rhizosphere soil the bacterial flora showed a decline in the monsoon months and an increase in the post monsoon months. In the rhizosphere, the fungi and actinomycetes counts also showed a similar pattern as the bacteria in the distribution. Bacterial density in the rhizoplane (on the root surface) ranged from 71.42 X 10^4 to 262.62 x 10^6/gm while in the rhizosphere soil it ranged from 72.5 x 10^4 to 226.53 x 10^6/gm. Rovira et. al. (1974) and Kaczmark (1974) reported that the bacterial density in the rhizosphere soil as 10^9/gm whereas direct microscopic counts has given numbers tenfold higher than either in rhizosphere soil or in the rhizoplane . The bacteria are not randomly distributed on the root surface, instead they appeared in profusion only at particular microsites. Rovira (1975) found that although plate counts of fungi was not

appreciably increased in the rhizosphere mycelium, biomass was found to be extensive about 12.14mm of hyphae/sqm of root surface and occupied 3% of surface area. Fischer (1894) encountered moulds in the marine environment far more frequently than either bacteria or yeast. With a very few exceptions he found numerous fungi fairly close to land. Waxman (1934) observed numerous fungi in some marine materials. Sparrow(1937) estimated true marine fungi in marine muds from Woodshole region.

There have been few reports of actinomycetes in the marine environment and usually only in the littoral zone and inshore localities (Grein and Meyer, 1958). Weyland (1969) collected mud samples from 107 stations in the various parts of Atlantic Ocean. The counts ranged from 23 to 290.9 actinomycetes/m^2. Very recently Chandramohan et. al.(1972) reported the occurrence and activities of actinomycetes in the sediments of Bay of Bengal. Their results clearly indicate that actinomycetes may occur not only as a part of marine and mangrove ecosystem but also may contribute significantly to the productivity of the ecosystem.

The bacterial population was predominated by gram negative forms and 96.6% of the isolates were found to be motile. Six genera of heterotrophic bacteria were identified from a total of 30 strains based on various morphological, physiological and biochemical characters. They are Alcaligenes, Flavobacterium, Cytophaga, Vibrio, Aeromonas, and Enterobacteriaceae. The characteristics of

bacteria isolated from mangrove environment had close similarity with the marine bacteria. More than 96.6% were gram negative motile forms. About 80% of the marine bacterial species catalogued by Zobell and Upham (1944) are gram negative rods. 26.4% of the total isolates were found to be pigmented. Relatively high percentage of occurence of pigmented bacteria has been reported from marine environment also. Zobell and Feltham (1934) reported 69.4% of the total isolates as pigment producers. Surendran (1985) found pigmented bacteria ranging from 36.1% to 41.7% in the three stations in the mangrove area of Cochin. Usually poor fermentative activity has been encountered in the marine bacteria whereas in the present study fermentative metabolism was predominant among the bacterial isolates (98.2%). This may indicate that the isolates from the rhizosphere were mostly facultative anaerobes. Alexander (1976) isolated Pseudomonas, Flavobacteriam, Alcaligenes and occassionally Agrobacterium in the rhizosphere. Except Agrobacterium and Pseudomonas all the other 3 genera were isolated in the present study. It is on these genera that the root effect appears to be most pronounced. Rouatt and Katznelson (1961)were able to show that Pseudomonas species were dominant in the rhizosphere while Arthobacter species were characteristic of root free soil. The presence of bacteria belonging to Bacillus, Cornebacterium, Micrococcus and Pseudomonas has been observed in the mangrove swamps of Thailand (Daengsbuba, 1979) and in coconut husk ret liquor (Bhat and Namboodiri, 1977). Such a similarity in the generic composition is not very surprising, as both the ecosystems foster the degradation of the plant materials in a damp estaurine marshy ecosystem. Eklundand and Gyllenberg (1974) have established the significance of these metabolically versatile microorganisms in

the plant decomposition. In the present study the members of each of the taxonomically distinct group showed the presence of series of enzymes implicated in the degradative processes.

It was interesting to note that about 70% of the isolates reduced nitrate and 80% hydrolysed starch and 60% liquified gelatin and 30% produced H_2S from sulphur containing aminoacids which accounts for the strong H_2S odour enountered from the mangrove swamps throughout the study period with an increased intensity in monsoon. The presence of similar physiologically active groups from mangrove swamps from Killai backwaters has been reported by Venkataraman and Ramamurthy. Sreenivasan and Venkataraman (1956) observed that only 5 species of marine bacteria are capable of dentrification. In the present study only 4 isolates seems to be active denitrifiers. Menon et. al. (1972) and Taylor (1940) found a decrease in population from 1m level to the 5cm level with the notable exception of nitrifying bacterial populations that increased by three orders of magnitude from the 1m to 5cm level. This increased population was always related to the high ammonifying population creating a ready nutrient source. The presence of the microorganisms with high degradative potential in the mangrove swamps of Murikumpadam and Cochin area reflect extensive microbial activities and continuous decomposition of foliage and detritus and the turnover of nutrients.

The fungi recorded from the rhizosphere belongs to the genera commonly found in land soils and can be easily recovered in the

spore form from the atmosphere. The majority were species of Pencillium, Aspergillus, Fusarium and Rhizopus. In marine and Woodshole region Sparrow (1937) found species of Pencillium, Aspergillus, Rhizopus, Trichoderma Cladosporium and he doubted them to be the true marine fungi. Though definitely able to live in the sea, most of them were exotic terrestrial species.

The maximum B:F and B:A ratios were observed during the month of October indicating the dominance of bacteria over fungi and actinomycetes in the post monsoon season. Such a type of dominance of bacteria over other microorganisms was reported by Last and Geighton (1965) in the surface of living leaves. Similar work was done by Balagopal and Oblisami (1972) in the rhizosphere of Phaseolus vulgaris.L. infected with Tobbacco Mosaic Virus. Detailed studies are needed to understand the interrelationship between microorganisms in the mangrove area.

Mangrove bacteria were more actively proteolytic than corresponding freshwater microbes. Ostroff and Henry (1939) investigated the ability of 15 proteolytic bacteria of marine origin to utilise 21 different nitrogenous compounds, out of which 10 compounds were readily utilised by proteolytic forms. Maximum count of proteolytic population was observed in both rhizoplane and rhizosphere during September and a minimum was encountered in August which indicated that the bacteria utilising root exudates were active in September. In the present study

60% of the bacteria utilised gelatin either as a source of nitrogen or energy or both. Vagnerova and Macura (1974) found that ammonifying and proteolytic actions are not substrate specific and their response mostly may be attributed to other enviromental factors in the rhizosphere. Selective enhancement of degradation of nitrogenous materials were solely done by bacteria utilising root exudates as energy source.

A gradual decrease in the lipolytic population was observed which reached a minimum in August and increased slightly in September-October, Murchelano and Brown (1970) found that in all seasons only fewer isolates hydrolysed starch than either Tween80 or gelatin and more isolates were proteolytic than lipolytic. They also found that during summer more isolates hydrolysed Tween80. In the present study among 5 zymogenous forms lowest count was encountered only in lipolytic population. Waxman et.al. (1933) Zobell and Anderson (1936) found chitinoclastic bacteria to be widely distributed in marine bottom deposits. In the present study chitinoclastic populations were found nearly in all months in the rhizoplane and rhizosphere. They were most numerous in rhizoplane, decreasing in abundance in the rhizosphere soil samples. Like the distribution of lipolytic population chitinoclastic bacteria also showed slight increase in post mansoon months. Out of the 31 pure cultures of chitinoclastic bacteria isolated from marine environment by Zobell and Rittenberg (1938) many produced yellow, orange, pink or violet pigments. Only red and brown

pigment forming chitinoclastic bacteria were isolated from the rhizosphere throughout the study period. The distribution of chitinoclastic bacteria in rhizoplane and rhizosphere soil indicates a relationship between this population and rhizosphere. From the Gulf of Mexico sediments, Cambell and Williams (1951) isolated 20 strains of aerobic chitin decomposing bacteria including species of Achromobacter, Flavobacterium, Micrococcus and Pseudomonas.

Caseinolytic populations drastically reduced in August in both rhizoplane and rhizosphere soil which were similar to the distribution of other zymogenous bacteria. This may be due to excessive rainfall or lowering of temperature. Chandrika (1983) found significant negative correlation between caseinolytic populations and phosphates in salvinia rich sediments collected from a depth of 30m off Cochin.

Ureolytic bacterial population showed a rise in August in rhizoplane and rhizosphere. This may be attributed to the alkaline pH values, in the rhizosphere. Urea decomposing bacteria were found by Bavendam (1932) in the mud around Bahama islands. He believed that the activities of such bacteria promote the precipitation of $CaCO_3$. Rubentshik (1925) found urea decomposing bacteria in all the mud samples taken from Odessalymans. Zobell and Feltham (1935) found surface mud to contain from 10 to1000 Urea decomposing bacteria/gm. From all these observations it has been

concluded that all heterotrophic aerobes, anaerobes and zymogenous aerobes with their ecological inter-relationship play a good role as potential decomposers or degraders of organic matter whether it is a leaf litter or root debris in the mangrove ecosystem. This is supported by the view that bacterial activity is related to sediment particulate matter (Iturriaga 1970).

Very little is known about the temperature requirements of soil microflora. Minimum, obtimum and maximum temperature from only laboratory experiments are known. The ambient temperature for bacteria was found to be 28 ± 2°C. There is a negative relationship between temperature and bacteria. Even though temperature decreased in August, the fungal and actinomycets counts showed an increase. This may be due to excessive rainfall and lowering of salinity as most of the isolates were exotic terrestrial species. Similar findings were obtained by Matondkar (1980) in the mangrove swamps of Goa. Ulken (1972) found that when temperature of the environment was about 20°C, the optimum temperature for plate count for fungi was about 25°C. This agrees well with the present result obtained during August.

The pH ranged from 7.3 to 7.7 during the period August-October in station 1 where as in station 2 it ranged from 6.4 to 7.2. Ulken (1972) found that, in experiments growth of the fungus begins at pH 6.2. In the result the maximum counts were obtained during August when the pH was recorded minimum. However in all the other months with optimum and alkaline pH normal counts were

obtained, thereby indicating less influence of pH on fungal growth.

The Eh in the rhizosphere was found to be significantly related to bacterial counts. Eh generally decreases with depth in sediments and Zobell & Feltham (1942) have found a parallel decline in bacterial population. Others have found no consistent pattern (Wood, 1959). Since bacteria were quite diverse in their response to Eh clearcut relationship between Eh and total bacteria would not necessarily be expected. Same pattern was observed for fungi and actinomycets.

Bacteria isolated from the observations were moderately halophillic as they survived low salinities and tolerated 5-20% saline media. Usually in experiments optimum growth of fungus occurs at 15% (Ulken, 1972) but here they survived in lower salinites. It is very doubtful whether they are true marine fungi or exotic forms. Though definitely able to live in the mangrove ecosystem most of them were terrestrial forms. The moulds which Fisher (1894) observed in marine environment were common species of terrestrial fungi primarily Fusarium, Pencillium and Aspergillus. The same can be applied to actinomycetes which are acclimatised to low salinities.

Zobell (1967), Carlouis (1974) reported that one of the important factors governing the distribution of bacteria in marine environment is availability of nutrients particularly phosphates and nitrates.

Statistical analysis revealed that, none of the physico chemical factors constituted significant relationship with bacterial population in both the stations. This may be due to the less number of observations as statistical validity needs more frequent observations. However, during monsoon decrease in phosphorus graphically coincided with the decrease in the bacterial counts. But fungi showed an increase in August whereas actinomycetes counts fluctuated during the monsoon months. The antagonistic nature of these isolates were not tested, however during routine work it was casually observed that cultures elaborated some antibacterial, antifungal and antibiotic substances which were active against Aspergillus sp. and Pencillium sp.

However, more detailed study on the physiology of this group, especially on the production of antibiotics from mangrove ecosystem could be of greater significance for the economy of the country.

The graphical representation of microflora along with physico-chemical factors revealed a relation to nutrient cycling. It was observed that the activities of rhizosphere microflora effect the circulation of nutrients in the mangrove ecosystem through their influence on Acanthus beds. The biochemical activity of rhizosphere microflora of Acanthus ilicifolius of soil revealed that:-

1. Rhizoplane microflora of Acanthus ilicifolius have greater ability than the rhizosphere soil population to effect rapid

biochemical changes like greater mineralization of organic matter. Certain kinds of substrates can be oxidised and fermented in the presence of mycorrhiza than in their absence.

2. The combined respiratory activity of microorganisms and roots of Acanthus ilicifolius result in greater carbon-dioxide production which dissolves in soil-water to form carbonic acid that leads to increased solubility of primary minerals in the Acanthus Beds.

3. The rhizosphere habitat stabilises the substrate availability in the mangrove, which is the major limiting factor for the heterotrophic soil microflora.

4. The loss of photosynthate as root exudates enhances growth of chelate producing microbes and facilitates the solubilisation of primary minerals which act through a positive feed back mechanism to increase the productivity of this ecosystem.

SUMMARY

1. Studies on the microbial flora in the rhizosphere of Acanthus illicifolius in mangrove ecosystem were conducted for a period of 5 months, (June-October, 1986). Two stations (station 1 and 2) with different ecological conditions, one in Murikumpadam area in Vypeen island and the other near Cochin backwaters in front of C.M.F.R.I. were selected and the distribution of bacteria, fungi and actinomycetes in rhizoplane and rhizosphere soil were studied both quantitatively and qualitatively along with physico-chemical characteristics. The results are given in the form of intensity charts and tables.

2. Samples from both the stations contained significant quantities of all the microflora. The distribution of microflora did not show much variation among the two stations but the seasonal cycle was very evident in station 2.

3. The bacterial population in the rhizoplane ranged from 71.42×10^4 to 262.62×10^6/g while in soil it ranged from 72.5×10^4 to 226.54×10^6/g. Similarly the fungal and actinomycetes ranged from 25.71×10^4 to 10^6/g and 2.98×10^4 to 21.42×10^5/g in root respectively. In the rhizosphere soil an average of 5×10^6/g and 3.23×10^5/g was encountered.

4. A total of 30 bacterial strains were isolated and six genera were identified from these isolates viz., Alcaligenes, Flavobacterium, Cytophaga, Vibrio, Aeromonas and Enterobacteriaceae.

5. Alcaligenes was found to be the predominant genus among the 30 isolates. Gram negative rods constituted 96.6% of the total isolates.

6. Fusarium was found to occur in maximum numbers in both the stations followed by Penecillium and Aspergillus and Rhizopus was found to occur in minimum numbers.

7 All the zymogenous populations showed seasonal variation during the study period. A decrease in the four zymogenous forms was observed during August in both rhizoplane and in the rhizosphere soil. The decrease was followed by a gradual increase during the month of September and October.

8. More isolates were proteolytic than lipolytic or amylolytic.

9. The inter-relationship among the microflora in the rhizosphere was tested and maximum B:F and B:A ratios were recorded during the month of October in station 1 and during July in Station 2, indicating the predominance of bacteria in both the stations.

10. All the three microflora dominated in the rhizoplane of Acanthus illicifolius than in the rhizosphere soil of both the stations.

11. 90% of the bacterial isolates produced H_2S from sulphur containing amino acids which indicated the predominant role of

these forms in the biogenic decomposition of albuminous compounds in the mangrove ecosystem. Purple sulphur bacteria and green sulphur bacteria were also encountered in the rhizosphere.

12. Statistical analysis revealed no significant relationship between microflora and chemical factors studied.

13. A significant negative correlation was observed between the bacteria and actinomycetes population.

14. Clustering of samples in some ranges of the counts of microflora were encountered. So the distribution of microflora is characterised by over dispersion.

15. The relationship between the physico-chemical parameters and bacterial counts revealed that station 1 and 2 are more or less homogenous.

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