ECOLOGICAL STUDIES ON PHYLLOPLANE FUNGI OF PADDY

Abstract

By

RAJEEVALOCHANA

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF

DOCTOR OF PHILOSOPHY IN BOTANY

To

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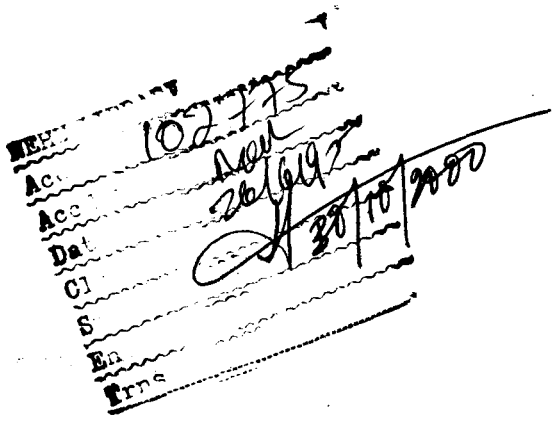


Microbial communities on the leaf surface of three varieties of paddy differing in resistance to the leaf spot pathogen, Helminthosporium oryzae, viz., Khonorullo (disease resistant), Ngoba (moderately susceptible) and Mirikrak (disease susceptible) were studied in detail using five different techniques of isolation (direct observation, moist chamber, leaf impression, washed leaves plating and dilution plate methods). The microflora of the phylloplane of the paddy plants is mainly influenced by varietal characters and also by the pathogen, H. oryzae. In addition, distribution of fungi on leaf surface was also dependent on leaf maturity and weather changes. Analysis of phylloplane mycoflora showed that in the resistant variety there was a significantly higher population of fungi as compared to the susceptible one. Further, the total number of fungal colonies was observed to increase significantly with the age of the plant and was independent of plant variety. Among the fungi isolated Cladosporium spp., Penicillium spp., Alternaria spp. and Aspergillus spp. dominated the leaf surface of both resistant and susceptible cultivars. Species diversity among fungal members as calculated by Sorenson's index was found to vary with plant age. A natural antagonism was found on the resistant varieties between Trichoderma viride and the leaf spot pathogen, H. oryzae.

From a study on the airspora of the three experimental plots where the paddy varieties were grown it was found that the fungal spores present in the air exerted a great

impact on the leaf surface of paddy. Most of the spores of air were also trapped on the leaf surface. Some of the forms however, maintained their specificity in the two environments viz., air and phylloplane. The spores of the pathogen were found in the air of all the three paddy varieties very early in the crop season but the visible symptom of the disease was observed later only on the leaf spot susceptible variety. T. viride, a possible antagonist of the pathogen was found only on the leaf surface of the resistant variety although it occurred in the air of all the paddy varieties.

Pathogenicity test was conducted by using Koch's postulates both in vitro and in vivo and it was confirmed that the causal organism for the disease occurring on the susceptible varieties was definitely **Helminthosporium oryzae** Breda de Haan which caused brown spot disease of paddy. This pathogen was also isolated in pure culture. The susceptibility to the pathogen increased with age which was clear from the pathogenicity studies done in vivo and in vitro. The pathogen spores from 15 day old cultures with a concentration of 2×10^5 spores/ml suspended in 0.01 M phosphate buffer at pH 6 if incubated at 35°C showed maximum spore germination and germ tube length.

From interaction studies done in vitro and in vivo between certain epiphytic fungi and the pathogen, it was observed that in vitro some selected fungal antagonists such

as Trichoderma viride may be used as a biological control agent forbrown spot disease of paddy although it was only partially successful in pot experiments. The exact mechanism of action of T. viride against the pathogen, H. oryzae could not be clearly understood. It can inhibit the pathogen either by hyperparasitism or by producing some antibiotics.

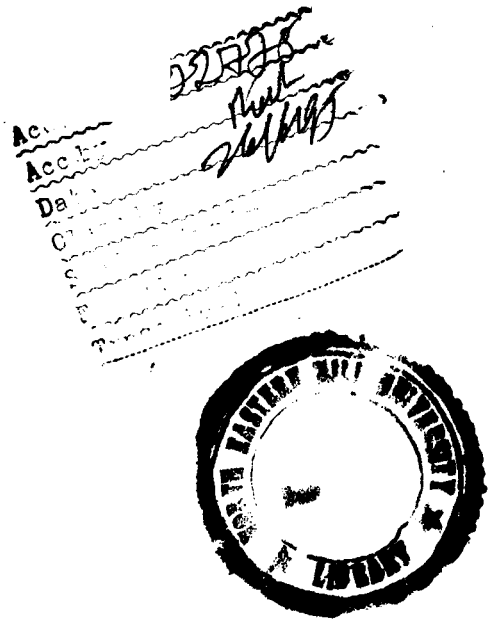
To see the competitive ability of some selected phylloplane fungi on the leaf surface of paddy varieties, spores of these fungi were germinated in leaf leachates, leaf extracts and on detached leaves of paddy and their growth performance was studied. The sampling for the experimentation has been done at young, mature and senescent stages of paddy varieties. The effect of the different treatments on the germination and growth performance of the fungi varied in the different experiments. Generally, the germination and growth performance of these fungi were enhanced in the leaf leachates, leaf extracts and on the surface of leaves independent of variety. But most of the antagonistic fungi of the pathogen such as T. viride were more stimulated in the leaf leachates, extracts and on the leaf surface of resistant varieties than on the susceptible one while the susceptible variety leachates, extracts and leaf surface stimulated the spore germination of the pathogen.

The results of the biochemical analysis of the leaf leachates indicate that eighteen amino acids, sixteen sugars,

twelve organic acids and nine phenols were recorded in leaf leachates of the three paddy varieties. The number of amino-acids, sugars, organic acids, and phenols increased as the plants grew older and more amino acids, sugars, organic acids and phenols were detected in the exudate of the resistant variety. The quantitative study of leaf extracts also indicate that more quantity of sugars, amino acids and phenols were detected in the resistant variety than in the susceptible one and the amount increased with plant age. The results of the biochemical analysis of the leaf leachates and extracts clearly confirm the fact that the colonization of fungi on the leaf surface is directly controlled by nutrient level of the leaf surface which changes with the release of substances from the leaf. In addition to high nutrient level in the resistant variety which enable this habitat to attract more saprophytic fungal colonization thereby forming a microbial barrier to foliar infection, some phenolic compounds may also provide resistance to the plant to brown spot disease of paddy.

Another factor which favours the development of saprophytes on older leaves during crop growth was found to be pollen. The presence of pollen on surfaces of leaves had marked influence on phylloplane fungal population. The total fungal population was highly stimulated on leaves with pollen especially on mature leaves and the effect was much pronounced on the leaves of resistant variety. In addition to change in

saprophytic flora the pathogen population also increased in the presence of pollen on the leaves of susceptible variety. This study suggests a possible role of pollen in pathogenesis and disease development.



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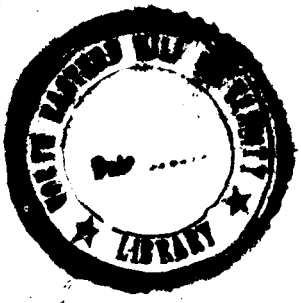

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I certify that the thesis entitled "Ecology of Phylloplane fungi of Paddy" submitted by Mrs. Rajeevalochana for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by her under my supervision. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. degree. This work has not been submitted for any degree of any other University.

R.R. Mishra

Supervisor

Place: Shillong
Date : September, 1983

Forwarded
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Rajeevalochana

Place: Shillong
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C O N T E N T S

GENERAL INTRODUCTION

Ever since the introduction of the term "phyllosphere" independently by Last (1955a,b) and Ruinen (1961) and subsequently by Dickinson (1965) using the term "phylloplane" for phyllosphere of Last, the study of leaf surface microflora has attracted the attention of different workers (Lamb and Brown, 1970; Sinha, 1971; Dickinson, 1971, 1976; Mishra and Srivastava, 1971b, 1974; Mishra and Tewari, 1976b; Sharma and Mukerji, 1976; Gupta and Mukerji, 1982; Gupta and Dixit, 1983).

Leaf surface holds a fascination especially for microbial ecologists because of its complex nutrient spectrum which supports a rich microflora. The study of the qualitative and quantitative composition of the epiphytic micro-organisms on leaf surface as well as the investigation of their activities constitutes an important problem concerning the inter-relations between plants and micro-organisms.

Considerable interest has developed recently (Narula and Mehrotra, 1981; Khara and Singh, 1981; Rao and Manoharachary, 1981; Pennycook and Newhook, 1981; Ahuja and Payak, 1981; Kumar and Singh, 1981; Miller and Roy, 1982, Gupta and Mukerji, 1982) in investigating the various types of micro-organisms which occur on leaf surface at various stages of plant growth and also during the changing environments.

An understanding of the nature, periodicity and density of the fungal propagules in the air is much helpful in making a

forecast regarding the occurrence of fungal diseases and the quantum of viable pathogenic propagules likely to cause infection. Very little work has been done on this aspect in India (Mishra and Srivastava, 1970b, 1971a, 1972; Mishra, 1972; Mishra and Tewari, 1976a; Kumar and Gupta, 1976, 1980; Dixit and Gupta, 1980). The effect of air fungal population over different fields in relation to the leaf surface fungi has not been studied in detail excepting a few workers (Mishra and Tewari, 1976a; Kumar and Gupta, 1980). By understanding a survey of the aero-mycoflora of different regions, some knowledge about the various human pathogens which are responsible for allergenic diseases may be obtained since 80% of them are caused by fungal spores (Agarwal et al., 1968).

Leaf surface is well known to harbour a definite microbial community by virtue of the presence of leachates (Godfrey, 1976; Irvine et al., 1978). The different microbial populations in such a community interact with one another by competing for space and nutrients, by production of secondary metabolites and also the antibiotics (Fokkema, 1973; Hudson, 1978). Earlier workers (Last, 1955a; Ruinen, 1956, 1961; Dickinson, 1965, 1967) studied the microflora of different plant species without laying much emphasis on the mutual interaction of the micro-organisms associated with leaf surfaces. Studies relating to mutual antagonism of the micro-organisms and the possibilities of biological control of

some pathogens opened a new field of investigation to the pathologists (Fokkema, 1973, 1978; Mishra and Tewari, 1976c; Sharma and Gupta, 1978, 1980; Rao et al., 1978; Tsuneda and Skoropad, 1980; Rai and Singh, 1980; Purkayastha and Bhattacharya, 1982; Papavizas et al., 1982). Thus the role of phylloplane saprophytic fungi in biological control in certain epidemic diseases may be important and if properly exploited it may offer an alternative to the synthetic fungicides (Srivastava et al., 1981; Purkayastha and Bhattacharya, 1982; Gupta and Dixit, 1982; Trutman et al., 1982; AbdEl,moity et al., 1982; Elad et al., 1983).

In addition, a large number of nitrogen fixing organisms are present on the leaves of plants growing on nitrogen deficient soil (Pillai and Sen, 1966). Supplied with moisture from the atmosphere and nutrients in the form of leaf exudates, such organisms may fix considerable amount of nitrogen. Recently, considerable interest has developed on this aspect and many workers are trying out different micro-organisms which might play a role in the growth and nitrogen nutrition of hostplants and in turn may increase the yield of plants (Kvasnikov et al., 1974; Blasco and Jordan, 1976; Remacle, 1977; Capone and Taylor, 1977; Banerjee and Chandra, 1978; Sadykov and Umarov, 1980a,b; Nandi and Sen, 1981, 1982; Sadykov, 1981; Sengupta et al., 1981; Nandi et al., 1982a, b).

Further, the studies on leaf surface mycoflora are also helpful in screening out the decomposers which are responsible for active turnover of minerals from leaf litter (Gupta, 1982). The potentialities of organic plant residues as fertilizers is well recognized (Whitehead, 1963; Chatterjee et al., 1979). However, the suitability of the organic material as fertilizer depends to a great extent on its rapidity of minerilization and quick liberation of the nutrients (Goluke et al., 1954). Much work has already been done on this aspect (Garrett, 1963, 1980; Harley, 1971; Kalekar et al., 1976; Wani and Shinde, 1980; Bhardwaj, 1980).

The excretion of nutrients by leaves has now been an established fact (Sinha, 1965; Godfrey, 1976; Irvine et al., 1978). The leaf leachates or exudates greatly influence the quality and quantity of micro-organisms occurring on the leaf surfaces (Tukey, 1971; Tyagi and Chauhan, 1982). Very little work has been done to study the biochemistry of the leaf leachates at various stages of growth of the plants (Tewari, 1973; Mishra and Tewari, 1976b, 1978; Tyagi and Chauhan, 1982). For explaining the population dynamics of the phylloplane mycoflora physico-chemical characteristics of the leaf surface environment should be properly understood (Mishra and Tewari, 1976b).

Further, stimulatory effect of leaf surface nutrients on the microflora has been reported by Brown (1922) and Mishra

and Srivastava (1970a), whereas Purkayastha and Deverall (1965) found that growth of Botrytis cinerea was inhibited by substances arising from the leaf cells. Detailed study on the overall effect of the leaf and leaf surface nutrients on the phylloplane fungi is, however, not available for most of the plants excepting a few reports in the literature (Mishra and Tewari, 1978; Tyagi and Chauhan, 1982). Thus an investigation of the spectrum of chemical compounds (aminoacids, sugars, organic acids and phenols) available on the leaves of different plants in relation to their effect on the spore germination of phylloplane fungi is warranted (Tyagi and Chauhan, 1982).

Also, the effect of pollen on the saprophytic mycoflora of the phylloplane has not been studied in detail. It has been observed by some workers (Ogawa and English, 1960; Bachelder and Orton, 1963; Chu-chou and Preece, 1968; Chu-chou, 1970; Fokkema, 1968, 1971; Warren, 1972, 1976; Garg and Sharma, 1982) that presence of pollen grains affects the establishment and infection of leaf-pathogens by changing the saprophytic mycoflora of the phylloplane. Any factor which might alter the balance between phylloplane micro-organisms and pathogens on leaf surface may be of significance in the understanding of disease-development (Garg and Sharma, 1982).

Relatively little work has been done to understand the ecology and role of naturally occurring microbes on the leaf

surface. Considering the significant role played by the phylloplane fungi in controlling diseases on crop plants, the present investigation was undertaken to study the various ecological aspects of the phylloplane fungi. The work was undertaken to find out the extent of host influence on building up of microbial communities under similar climatic conditions.

In North-Eastern part of India where rice is the staple food for the people, paddy constitutes one of the most important crop. Many epidemic diseases are reported on paddy from this region. Considerable loss in yield was noted during the great famine of Bengal, in 1942-1943 caused by brown spot disease of rice. There was almost failure of the paddy crop and the losses in yield upto 90% in Bengal (Ghose *et al.*, 1960) and in weight of grains ranging from 4.6 to 29% in Punjab (Bedi and Gill, 1960) were recorded due to infection by *H. oryzae*.

The present study was undertaken on three varieties of paddy which are commonly grown in North-Eastern region of India. The three varieties exhibit different degree of response in relation to *Helminthosporium oryzae*: Khonorullo is a disease resistant variety; Ngoba, a local variety is moderately resistant and Mirikrak, is a disease susceptible variety.

Therefore, work was taken up to screen different saprophytic fungi which may be exploited for the biological control of the pathogen *H. oryzae* which causes brown spot

disease of rice. The present study was designed to follow the pattern of fungal colonization on paddy leaves from initial expansion stage through maturity and upto senescence.

It is now well recognised that studies on ecology of micro-organisms depend on the careful selection and application of proper techniques of isolation (Dickinson, 1971). For proper studies of phylloplane, simultaneous use of several techniques have been strongly recommended for getting a complete spectrum of the fungal flora (Sharma et al., 1974; Dickinson and Wallace, 1976; Gupta, 1982). Therefore, several complementary techniques, viz., direct observation (Edward and Hartman, 1952; Masurovsky and Jordon, 1960), moist chamber (Keyworth, 1951), leaf impression plate (Potter, 1910), dilution plate (Dickinson, 1971) and washed leaves plating (Macauley and Thrower, 1966) were used simultaneously. Further, nutrient medium used also influences the isolation of different fungi (Tsao, 1970). Hence several media viz., Czapek's Dox agar, Potato dextrose agar and cellulose agar were tried to find out best combination of media on which maximum number of fungi could be isolated.

Most of the earlier studies on biological control have been done in vitro, adopting dual culture method by observing the imhibition potentiality of the pathogen by different test fungi. These studies often ended with doubts about their success in controlling the pathogens in vivo as a result, there is a lot of discrepancy between laboratory and field studies

(Heuvel, 1970; Roy, 1977; Rai and Singh, 1980; Purkayastha and Bhattacharyya, 1982). Thus in the present studies, after screening the potential antagonists of the pathogen, H. oryzae in vitro their success in controlling the brown spot disease of paddy in pot experiments were also tried.

There is still ample scope for further research on this important field of microbial ecology which may open up many unexplored vistas which will in turn help in solving the various ecological problems.

The studies during the present investigation has been classified into following 7 chapters and each aspect will be discussed separately.

I. Assessment of the fungal flora from the phylloplane regions at different developmental stages of the plant.

II. Survey of air fungi of the site where the three varieties of paddy were growing and the evaluation of their impact on the leaf surface mycoflora.

III. Screening and isolation of the pathogen from the leaf surface and the studies on the role of different factors which control the occurrence of the pathogen (H. oryzae).

IV. Interaction studies in vitro and in vivo between certain epiphytic fungi and the pathogen obtained from the phylloplane of paddy plants and also the exploitation of the effective epiphytes in the biological control of the pathogen.

V. Competitive ability of the phylloplane fungi on the leaf surface and their germination in leachates obtained from different ages of the three varieties of paddy plants.

VI. Biochemical analysis of the leaf leachates and leaf extracts and their role on the colonization of the phylloplane fungi.

VII. Effect of pollen on the saprophytic and pathogenic mycoflora of the phylloplane.

REVIEW OF LITERATURE

The presence of fungi on the surface of plants has been recognized as early as in 1866 by Debary. He described Dematium pullulans as a fungus commonly occurring under such situation. It has long been known that not only pathogenic micro-organisms but also populations of non-pathogenic micro-organisms may develop on the surface of living leaves and other aerial parts of plants.

The term 'Phyllosphere' was proposed by Last (1955) and Ruinen (1956) to describe the mileu on leaf surfaces. Later, Dickinson (1965) and Last and Deighton (1965) preferred to restrict the term to the zone near leaves and to use 'Phylloplane' when referring to actual leaf surfaces.

Extensive reviews of investigations on the epiphytic microflora of living leaves are given by Ruinen (1961), Last and Deighton (1965), Leben (1965), Sinha (1965), Mangenot (1966) and Sharma and Mukerji (1974).

Since the mid 1950's the study of micro-organisms on leaf surfaces has become a recognised field of investigation. By 1970 it was felt to hold an international symposium on the subject and the proceedings were published in 1971 (Preece and Dickinson, 1971). Five years later a second symposium was held (Dickinson and Preece, 1976) which reflects the increasing amount of research being carried out in the area. Subsequently,

in 1980 a third international symposium on the microbiology of leaf surfaces was held (Blakeman, 1981). As such during the last few years considerable progress has been made with many investigations since the existence of a definite phylloplane microflora was first described by Last (1955), Ruinen (1956) and Kerling (1958).

Leaf Surface Microflora

By critical examination of the literature it is found that much information is available on the leaf surface of plants and the organisms which live there especially the phylloplane fungi.

The leaf surface forms a unique habitat since it supports a definite microbial community by providing a complex of niches to various organisms viz., bacteria, actinomycetes, yeasts and fungi.

Burri (1903) and Dugeli (1904a,b) reported distinct bacterial flora on plants which differed from air and soil. Potter (1910) recorded bacteria and fungi from leaf impression cultures and Derx (1930) reported the association of Sporobolomyces and Telletiopsis with wheat leaves. Keener (1951) observed that the leaf soon after it unfolds from the bud is relatively a clear sheet which later becomes the seat of various microbial activities.

Last (1955a) studied seasonal incidence of Sporobolomyces on leaves of three crops viz., winter and spring sown wheat and spring sown barley.

Ruinen (1961) while studying the microbiology of phyllosphere of the humid tropic vegetation, observed divergent microflora including algae, fungi and bacteria, and recorded maximum population on senescent foliage. She noticed oligonitrophilic and nitrogen fixing bacteria as first colonizers of the leaf surface. Kerling (1964) studied leaf surface fungi on rye and strawberry and observed that the population of Botrytis cinerea increased rapidly as the leaves of strawberry approached to senescence.

According to Hudson (1962) the micro-organisms which are present on the leaf surface in active state are known as colonizers and he classified the latter into two types: (i) common primary saprophytes which are also present in the air so refered as field fungi, viz., Aspergillus sp., Penicillium spp. (i) Restricted primary saprophytes which are specific to the host plant.

Last and Deighton (1965) pointed out that bacteria and yeast like fungi were more abundant on the leaves than the hyphomycetes. Members of Sporobolomycetaceae dominated the surface of diseased leaves infected by fungi, nematodes and mites.

Dickinson (1965) distinguished three groups of leaf surface fungi, viz., the transient fungi present on the leaf surface including yeasts and other fungal propagules which are capable of sporulating on leaf surface but are not isolated from washed discs. The second group of fungi dominated by Cladosporium herbarum were recorded from both leaf surface washings and washed discs. The third consisted of the forms growing vegetatively on leaf surface but pycnidia were formed only on moribund leaves.

According to Leben (1965) among the epiphytic leaf micro-organisms 'casuals' and 'residents' may be distinguished, the former remaining inactive or developing only on organic debris fallen on to the leaf from elsewhere, the latter growing actively on the leaf surface and using nutrients excreted by the leaf. There may also exist intermediate type of micro-organisms.

Hogg and Hudson (1966) also recognised three distinct patterns of fungal distribution on the leaves of birch. They reported Cladosporium herbarum to be the primary colonizer. Pady et al. (1969) observed that many dead leaves of wheat due to rust infection (Puccinia recondida) favoured the growth of Cladosporium which sporulated heavily on dead leaves.

Holoman (1967) investigated leaf surface mycoflora of three potato varieties where Aureobasidium pullulans and

Cladosporium herbarum were the usual inhabitants. He placed the isolates obtained from washing technique in one of the four groups i.e., (i) Aureobasidium pullulans (ii) Cladosporium herbarum (iii) Alternaria tenuis, Botrytis cinerea and Fusarium spp., (iv) Miscellaneous.

Dickinson (1967) pointed out that forms like Cladosporium, Stemphylium, and Alternaria were of frequent occurrence on the leaves of Pisum sativum. Dickinson (1967) further worked on fungal colonization of Pisum leaves and found that not all the fungi recorded by moist chamber technique were phylloplane saprophytes as several species viz., Penicillium spp. and Aspergillus fumigatus constitute a group of casual inhabitants of the phylloplane and their presence may merely reflect the relative abundance of their spores in the atmosphere.

Later on, Lamb and Brown (1970) also confirmed that the microflora present on leaf surfaces may be divided into two groups (i) the residents which are the actively growing saprophytic forms (ii) the transient species which are the inactive forms and are deposited on the surfaces of the leaves as wind-borne propagules. The residents are the epiphytic organisms which grow and reproduce as saprophytes on the leaf surface whereas transient organisms are present merely by chance on the leaf surface and active growth and reproduction in such forms do not occur.

Mishra and Srivastava (1971b), however, differentiated the phyllosphere and phylloplane and retained both the terms to denote two different regions of leaf surface as rhizosphere and rhizoplane regions of root.

Although in recent years workers use either of the terms and they consider them more or less synonym (Dickinson, 1976).

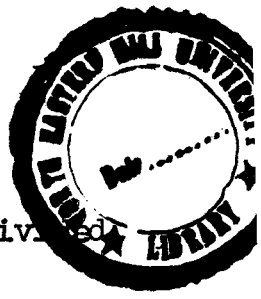
The colonization of leaf surfaces by fungi presents an interesting study with regard to substrate relationships (Pugh and Buckley, 1971). The fungal colonizers on leaf surface come either from the air or from soil. The occurrence of many fungi on aerial surfaces may be directly related to inoculations from the atmosphere, which in turn are related to the production of the deciduous propagules elsewhere (Dickinson, 1976). Sometimes seed may also act as a source for the epiphytic microflora.

Factors affecting the microbial colonization on leaf surface

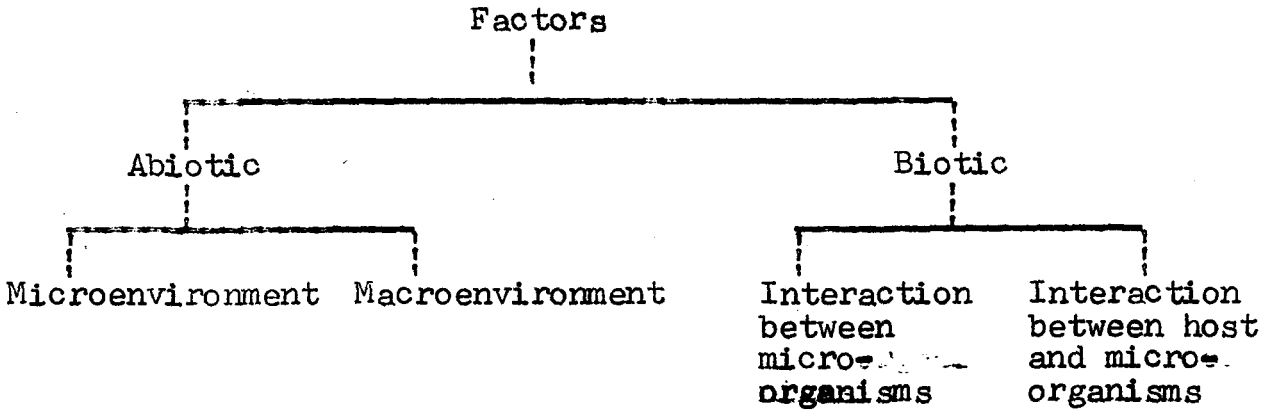
The factors may broadly be classified as under:

1) Abiotic factors,

2) Biotic factors.



The above two major groups may further be sub-divided into the following:



Abiotic factors

1) Microenvironment or the leaf surface:

The microfloral colonization on leaf surface is directly dependent on the host species. These factors determine the periodicity and affect the trapping of spores on leaf surfaces (Gregory, 1950, 1961; Hirst and Stedman, 1963).

The microbial pattern of the phyllosphere is characteristic of a particular plant species (Pillai and Sen, 1966; Garg et al., 1978). From the work of Kerling (1964) and Singh and Sinha (1962) host specificity is very important and it is controlled by the physical and chemical nature of the leaf.

Factors affecting microbial activity:

1) Physical factors,
2) Chemical factors.

1) Physical factors

i) Morphological nature of the leaf
ii) Plant maturity or Plant age

iii) Plant height

iv) Leaf surface temperature and moisture

v) pH of the leaf

vi) Area of the leaf.

2. Chemical factors

i) Silica, waxes

ii) Leaf leachates.

Physical factors

1) Morphological nature of the leaf:

The leaf surface traps different spores present in the air through various devices, viz., hairs, sticky surfaces etc. The host species themselves play a very important role in microbial colonization by providing different ecological niches to the micro-organisms (Dickinson, 1981). The ecological habitat of the plant surface is of different nature. Cuticle, epidermis etc. of different plants vary. Cuticle may be thick or thin, may be associated with different types of hair, openings on the leaf surface may also vary depending on the plant species. The leaves of different species of plants support a distinctive microbial population (Lamb and Brown, 1970). Host specificity is known to be controlled both by the physical nature of the leaf surfaces and also the chemical nature of exudates.

2) Maturity of the leaf:

This factor is found to be another major parameter influencing the composition of micro-organisms (Sinha, 1971). Invariably the numbers of micro-organisms increase with advancing age of leaf (Marshall and Walkley, 1951; Ruinen, 1956; Pugh, 1958; Kerling, 1958, 1964; Hudson, 1962; Sinha, 1971; Bakshi, 1981; Narula and Mehrotra, 1981; Kumar and Singh, 1981).

The gradual increase in number of fungi may reflect the continuous deposition of propagules of the air spora during prolonged exposure or it may also be due to multiplication of micro-organisms on the phylloplane of old leaves (Marshall and Walkely, 1951; Last, 1955; Ruinen, 1956; Kerling, 1958; Tukey, 1958, 1965; Stout, 1960; Orellana and Thomas, 1962; Chand, 1963; Mishra and Tewari, 1976a, b). As larger amounts of nutrients are leached from ageing leaves than the young ones (Kerling, 1964) the exudates of the former favour increased colonization of saprophytic micro-organisms (Last, 1970; Pati and Chandra, 1980; Sharma et al., 1980).

3) Plant height:

Plant height is an important factor for microbial colonization on phylloplane (Mishra and Tewari, 1969, 1976a; Dwivedi and Kumar, 1981). The mycopopulation usually increases from top to the bottom of the plants, i.e. leaves at lower nodes near the soil have larger microbial population as compared to

those at higher nodes of the plants (Mishra and Tewari, 1969; Lamb and Brown, 1970; Gupta and Mukerji, 1982). The maximum population at lower height both in quality and quantity may be accounted primarily due to its closeness to soil since soil contributes a number of fungi to the leaves through air current or rain splashes. The soil further helps in maintaining more favourable micro-environment by regulating the moisture and temperature regimes at lower region (Mishra and Tewari, 1976a). Plants develop a short canopy at the different levels and lower regions near the ground are more favourable for the growth of the various microbes on the leaf surface. Thus a possible inter-relationship may, therefore, be expected between soil and phyllosphere-mycoflora particularly at lower height of the plants and this explains the high fungal counts from leaf surface at lower heights (Lamb and Brown, 1970 and Mishra and Tewari, 1976a).

4) Leaf surface temperature and moisture:

Sutton (1953) and Burrage (1971) observed that each leaf is surrounded by so called a boundary layer in which the leaf temperature, moisture and wind speed is influenced by the leaf. According to Mishra and Tewari (1976b) the microenvironment of the boundary layer may not be suitable for all the microorganisms trapped on the leaf surface and most of the forms casually present on the surface do not thrive well. Moisture content and temperature differ on different leaves and even on

the same leaf variation is possible which affects the microbial colonization on the leaf.

5) pH of the leaf surface:

Besides temperature and moisture, pH of leaf surface also varies (Barlocher et al., 1978). Different plants may have different pH and only those leaf surfaces with favourable pH for germination and growth of a particular fungus or a group of related species will encourage the colonization (Blakeman, 1971; Kumar and Gupta, 1976; Barlocher et al., 1978).

6) Area of the leaf:

According to Pugh and Buckley (1971) when leaves are placed in moist chambers fungi which appear first are seen near the veins. It has been seen that more fungi are isolated at the leaf tips because dew drops get collected at the tips which provide nutrition to the fungi occurring there. Thus the microflora may vary on different regions of the same leaf. There may be more microorganisms near the veins or the petioles. This may be because even on the same leaf, temperature, moisture content, leaf exudation may vary at different sites (Mishra and Tewari, 1976b; Andrews et al., 1980).

Chemical factors

1) Silica, waxes:

The presence and nature of the waxy coatings on leaves

are important factors in microbial colonization (Holloway, 1970, 1971; Hallam and Juniper, 1971; Forester, 1977; Merrall, 1981). The leaf surfaces of higher plants are covered by non-cellular cuticles which are heterogenous in chemical composition. The major chemical components are lipids, waxes and cutin. The wax and silica deposition is different on different leaves of the plant species and they greatly influence the microbial colonization and penetration (Holloway, 1971).

2) Leaf leachates

Mann and Wallace (1925) for the first time used the term 'leaching' to describe the removal of mineral nutrients from leaves soaked in water.

Colodny (1932) pointed out that wheat and barley were more susceptible to leaching than rye.

Inorganic nutrients like K, Ca, Mg, and Mn usually are leached in greater amount (Arens, 1934). He also reported greater loss of nutrients in the light than in the dark. Tukey et al. (1957) reported that loss of carbohydrates from leaves was directly controlled by light intensity. Phillis and Mason (1942) and Sharpe (1955) reported an abundance of K and traces of Ca in dew on the surface of cotton leaves. Thus dew plays an important role as a leaching agent.

Fujiwara and Iida (1956) recorded a decrease in the K content of rice leaves after a heavy rain.

Schoch (1955) reported that differences in quantity and quality of the leached substances were not only among species but also among individual leaves of the same crop and even for the same plant depending upon the physiological age of the leaf. Young leaves are hydrophobic and are wetted with difficulty (Linskens, 1952; Holloway, 1971) and this in turn retards the leaching. Thus young leaves are less susceptible to leaching whereas leaves approaching senescence are more (Sinha, 1971).

Leaves injured by biological, physiological or by mechanical sources are more susceptible to leaching than the healthy ones (Helder, 1956; Tukey Jr. and Morgan, 1963; Tukey Jr., 1971).

Ruinen (1961) pointed out that leaf surface has its own environment and nutrient available to the microbes on leaves are mostly through the secretion/excretion of leaves. The composition of the nutrients varies in case of different plants.

Temperature also effects the loss of metabolites by leaching. Mitchell (1968) reported leaching of some carbohydrates at higher temperature.

According to Tukey Jr. (1971) leaching is removal of substances from the plants due to the action of aqueous solution, rain, dew, mist and fog.

Leaf leachates or exudates greatly influence the quality and quantity of microorganisms occurring on the leaf surfaces (Godfrey, 1976).

Substances leached from leaves include a great variety of materials including both inorganic and organic substances (Morgan and Tukey, 1964).

Inorganic nutrients leached include all the essential minerals and some other elements found in plants including both the macro- and micro-elements. Organic substances which include free sugars, pectic substances, alcohols, amino acids, organic acids are detected from leaf leachates (Tukey, 1971). Kozel and Tukey (1968) have shown that growth regulating chemicals such as the gibberellins as well as vitamins (Wasicky, 1958), alkaloids (Bode, 1958) and phenolic substances (Del Moral and Muller, 1964; Kozel and Tukey, 1968) are leached from plants.

No authentic information is available regarding the quantity of amino acids and growth regulators leached from the leaf surface (Tewari, 1973).

Thus the phyllosphere population seems to be directly governed by leaf exudation (Mishra and Tewari, 1976).

Macroenvironment

1) Meterological factors:

Many workers (Gregory, 1950, 1961; Last, 1955a; Hirst and Stedman, 1963; Mishra, 1968; Heuvel, 1970; Sinha, 1971; Ahuja and Payak, 1981; Kumar and Singh, 1981; Pennycook and Newhook, 1981) have found that meterological factors such as atmospheric temperature, humdity, wind, light and rain are important factors influencing the micro-organisms on the leaf surface. The total number of micro-organisms and also the specific composition of the microflora were affected by the weather conditions some being present throughout the growing period whereas others were exclusively associated with a particular set of climatic factors (Mishra and Srivastava, 1970a; Sinha, 1971; Sharma and Mukerji, 1976; Garg et al., 1978; Khara and Singh, 1981; Hayes, 1982).

2) Leaf surface microorganisms in relation to air spora:

There is a close correlation between the air spora of a locality and the leaf surface fungi of plants growing in the area (Mishra and Tewari, 1976a).

The investigation in the aerobiology of India dates back to the researches of Cunningham (1873) who reported changes in atmospheric spore content of Calcutta prison.

Grainger (1954) recorded higher concentration of Helminthosporium avenae in air at lower level of infected oat crop.

Last (1955b) studied the air spora within and above the mildew infected cereal crops and recorded higher population near the ground. Gregory (1950, 1957, 1961) also observed a direct correlation between air spora and the microflora of leaf surface. Rishbeth (1959) estimated the spore trapping capacity of conifers. The number decreases only by death and washing off in rains but is continuously renewed by arrival of fresh spores. Pady et al. (1967) suggested that many of the air borne fungal hyphae are conidiospores which were probably released by the wind current from dead leaves of crops. According to Pady (1971) the leaf is thus an admirable site for both saprophytic and parasitic fungi and under favourable conditions spores are produced in tremendous number and are released into the air.

Spores of various foliar obligate pathogens such as rusts, smuts were found to be usually present in maximum number in the air and they exhibit a characteristic release-pattern with varying peak hours (Gregory, 1961). Gregory (1971) suggested three main ways for arrivals of spores: 1) dry wind borne route, 2) in rain drop and 3) in rain splash droplets.

An understanding of the nature, periodicity and density of the fungal propagules in the air is much helpful in making a forecast regarding the occurrence of fungal diseases and the quantum of viable pathogenic propagules likely to cause infection (Mishra and Tewari, 1976a).

The leaf surface releases fungal spores which largely contribute to the air spora of the locality. Mishra and Srivastava (1971a) reported that a sort of cyclic phenomenon exists between fungal spores of air, soil and plant surface and some of the forms, maintain their specificity in the specialized environment. Recently, many workers (Lamb and Brown, 1970; Mishra and Srivastava, 1970a,b, 1971a, 1972; Sinha, 1971; Burrage, 1976; Mishra and Tewari, 1976a; Bovalluis et al., 1978; Kumar and Gupta, 1980; Dixit and Gupta, 1980) have contributed to the comparative studies between phylloplane and air spora of different fields. They observed that number of spores deposited on the leaf surface is nearly proportional to the number of spores in the air. There are, however, few species which are either specific to air or to phylloplane (Lamb and Brown, 1970; Sinha, 1971; Mishra and Tewari, 1976a).

3) Influence of pollen on leaf surface microflora

The development of saprophytic fungi on rye leaves shows a sudden increase shortly after flowering due to the presence of pollen that has fallen on leaves (Kerling, 1964). Fokkema (1968, 1971) demonstrated that addition of pollen to inoculum of Cladosporium herbarum enhanced the colonization of the fungus on leaves. Warren (1972) also observed that main colonizers reached higher numbers on leaves with pollen deposit. Warren (1976) observed an increase in number of yeasts, Aureobasidium pullulans and Cladosporium spp. in presence of pollen on leaves of lime and

barch. Recently, Garg and Sharma (1982) have observed that the presence of pollen on surfaces of flag leaves of Triticale had marked influence on populations of phylloplane microfungi. Total fungal population was exceedingly higher on leaves with pollen, the effect being much pronounced on mature leaves. It has been demonstrated by different workers (Ogawa and English, 1960; Bachelder and Orton, 1963; Chu-chou and Preece, 1968; Chou-chou, 1970; Fokkema, 1971; Warren, 1972, 1976; Garg and Sharma, 1982) that presence of pollen affected infection of leaves by parasites. Thus any factor which might alter the balance between sporophytes and pathogens on leaf surface should be of significance in disease development. Recently, Olivier (1978, 1983) has also worked on some phyllosphere fungi which capture wind borne pollen grains.

4) Effect of atmospheric pollution on leaf surface mycoflora

Pollution can be defined as the significant alteration of an environment by the introduction of an alien body or compound. The alien factor is almost assumed to be a toxic chemical compound (Saunders, 1971). Most industrial and domestic effluents although they are usually apparently harmless may cause damage to plants and leaf surface mycoflora at high concentrations and under specific conditions. The effects of pollutants upon the leaf surface and its environment are similar in some respects to the effects of fungicides and pesticides. Information on the effects of pollution upon

microorganisms especially upon the microflora of the leaf surface is very scanty (Vas and Ingram, 1949; Couey and Uota, 1961; Saunders, 1971; Smith, 1976, 1977).

The atmospheric pollutants consist of soot, smoke, dust, carbon monoxide, sulphur dioxide, sulphuric acid droplets, flourine gas, H_2S gas, flourides, chlorine, chlorides, iodides, bromine, herbicides, fungicides, fertilizers and various photochemical compounds in the smoke viz., smog, ozone, peroxyacetyl nitrate, oxides of nitrogen, al hydes, petroleum gases etc. A few instances of inhibition or stimulation of elements of the microflora of leaf surface by pollutants are known (Saunders, 1971), most of them in relation to sulphur dioxide. The selective sterilisation of the leaf surface by SO_2 is similar to that caused by some fungicides and insecticides because it alters the composition of the microflora by eliminating the sensitive members of the community and by providing additional living space for the resistant members.

5) Effect of foliar spraying of fungicides, antibiotics, nutrients and other chemicals on leaf surface mycoflora:

Hislop and Cox (1969) reported 90% decrease in fungal population after spraying of Captan. Stott (1971) used Captan and fungex and observed that two days after first spraying, fungal population was decreased to about 60% but the differences diasppeared two weeks after the first spraying.

Tewari (1973) also studied the effect of foliar spraying of two fungicides viz., captan and ziram and two antibiotics viz., Streptomycin and Penicillin on the phylloplane fungal population of two plants and observed that the fungicides and antibiotics spraying reduced the quantity of the fungal flora significantly. The effect, however, was not long lasting and gradually diminished with increased interval of sprayings. Recently, there are many reports regarding the effect of fungicides and pesticides application on phylloplane mycoflora (Bainbridge and Dickinson, 1972; Dickinson, 1973; Hislop, 1976; Mishra and Tewari, 1979; Andrews, 1981; Mehan and Chohan, 1981; Fitzell, 1981). Most fungicides are to some extent nonspecific in relation to the variety of organisms affected and they may alter the microbial balance with usually unknown consequences. The disease may be cured or prevented but in eliminating some harmless saprophytes, other micro-organisms with which they were in competition may become dominant and pathogenic (Hislop, 1976).

Foliar application of sugars reduced their susceptibility to downy mildew in sugar beet (Russell, 1968). Cobalt chloride sprayed on leaf surface to certain extent stimulated the mycoflora, higher concentration, however, proved detrimental to fungi (Mishra and Kanaujia, 1971), whereas, magnesium chloride foliar spraying always favoured fungal colonization on phylloplane in both healthy and diseased plants of tomato

(Mishra and Kanaujia, 1974). When bean leaflets were pretreated with decenylsuccinic acid, numbers of lesions caused by Botrytis fabae increased (Sol, 1968; 1969). It has been further observed by Sol (1966, 1967, 1968, 1969) that foliar application of certain substances effect the germination and infection of leaf pathogens.

Biotic factors

1) Interaction between leaf surface microorganisms:

Antagonism

Antagonism commonly means a relation between organisms in which one organism, the antagonist creates adverse condition for the other.

Several papers have appeared mentioning effects of non-pathogenic microorganisms on infection of aerial parts of plants by fungal or bacterial pathogens. Several reviews have come out in recent years (Leben, 1965; Sinha, 1965; Heuvel, 1970, 1971; Crosse, 1971; McBride, 1971; Fokkema, 1976; Skidmore, 1976; Blakeman and Brodie, 1976; Mishra and Tewari, 1976c, Reinecke, 1981; Spurr, 1981a, b; Dubos and Bulit, 1981; Kranz, 1981) on the interactions between microorganisms and a posibility of biological control of some plant pathogens.

The microorganisms interact and the interaction leads to the suppression or stimulation of one or the other, bringing

in antagonistic or associative effects (Sinha, 1965). This complex problem of the phylloplane has a profound influence on the course of events in the infection of hosts and is intimately related to the formulation of methods of disease control.

One of the earliest references to interaction between microorganisms in phyllosphere is by Last (1955a) who reported occurrence of Sporobolomyces on cereal leaves where the colonies were greatly decreased by Tilletiopsis sp.

Newhook (1957) used Cladosporium herbarum and Penicillium sp. antagonistic to Botrytis cinerea to the tomato petals. Simard et al. (1958) showed antagonism between some microorganisms isolated from the surface of dead apple leaves and the apple scab fungus, Venturia inequalis. Morton and Peterson (1960) reported that early infection of Helminthosporium sativum inhibited the development of Septoria passerinii in leaf sheath. Wibe and Morton (1962) demonstrated that cell free extract of certain fungi particularly that of H. sativum was inhibitory to S. passerinii. Voznyakovskaya and Shirokov (1961) isolated many epiphytic microorganism on strawberry out of which 13 isolates were antagonistic to Botrytis cinerea. Bier and Rowat (1962a, b) found that two common saprophytes were inhibitory to Hypoxylon prinatum, causal organism of canker disease. Bhatt and Vaughan (1963) treated strawberry plants at the late bloom stage with spore

suspension of Cladosporium herbarum, Aspergillus sp. and Penicillium sp. isolated from their fruits and recorded some control of B. cinerea. Leben (1964) observed that some bacterial isolates decreased the occurrence of cucumber anthracnose.

Das and Pal (1968) reported that Rhizopus nigricans decreased the lesions caused by Alternaria solani on potato leaves and inhibited spore germination and mycelial growth of the pathogen in vitro. Akai and Kuramato (1968) observed Candida sp. to be responsible for decreasing leaf spot caused by Cochiobolus miyabeanus on rice leaves but failed to inhibit the spore germination or mycelial growth of pathogen in vitro. Kapooria and Sinha (1969) reported parallel results both in vivo and vitro when there was reduction of Puccinia penniseti on leaves of pearl millet by several phyllospheric fungi viz., Chaetomium globosum, Aspergillus japonicus and Fusarium oxysporum. Heuvel (1969, 1971) showed that some isolates of Aureobasidium pullulans disfavoured the development of leaf lesions formed by Alternaria zinnae.

There are many reports of biological control of leaf pathogens in vitro but only very few reports are available showing their success in vivo (Heuvel, 1969; Mitchell, 1973; Mishra and Tewari, 1976c; Rao et al., 1978; Purkayastha and Bhattacharyya, 1982; Sharma and Gupta, 1980; Rai and Singh, 1980; Sharma et al., 1981; Chet et al., 1979, 1981; Adams and

Ayers, 1982; Howell, 1982; Gupta and Dixit, 1982; Trutman et al., 1982).

Biological control is an area currently of widespread interest, and the field is represented by many recent papers on hyperparasites, filamentous fungi and bacteria as biocontrol agents (Endo et al., 1973; Roy, 1977; Sharma and Gupta, 1978, 1980; Dumitras and Sesan, 1979; Pineau, 1979; Fokkema et al., 1979; Peresse and LePicard, 1980; Lundborg and Unestam, 1980; Staub and Kuc, 1980; Tsuneda and Skoropad, 1980; Upadhyay and Rai, 1980; Sivasithamparam and Parker, 1980; Chet and Baker, 1981; Dickinson et al., 1981; Srivastava et al., 1981; Schulz, 1981; Sharma and Heather, 1981; Papavizas et al., 1982; Willoughby, 1983; Bhattacharyya and Purkayastha, 1982; Trutman et al., 1982; Gupta and Dixit, 1982).

Interaction between the host and the microorganisms

1) Inhibitory substances released from leaf surface:

Bernard (1909) for the first time put forth the idea of the production of inhibitory substances by host due to the infection of saprophytes which inhibited the parasitic invasion. Muller and Borger (1940) used the term phytoalexin and defined it as an "antibiotic which is produced as a result of the interaction of two metabolic systems, the host and the parasite and which inhibits the growth of microorganisms pathogenic to the host." He observed that the interaction between potato and

an avirulent strain of Phytophthora promoted the activity of a factor which inhibited the growth of a virulent strain of the pathogen. Several other workers (Uritani and Akazawa, 1955; Cruickshank and Perrin, 1961, 1963, 1965) have also reported the occurrence of such phenomenon. Topps and Wain (1957) noted that the concentrated leaf washings from a range of woodland trees caused limitation of germ tube growth in Botrytis cinerea. Phenols are most commonly responsible for inhibition (Kirkham, 1954; Topps and Wain, 1957; Shepherd and Mandryk, 1963; Brillova, 1971; Van Lelyveld, 1974; Edreva, 1976; Carrasco et al., 1978; Van Lelyveld et al., 1981; Alfenas et al., 1982). Recently many workers (Mahadevan, 1967; Bailey, 1971; Mace and Beech, 1973; Dix, 1974; Irvine et al., 1978; Iwata et al., 1980) have reported that the resistance to diseases is obtained by production of inhibitory substances by the leaves.

Sinha (1965) also reported that host tissues are known to exude phytonocides which are inhibitory to the invading fungi and bacteria and some of the leaf surface microorganisms may cause the production of phytoalexins in the host and bring about changes in the reaction of the host to parasites.

Afifi (1975); Mishra and Tewari (1978); Singh and Rai (1981); Sinha and Prasad (1981); Vishunavat and Shukla (1981) and Rathore et al. (1982) have studied the effect of leaf exudates and extracts of different plants on growth behaviour of some microfungi occurring on phylloplane and they observed that some pathogens are inhibited in the leaf exudates and leaf

extracts because of inhibitory substances present in them.

2) Biologically active substances produced by the microorganisms in the presence of the host:

Epiphytic microorganisms being in close contact with the plant during the whole vegetative period, in the process of their vital activity secrete a number of metabolic products. Some of the substances like group B vitamins, indole auxins, gibberellin-like substances are biologically active and they penetrate into plant tissues and although present in negligible concentrations may have a considerable effect upon the metabolism of the plant which in turn will influence the leaf surface mycoflora (Klincare et al., 1971).

3) Biological state of the plant:

Many reports are there in the literature where workers have studied the microflora of both healthy and infected leaves of different plants (Last, 1970; Sadasivan and Prasad, 1973; Sharma and Tewari, 1981). The total microbial population was found higher on healthy leaves than the diseased ones (Sharma and Tewari, 1981) although maximum filamentous fungi are found on diseased leaves (Last, 1970; Sadasivan and Prasad, 1973; Sharma and Garg, 1979; Garg and Sharma, 1980; Pal, 1981, 1982). It may be noted that more nutrients are leached through the breakage of the leaf tissue during infection which is the reason for the increase in fungal population both qualitatively

and quantitatively on infected leaves (Last, 1970).

4) Nitrogen fixers in phylloplane:

There are many reports of microorganisms especially bacteria which are present on the phylloplane and they help in nitrogen-fixation (Kvasnikov et al., 1974; Blasco and Jordan, 1976; Remacle, 1977; Capone and Taylor, 1977; Banerjee and Chandra, 1978; Sadykov and Umarov, 1980a, b; Sengupta et al., 1981; Sadykov, 1981; Nandi and Sen, 1981, 1982; Nandi et al., 1982a, b).

Thus the study of phylloplane is a complex problem where many factors are involved and great phytopathological possibilities are bound to unfold as work proceeds (Sinha, 1965). There is ample scope for biological control of pathogens by studying various interactions between plant pathogens and epiphytic microorganisms on the aerial parts of plants (Dickinson and Preece, 1976; Blakeman, 1981).

Thus the present review provides evidences for the fact that leaf surface of plants is a nutrient rich habitat. This habitat provides a complex of niches which harbours a variety of microorganisms. Further, the community structure is affected by several physical, chemical and biological factors viz., the environment, the substrate and the interactions between microorganisms respectively.

The present study was carried out at Shillong, the capital of Meghalaya, which is located at 24°34' N latitude and 91°56'E longitude (Fig. 1). For the general field study, a plot in the Botanical garden in the University campus which is located at an altitude of 1540 m was selected. Physiographically the area is hilly covered with pine forest (Pinus kesiya).

The soil is red laterite under red loam or brown loam soil type. The sand content of the soil is upto 90% at some places and it is usually acidic in reaction. The soil is rich in nitrogen content. However, the amount of phosphorus is relatively low ranging between 20 kg/acre to 50 kg/acre. The soils are poor in available muriate of potash (Zimba, 1978).

The climate of Shillong is very much controlled by the seasonal winds as in the other parts of the country. The seasonal winds are the South-west monsoons and the North-east winter wind.

Hence the year may be divided into four seasons:-

1. Spring season - March and April.
2. Summer (rainy) season - May to September.
3. Autumn season - October and November.
4. Winter season - December to February.

Fig. 1: Map of Meghalaya showing the location of Shillong.

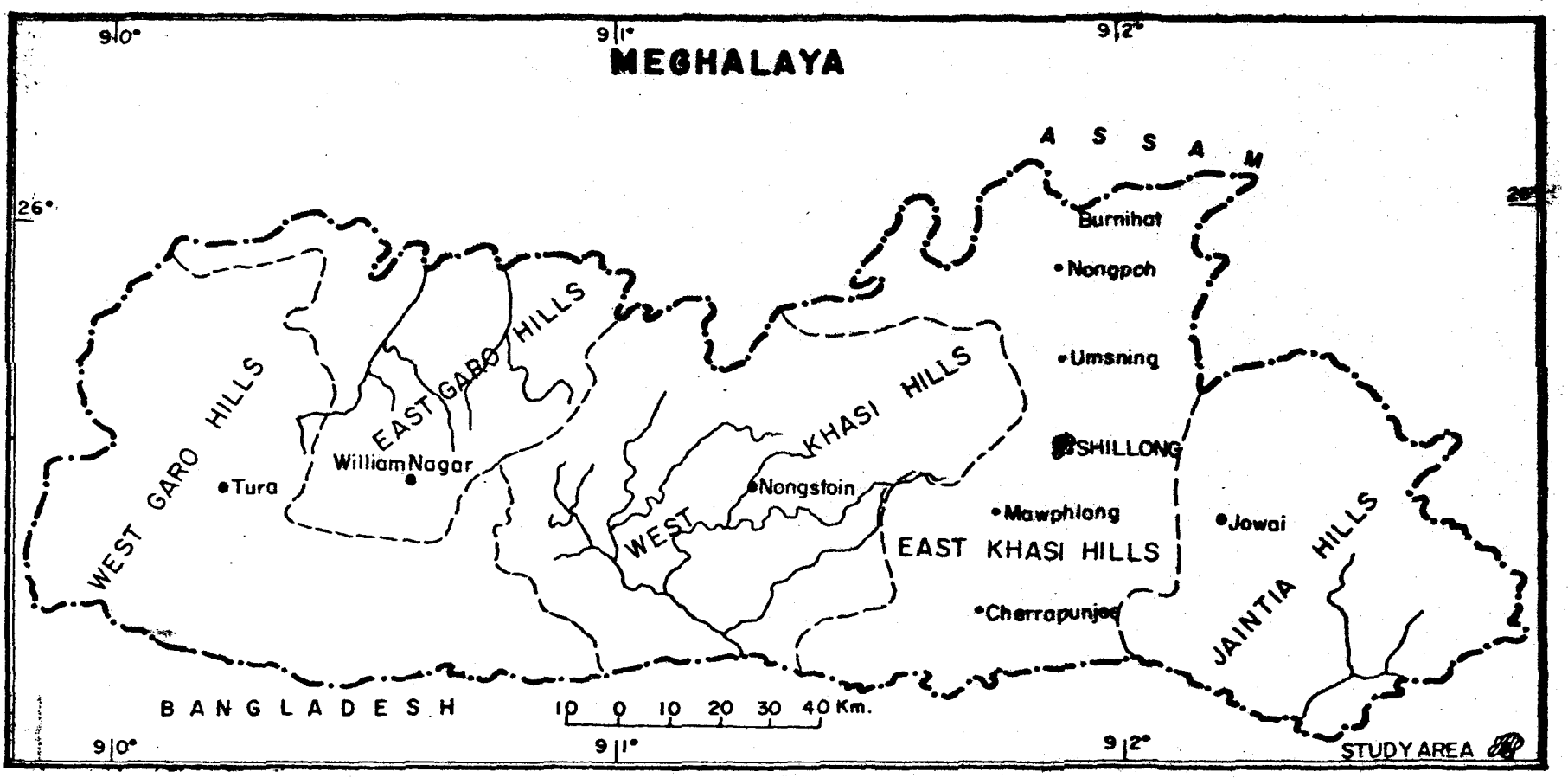

MEGHALAYA
9|0°
9|1°
9|2°
26°
ASSAM
Burnihat
Nongpoh
Umsning
SHILLONG
Mawphlang
Jowai
Cherrapunjee
Nongstoin
Tura
William Nagar
WEST GARO HILLS
EAST GARO HILLS
KHASI HILLS
WEST
EAST KHASI HILLS
JAINTIA HILLS
BANGLADESH
10 0 10 20 30 40 Km.
STUDY AREA

Fig. 1

Fig. 1.1: Meteorological data for minimum, maximum temperature (in °C), relative humidity (in percentage) and rainfall (in mm) of two years (1981 and 1982) of Shillong.

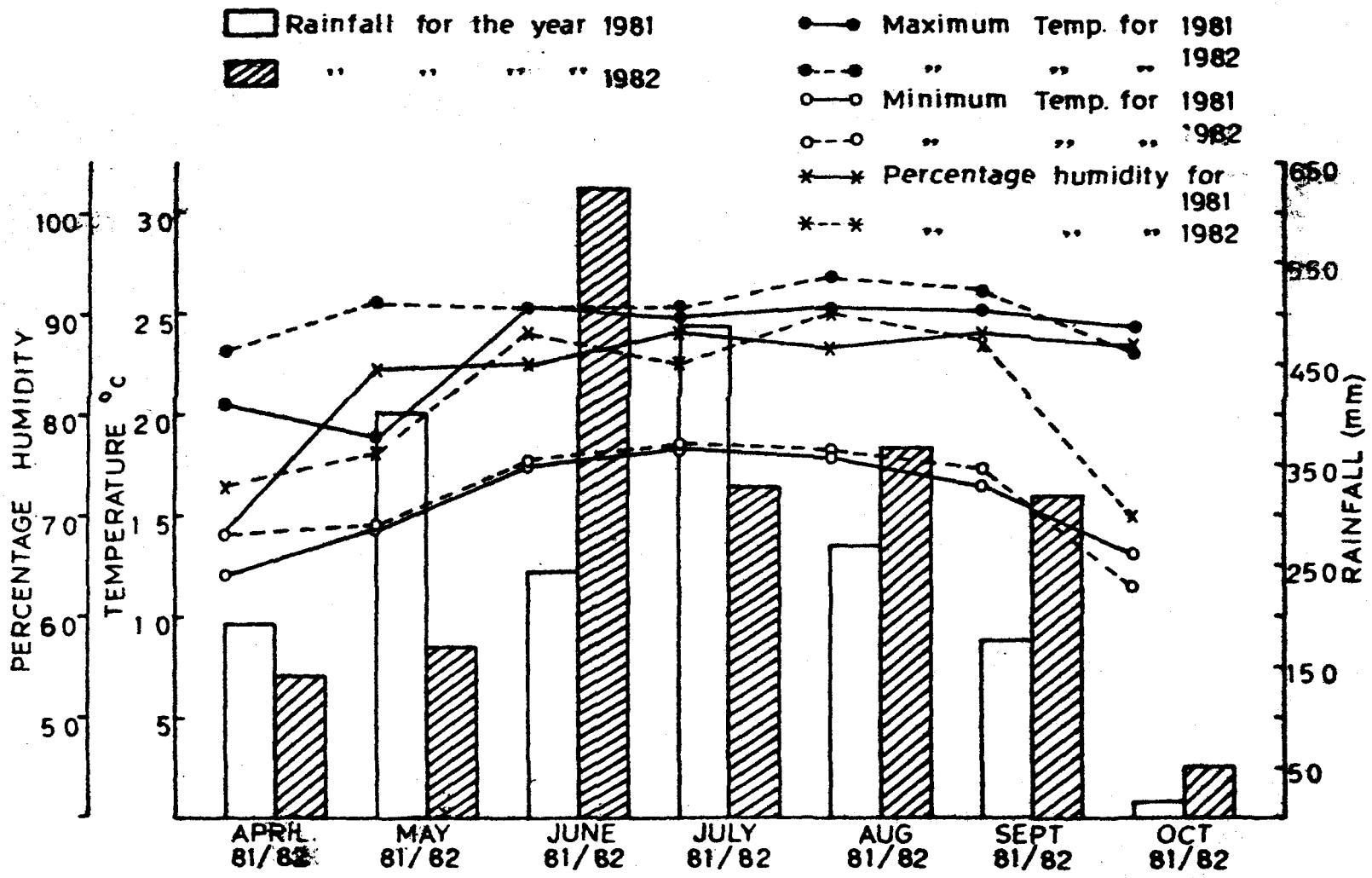

Rainfall for the year 1981
" " " " 1982
Maximum Temp. for 1981
" " " 1982
Minimum Temp. for 1981
" " " 1982
Percentage humidity for 1981
" " " 1982
PERCENTAGE HUMIDITY
TEMPERATURE °C
RAINFALL (mm)
100
90
80
70
60
50
30
25
20
15
10
5
650
550
450
350
250
150
50
APRIL 81/82
MAY 81/82
JUNE 81/82
JULY 81/82
AUG 81/82
SEPT 81/82
OCT 81/82
MONTHS

Fig. 1.1

During March and April, the atmosphere gradually warms up. From the middle of April to the middle of May, the temperature reaches the maximum. The maximum temperature recorded at Shillong during the period was 26°C. The average minimum temperature was 12.77°C. The rain usually starts by the third week of April and continues upto the end of September and sometimes even upto the middle of October after which it gradually stops. The average rainfall is as high as 271.57 mm. Similarly, the average humidity is also very high ranging between 71.38 to 84.21 in a diurnal cycle. October and November are two months when the climate is cool and temperate. As the month of November approaches the temperature slowly comes down and the climate gradually changes from cool to a cold one. After November, winter season sets in, which continues upto the end of February. During these months, the climate is very cold and the temperature falls down to 4-5°C. The low temperature of the winter results into frost which may be seen sometime early in the morning during December and January.

The data of the temperature, both minimum and maximum, percentage humidity and rainfall during the two crops seasons (1981 and 1982) in the present investigation has been presented in Fig. 1.1.

CHAPTER I

ASSESSMENT OF THE FUNGAL FLORA FROM THE PHYLLOPLANE REGION

INTRODUCTION

Leaf surface which harbours a definite microbial community of various pathogens and saprophytes has been termed as phylloplane analogous to rhizoplane of roots (Kerling, 1964). It was noted as early as in 1900's (Burri, 1903; Dugeli, 1904a,b; Potter, 1910) that aerial parts of plants have their characteristic flora which differs from that of air and soil.

Leben (1965) classified the phylloplane fungi as residents and casuals. The residents usually colonise and multiply on the leaf surface while the casuals reach the plant body accidentally and can only grow preferably on dead plant parts. Later on, Lamb and Brown (1970) used transients for casuals.

The studies on phylloplane mycoflora are important because of their role in biolgical control of certain epidemic diseases, active decomposition and enhancement of activity of phylloplane nitrogen fixers (Singh and Sinha, 1962).

Considerable interest lies in investigating the types of microorganisms which occur on leaf surfaces during the changing meteorological environments at various periods of plant growth (Sinha, 1971). The various factors such as host species, leaf maturity and weather changes may play important

role in determining the distribution of fungi on leaf surface both quantitatively and qualitatively (Rao and Manoharachary, 1981).

Recently, much work has been done on the ecology of phylloplane fungi of different plants especially in relation to nature of the leaf, age of the plant, changing environmental factors and also the leaf leachates (Dickinson, 1971, Narula and Mehrotra, 1981). However, only few workers (Sharma and Sinha, 1972; Balagopal and Oblisami, 1973; Kumar and Gupta, 1974; Pace and Campbell, 1974; Kumar and Balasubramanian, 1981; Tyagi and Chauhan, 1982) have studied the changes in the microflora of phylloplane of crop plants influenced by varietal characters and also by the plant pathogens.

The microflora present on the phylloplane is recognised as the fourth dimension (Last, 1971) and dictates the development of a disease in addition to the virulent and aggressive pathogen, susceptible host and favourable environmental conditions.

Relatively little work has been done to understand the ecology and role of naturally occurring microbes in the communities formed by the phylloplane fungi. Thus the work was taken up to find out the extent of host influence on building up of fungal communities out of the same source of inoculum under changing meterological environments. The present study was

taken up on leaves of three varieties of paddy differing in their resistance to brown spot disease.

MATERIALS AND METHODS

Plant material

Three varieties of paddy (*Oryza sativa* Linn.) viz., Khonorullo (Disease resistant), Ngoba (Moderately susceptible) and Mirikrak (Disease susceptible) were chosen as experimental plants. Seeds of these plants obtained from ICAR research complex, Shillong, were sown in the experimental plots of the Botany Department of the University on 26th April each of 1981 and 1982.

Paddy is the most important crop of the north eastern region of India and is grown in 8,45,000 hectares in various states of the region which represents 70.4 percent of the total cultivated area. It is the staple food of the population and is grown from valleys to hill tops. The paddy crop is a summer season crop and is grown from last week of April to last week of October in Meghalaya. Harvesting starts in the beginning of October.

The plants during the present investigation were sown on dry terraces with no water stagnation. Fertilizer in the form of NPK was applied at sowing time. The crops were grown under rainfed conditions as paddy is usually grown in this

PLATE 1.1

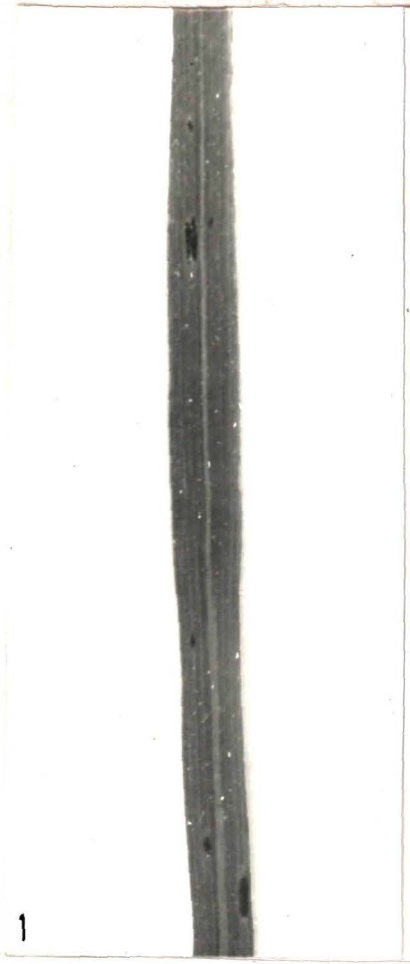
1. A plant of Khonorullo variety of paddy
2. A plant of Ngoba variety of paddy
3. A plant of Mirikrak variety of paddy.

PLATE- 1·1

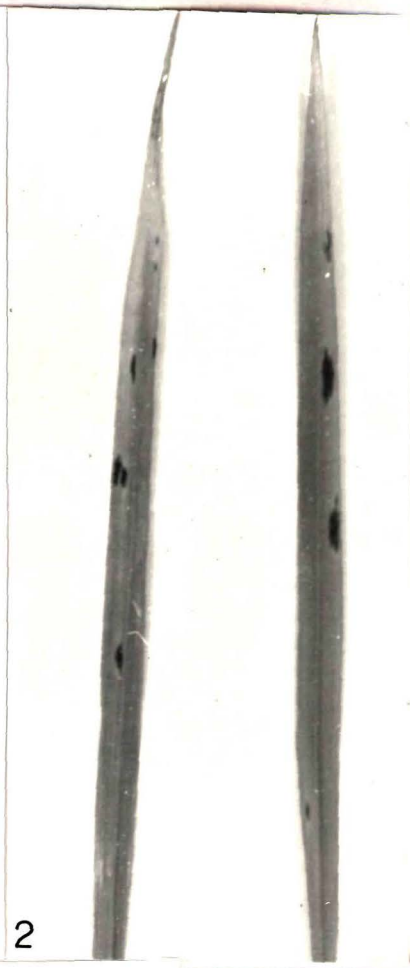
PLATE 1.2

1. A leaf of Ngoba variety of paddy showing leaf spot disease symptoms.
2. Leaves of Mirikrak variety of paddy showing distinct leaf spot disease symptoms.
3. Infected ear of Ngoba variety of paddy
4. Infected ear of Mirikrak variety of paddy
5. An enlarged infected ear of Mirikrak variety of paddy showing distinct brown spot disease symptoms on the grains.

PLATE - 1·2

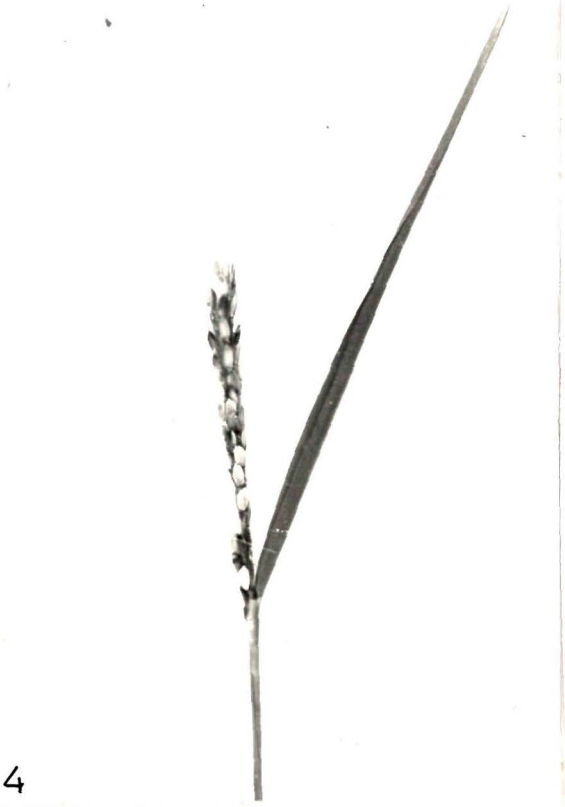


1



2

3



4

5

region during the rainy season.

The three varieties of paddy, viz., Khonorullo, Ngoba and Mirikrak which were used as the plant material throughout the course of the present investigation differed mainly in disease resistance towards the pathogen, Helminthosporium oryzae Breda de Haan. In addition, in Khonorullo variety the plants were tall with large leaves medium to bold grains, medium to long panicle length, medium to high number of leaves on main culm and with low tillering (Plate 1.1;1). Whereas, in Ngoba variety, the plants were dwarf with medium leaf length, medium grain size, medium number of leaves on main culm and high tillering (Plate 1.1;2) and in Mirikrak variety, on the other hand, the plants were of medium height with small to medium leaf length, small to medium grain size, medium panicle length, small to medium number of grains per panicle, medium leaf number on main culm and exhibited medium tillering (Plate 1.1;3).

Collection of samples

The collection of leaf samples was done regularly at fortnightly intervals from the time the leaf bud unfolded till the senescent stage as shown in Table 1.1. Leaves were collected at random in such a way that each collection had young as well as old leaves of each plant. Such leaves were collected in sterilized polythene bags with the help of sterilized scissors and forceps.

The leaf surface was examined for mycoflora by different techniques.

Techniques

The following techniques were employed for the study of mycoflora on the leaf surface on the three varieties of paddy at different growth stages of the plant.

1. Direct observation:

i) Cellotape impression technique (Edward and Hartman, 1952) was employed in which strips of cellotape was pressed gently against the leaf surfaces and the cellotape strips with the impression of the mycoflora were stained in cotton blue - lactophenol and the total number of spores and mycelia were recorded in five different fields of microscope and average values were calculated.

ii) Nail polish impression (Masurovsky and Jordan, 1960) technique was employed in which nail polish of a light colour was coated smoothly on the leaf surface and when this layer dried it was peeled off and mounted in lactophenol with 0.10% acid fuschin. Such peels containing the impressions of leaf surface mycoflora were observed under a microscope and the total number of spores and mycelial were recorded under five different fields of microscope and their average values were calculated.

2. Moist chamber (Keyworth, 1951):

Ten leaves were kept in moist chambers prepared by moisting 3-4 filter papers kept in petri plates with sterile distilled water. These were incubated at 25±1°C and observations taken at intervals of 5 days till one month.

3. Impression plate (Potter, 1910):

In this technique leaves were gently pressed against nutrient agar in petri plates with the help of a sterilized forceps. The leaf was subsequently removed leaving its impression on the nutrient agar (Czapek's Dox agar and cellulose agar medium) contained in a petri plate. The surface of the leaves of different varieties were pressed separately in different petri plates containing the nutrient agar. Five relicates were maintained for each variety. These petri plates were incubated at 25±1°C for 6 days and observations were taken by counting the number of colonies of different fungi occurring on the plates.

4. Dilution plate (Dickinson, 1971):

This method was employed for quantitative estimation of fungi (Dickinson, 1971; Sharma et al., 1974). The colonies were counted irrespective of their origin from a spore or mycelium. 1 gm of leaves were weighed and they were cut into small segments with the help of a sterilized scissor and the leaf segments were put in 250 ml conical flask containing 99 ml of

sterilized distilled water and then kept on a mechanical shaker for 30 mins. to get a uniform shaking. 1 ml of this washing was taken and diluted 10 times by adding sterilized distilled water. The plating of 10^{-3} dilution was found to be appropriate for colony counting. 1 ml of this aliquot was pipetted on nutrient agar (Czapek's Dox agar and Cellulose agar mediums). Five replicates were maintained for each variety. Plates were incubated at 25±1°C for 6 days and the fungal colonies were counted.

5. Washed leaves plating:

The leaf segments which were washed as given in the dilution plate technique were taken out and transferred into another 250 ml flask containing sterilized distilled water and again were given thorough 20 washings of sterilized distilled water. These leaf pieces were teased into smaller fragments with the help of sterilized forceps and needle and dried on sterilized blotting paper. Five such pieces were inoculated in each petri plate containing nutrient agar (Czapek's Dox agar and Cellulose agar mediums). Five replicates were maintained for each variety. The petri plates were incubated at 25±1°C for 6 days and the leaf segments were examined for fungal colonies occurring on them and the observations were taken till no new forms appeared.

Nutrient media used for isolation of fungi

Several media were employed during the present investigation such as potato dextrose agar, Czapek's Dox agar, cellulose agar to achieve isolation of maximum number of fungi.

1. Potato dextrose agar:

Potato peeled and sliced - 200 gm, D-Glucose - 20 gm, Agar - 15 gm, Tap water - 1 litre.

Peeled sliced potatoes were boiled for 1 hr in one litre of water. This was filtered through cheese cloth and Glucose was added to it and the medium was sterilized at 15 lb for 15 mins.

2. Czapek's Dox agar (Raper and Thom, 1949):

Agar - 15 gm, Sucrose - 30 gm, $NaNO_3$ - 3 gm, K_2HPO_4 - 1 gm, $MgSO_4 7H_2O$ - 0.5 gm, Kcl - 0.5 gm, $FeSO_4 7H_2O$ - 10 mg, Distilled water - 1 litre, Streptomycin - 30 mg.

3. Cellulose Agar (Eggins and Pugh, 1962):

Agar - 20 gm, Cellulose (powdered) - 10 gm, $(NH_4)_2SO_4$ - 0.5 gm, L-Asparagine - 0.5 gm, KH_2PO_4 - 1 gm, Kcl - 0.5 gm, $MgSO_4 7H_2O$ - 0.2 gm, $CaCl_2$ - 0.1 gm, Distilled water - 1 litre, Streptomycin - 30 mg.

Isolation of fungi

The plates were examined regularly and slow growing forms were transferred to fresh nutrient plates to avoid over

running of fast growing ones. Efforts were made to get pure cultures of various fungi occurring in moist chambers, impression plates, dilution plates and washed leaves plating techniques and these were raised on tube slants containing Czapek's Dox agar, cellulose agar and potato dextrose agar media. The fungi were identified from pure cultures maintained.

Physical analysis of the leaves

1. pH:

pH of the leaves was determined by electric pH meter. 10 gm of fresh leaves were crushed in 25 ml of double distilled water and filtered. The filtrate was then used for determining pH.

2. Moisture content:

Moisture content of the leaves in terms of percentage dry weight was determined by drying 10 gm of fresh leaves in hot air oven at 105°C for 24 hours, cooled at room temperature and then weighed to get the dry weight of leaves. The process was repeated till the constant weight was obtained.

Interpretation of the data

The results have been expressed as an average of isolations done during two growing seasons (1981 and 1982).

1. Qualitative estimation:

i) The qualitative estimations were expressed as percentage frequency of occurrence. The latter was calculated

for moist chamber, impression plate and washed leaves plating techniques.

The percentage frequency of occurrence was calculated for each fungus based on the formula given by Tresner et al. (1954).

$$\text{Perdentage frequency of occurrence} = \frac{\text{Number of samples of occurrence}}{\text{Total No. of samples}} \times 100$$

ii) The qualitative differences in mycoflora were correlated with plant age and variety. The communities formed by the three varieties of paddy were compared with respect to the similarity of their species composition with age of the plants using Sorenson's similarity index (S) as given by Sorenson (1948).

$$S = \frac{2 \times \text{number of species common to both communities A \& B}}{\text{Total number of species in community A} + \text{Total number of species in community B}}$$

where A and B are two communities (two varieties) compared together.

2. Quantitative estimation:

For quantitative estimations the number of fungal propagules was expressed as fungal population/gm fresh weight of leaves. It was calculated as:

$$\frac{\text{Average No. of colonies per petri plates x dilution of the aliquot } (10^3)}{\text{Fresh weight of leaves (1 gm).}}$$

The fungal population was calculated in case of dilution plate technique where colony numbers could be counted easily.

The data collected during the present investigation by dilution plate technique was statistically analysed by using student's 't' test and analysis of variance done by 'F' test.

Further, pH and moisture content of the leaves were correlated with the mycopopulation at different growth stages of the plant by using Pearson's product moment correlation coefficient (r).

RESULTS

Methodology

A total of 88 species belonging to 53 genera of fungi were isolated from the phylloplane of three varieties of paddy viz., Khonorullo, Ngoba and Mirikrak by using five different techniques of isolation namely Direct observation, impression plate, moist chamber, dilution plate and washed leaves plating techniques at different growth-stages of the plant during two crops seasons, i.e. 1981 and 1982 (Table 1.2). Table 1.2 shows a comparison of the five different techniques for

Table 1.1: Showing developmental stages of paddy plant at different ages of the plant at different sampling dates: Sowing date - 26th April 1981/1982.

Sampling dates	Age of the plant	Developmental stage of the plant
11th May 1981/1982	15 days	Seedling stage
26th May 1981/1982	30 days	Very young stage
10th June 1981/1982	45 days	Young (beginning of tilling)
25th June 1981/1982	60 days	Tilling
10th July 1981/1982	75 days	Tilling
25th July 1981/1982	90 days	Pre-flowering stage (Head begins to swell)
9th Aug. 1981/1982	105 days	Flowering begins
24th Aug. 1981/1982	120 days	Flowering stage
8th Sep. 1981/1982	135 days	Post-flowering (Milky stage)
23rd Sep. 1981/1982	150 days	Post-flowering (Kernel formed)
8th Oct. 1981/1982	165 days	Before harvest (drying stage)
23rd Oct. 1981/1982	180 days	Ripe harvesting stage

Table 1.2: Showing the comparison of the five techniques used for isolation of mycoflora.

Fungi	D.O	I.P	W.L	M.C	D.P
PHYCOMYCETES					
MUCORALES					
Absidia glauca					+
Absidia sp.				+	
Cunninghamella echinulata				+	
Mucor alternans		+		+	
M. basiliformes			+		+
M. hiemalis			+		+
Rhizopus nigricans		+	+	+	+
OOMYCETES					
Pythium sp.		+	+		+
ASCOMYCETES					
Ascochyta sp.	+				
Chaetomium bostrychodes		+	+	+	+
C. funiculosum				+	
C. fusiforme				+	
C. globosum		+		+	+
C. succineum				+	
Cochliobolus miyabeanus				+	
Colletotrichum capsicum				+	
Coniothyrium indicum		+	+	+	
Gelasinospora tetraspora				+	
Masoniella griseum				+	
Melanospora zamae		+	+	+	+
Pyrenochaeta decipiens				+	
Sordaria humana				+	
S. macrospora			+	+	+
Triangularia obliqua				+	
HYPHOMYCETES					
SPHAEROPSIDALES					
Phoma glomerata		+	+	+	+

Fungi	D.O	I.P	W.L	M.C	D.P
P. hibernica		+		+	+
Macrophomina phaseolina				+	+
Rhizoctonia solani		+		+	
MONILIALES					
Acremonium indicum				+	
A. persicinum					+
Alternaria alternata	+	+		+	+
A. solani		+	+		+
Arthrinium sp.		+	+	+	+
Aspegillus candidus					+
A. clavatus					+
A. flavus	+		+	+	+
A. fumigatus					+
A. nidulans	+		+	+	+
A. niger	+	+	+	+	+
A. ochraceous				+	+
A. sydowi		+	+		+
A. terreus					+
A. versicolor					+
Aureobasidium pullulans		+	+		+
Botrytis cinerea		+	+		+
Candida albicans			+		+
Chrysosporium pruinosum		+	+	+	+
Cladosporium cladosporoides		+	+	+	+
C. herbarum	+	+	+	+	+
C. sphaerospermum					+
Curvularia lunata	+	+	+	+	+
C. pullescens				+	
Dreschlera graminea		+	+	+	
D. rostrata					+
Epicoccum nigrum	+	+	+	+	+
E. purpurascens					+
Fusarium oxysporum	+				
F. moniliforme		+	+	+	+
Gliocladium penicilloides		+	+	+	+
Didymostilbe ellisii			+		

Fungi	D.O	I.P	W.L	M.C	D.P
Harphographium fasciculatum				+	
Helminthosporium oryzae	+	+	+	+	+
Humicola prisea		+	+	+	+
Memnoniella echinata		+		+	+
Monilia sitophylla			+		
Neurospora sp.		+			
Nigrospora oryzae	+	+	+	+	+
N. sphaerica				+	
Oedocephalum glomerulosum				+	
Papulospora sp.					+
Paecilomyces veruta				+	
Penicillium funiculosum	+	+	+	+	+
P. chrysogenum		+	+	+	+
P. glaucum					+
P. stipitatum				+	
Stachybotrys atra		+		+	
Starkeomyces koorchalomoides		+	+	+	+
Stemphyllium botryosum				+	+
Torula herbarum		+		+	+
Trichoderma viride	+	+	+	+	+
Trichothecium roseum		+	+	+	+
Verticillium albo-atrum		+	+	+	+
MELANCONIALES					
Pestalotia monorhynca		+	+		+
White sterile mycelia	+	+	+	+	+
Brown sterile mycelia		+	+		+
Orange sterile mycelia				+	
Yellow sterile mycelia		+	+	+	+
Black sterile mycelia	+			+	

D.O = Direct observation technique; I.P = Impression plate technique; W.L = Washed leaves plating technique; M.C = Moist chamber technique; D.P = Dilution plate technique.

Table 1.3: Showing presence of different fungi on three varieties of paddy leaves by using direct observation technique (both nail-polish impression and cellotape impression techniques) (average of two years observations) at different growth stages of the plant.

Fungi	AGE OF THE PLANT IN DAYS																																			
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
ASCOMYCETES																																				
Aschochyta sp.																+																				
HYPHOMYCETES MONILIALES																																				
Alternaria sp.	+			+	+	+	+	+	+		+		+		+	+				+	+	+		+	+						+			+	+	+
Aspergillus niger							+									+	+					+	+	+			+				+		+		+	
Cladosporium herbarum				+		+	+	+		+	+	+	+		+	+	+	+			+	+		+	+		+						+			
Curvularia lunata	+						+	+	+				+	+					+						+			+		+	+			+	+	
Epicoccum nigrum							+	+	+		+					+		+			+	+		+		+	+	+		+		+		+		
Fusarium oxysporum				+	+																	+		+					+			+		+		
Helminthosporium oryzae																					+		+	+		+	+			+			+			+
Nigrospora oryzae							+						+								+	+														
Penicillium sp.														+								+														
Trichoderma viride																			+																	
White sterile mycelia	+		+				+	+					+	+					+										+	+			+			
Black sterile mycelia							+																													

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety.

Total species isolated = 13.

Table 1.4: Showing the presence of fungi of three varieties of paddy isolated by impression plate technique (average of 2 years observations) at different growth stages of the plant.

Fungi	AGE OF THE PLANT IN DAYS																							
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
PHYCOMYCETES																																				
MUCORALES																																				
Mucor alternans	+			+	+											+			+	+		+			+	+	+	+			+			+		
Rhizopus nigricans					+																							+	+		+	+	+			
OOMYCETES																																				
Pythium sp.				+	+		+	+	+							+		+				+									+			+		
ASCOMYCETES																																				
Chaetomium bostrychodes							+																								+			+		
C. globosum													+																		+	+		+	+	
Coniothyrium indicum							+	+																												
Melanospora zamae				+												+																				
HYPHOMYCETES																																				
SPHAEROPSIDALES																																				
Phoma glomerata										+	+		+		+							+	+	+												
P. hibernica					+											+												+		+	+		+	+		+
Rhizoctonia solani				+									+																							
MONILIALES																																				
Alternaria alternata	+				+	+	+		+				+	+		+	+	+	+	+	+	+	+	+	+	+		+			+	+	+	+		
A. solani		+					+	+		+	+		+		+	+			+	+		+			+			+			+	+				
Arthrinium sp.				+	+	+	+		+			+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		

Fungi	AGE OF THE PLANT IN DAYS																																			
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
Aspergillus nidulans							+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A. niger				+		+				+		+	+		+	+			+	+	+	+	+	+	+	+	+	+	+	+	+					
A. flavus										+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
A. sydowi														+																						
Botrytis cinerea													+				+																			
Chrysosporium pruinosum													+																							
Cladosporium cladosporoides			+				+											+	+	+		+					+			+			+			+
C. herbarum	+	+		+		+	+	+			+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Curvularia lunata									+			+			+	+	+	+	+			+	+		+		+		+		+					
Dreschlera graminea										+	+		+																							
Aureobasidium pullulans																									+											
Epicoccum nigrum					+			+		+																										
Fusarium moniliforme	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+				+	+	+	+				+		+
Gliocladium pencilloides														+									+						+	+						
Helminthosporium oryzae		+						+	+					+	+		+	+		+	+		+	+			+		+	+			+		+	+
Humicola prisea										+		+																								
Memnoniella echinata																+	+	+	+	+	+	+		+	+	+	+	+	+		+	+		+		
Nigrospora oryzae						+						+				+							+					+				+		+		
Neurospora sp.									+																											
Penicillium funiculosum	+			+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Fungi	AGE OF THE PLANT IN DAYS																							
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
P. chrysogenum				+			+			+			+			+			+			+			+			+			+			+	+	
Stachybotrys atra										+	+	+	+		+																				
Starkeomyces koorchalomoides							+			+				+							+		+	+				+			+		+	+	+
Torula herbarum																									+											
Trichoderma viride							+			+	+		+	+		+			+							+			+	+		+				
Trichothecium roseum							+	+	+	+			+	+	+	+											+			+	+	+	+			
Verticillium albo-atrum	+	+					+			+			+	+		+	+		+			+	+	+				+			+			+		
MELANCONIALES																																				
Pestalotia monorhynca										+	+	+	+		+							+		+											
White sterile mycelia				+				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+		+	+	+			+		
Brown sterile mycelia							+	+	+	+		+	+	+	+	+	+	+	+	+		+			+	+	+									
Orange sterile mycelia		+			+		+		+				+			+		+	+	+	+	+	+	+	+	+									
Yellow sterile mycelia																+	+	+	+		+			+						+		+				

+ = presence of fungi; K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety.

Table 1.5: Showing the presence of fungi on phylloplane of three varieties of paddy at different growth stages isolated by washed leaves plating method (average of two years observations).

Fungi	AGE OF THE PLANT IN DAYS																							
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
PHYCOMYCETES																																				
MUCORALES																																				
Mucor hiemalis	+						+	+		+	+	+	+	+	+	+	+	+	+											+			+		
M. basiliformis														+							+														
Rhizopus nigricans													+		+	+		+	+					+			+			+					
OOMYCETES																																				
Pythium sp.				+						+		+	+			+	+		+			+			+	+	+	+		+		+			+	
ASCOMYCETES																																				
Chaetomium bostrychodes																		+			+														
Coniothyrium indicum												+																								
Melanospora zamae																	+		+	+					+											
Sordaria macrospora							+				+																									
HYPHOMYCETES																																				
SPHAEROPSIDALES																																				
Phoma sp.				+						+	+	+				+	+								+			+			+			+	
MONILIALES																																				
Alternaria solani	+			+	+	+				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+	+	+	+
Arthrinium sp.																			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus flavus	+	+	+				+	+		+	+		+	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
A. nidulans										+	+		+	+	+	+	+	+	+	+	+	+		+				+	+		+	+	+	+	+	+

Fungi	AGE OF THE PLANT IN DAYS																																		
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
A. niger	+	+	+	+	+	+	+	+	+	+			+	+	+	+				+						+	+	+	+	+	+			+		+
Botrytis cinerea																							+		+											
Aureobasidium pullulans																							+		+	+			+			+		+	+	
Candida albicans																						+			+	+		+	+	+						
Chrysosporium pruinosum																	+	+							+	+		+		+	+			+		
Cladosporium cladosporoides													+			+			+		+				+			+			+					
C. herbarum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Curvularia lunata										+	+		+	+	+	+	+				+	+	+	+	+	+		+	+	+	+			+		
C. pullescens														+								+			+											
Dreschlera graminea																						+			+		+									
Epicoccum nigrum								+		+							+	+			+	+			+	+	+	+	+		+	+	+			
Fusarium moniliforme	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																				
Didymostilbe ellisii																						+														
Gliocladium pencilloides																+		+										+			+					
Helminthosporium oryzae											+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+
Humicola prisea														+												+	+									
Monilia sitophylla											+			+	+					+									+		+	+		+	+	
Nigrospora oryzae																						+	+	+	+			+			+	+				
Penicillium funiculosum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. chrysogenum																						+	+		+			+								
Starkeomyces koorchalomoides	+	+			+					+	+	+										+														

Fungi	AGE OF THE PLANT IN DAYS																							
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
Trichoderma viride				+			+			+			+			+			+			+	+		+			+	+		+			+		
Trichothecium roseum					+		+	+	+	+			+			+	+		+	+	+	+	+		+			+			+	+		+	
Verticillium albo-atrum				+			+						+			+		+	+	+	+				+	+	+	+			+	+		+	+	+
MELANCONIALES																																				
Pestalotia monorhynca													+			+			+						+			+	+							
White sterile mycelia				+			+	+		+		+	+	+	+	+	+		+		+				+			+	+	+	+	+				
Yellow sterile mycelia										+		+							+						+			+	+	+				+	+	
Brown sterile mycelia													+	+	+	+			+						+	+		+	+		+					+

+ = presence of a fungus; K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety.

Table 1.6: Showing percentage frequency of occurrence (average of two years observations) of phylloplane fungi of three varieties of paddy at different growth stages of the plant isolated by moist chamber technique

Fungi	AGE OF THE PLANT IN DAYS: 15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
PHYCOMYCETES																																				
MUCORALES																																				
Absidia sp.																20	20															20				
Cunninghamella echinulata																				20																
Mucor alternans										20	20	40				20	20		40	20		20			20	20	30	30	30	20	20	20	30	30	40	30
M. hiemalis																										20	20									
Rhizopus nigricans	10		10		10	10	40		40	40	40		20	40	20	20	20	20	20		20	20			20	20		40	40		50	60	20	60	50	50
ASCOMYCETES																																				
PYRENOMYCETES																																				
SPHAERIALES																																				
Chaetomium bostrychodes													50			60		60	50	60	60	70	80	80	90	80	80	90	90	90	100	100	100	100	100	100
C. funiculosum														10			20						20	20	30											
C. fusiforme																					30		20	30												
C. globosum							20	40	50	50	50	50	60	50	50	60	60	60	50	50	50	75	75	80	100	90	90	100	90	90	100	100	100	90	90	80
C. succineum																						20														
Cochliobolus miyabeanus																								10			10			20			30			
Colletotrichum capsicum																	10						10													
Coniothyrium indicum								30		50	30														80			70	20							
Gelasinospora tetrasperma											20												20	20								30				
Magoniella griseum																				10																
Melanospora zamae											20			20	20					20		30	20	20												
Pyrenochaeta decipiens																							10													
Sordaria humana																									10											
S. macrospora																20	20								30	20										
Triangularia obliqua																10																				
HYPHOMYCETES																																				
SPHAEROPSIDALES																																				
Phoma glomerata								40			50	40	40	50	50	50	60		50	60	60	60	70	60	70	70	60	90			100					
P. hibernica													10																							
Macrophomina phaseolina																												20			20	20				
Rhizoctonia solani										10	10											20			30	20			20					30		
MONILIALES																																				
Acremonium indicum																				10																
Alternaria alternata							10		20	30	30	40	60	80	80	90	60	90				80	90					80	60	80			50			
Arthrinium sp.							10	10								20	20	10					30	20	20						20	30	20	20	20	20
Aspergillus flavus					10	30	20	30	10	10	10							30	20	20	30	30	20		20			20			30	40		30	30	10
Aspergillus nidulans							20	10	10	10	10			10	10	10	10		20	20		10	10	10	20		30	20		20		20	30	30	20	10
A. niger			10	10			20	20	10	10	20	20	20				20	20			20	20	10				20	30	50	30	20	20			20	
A. ochraceus																				10					10	10										
A. sydowi																									10						10					
Chrysosporium pruinosum																							20			30	20									
Cladosporium cladosporoides																10	10	10	30	40	50				50			80	50	90	80	90	90	100	30	10
C. herbarum													10	30	20																					
Curvularia lunata	20	20		20			20		10	20	20					50	30				30	50			50			80			30	10		10		
C. pallescens																															10	10				
Drechslera graminea						10		20	10								10								10											10
Epicoccum nigrum																									10									20		
Fusarium moniliforme							10	10	10					10	20				10	10	10							10	20	10	20	30	30	40	30	30
Gliocladium pencilloides																						20														
Harpographium fasciculatum													20																							
Helminthosporium oryzae																		20		10	20		20	50		30	50			50			50			50
Humicola grisea										10																		20	20					10		
Memnoniella echinata				10	10	10	10	10	10				30	30	40	50	40	50	40	50	60			50							50	40	30	60	50	40
Nigrospora oryzae										10	10		20	10	10	20	20	30	30	20	20			10	30			10			30	30				
N. sphaerica																						10														
Oedocephalum glomerulosum					10																															
Paecilomyces veruta																		10								10	10		10	10					10	10
Botrytis cinerea																						10		10					20	20			20			30
Penicillium chrysogenum																				10			10			20	10									
P. funiculosum	20			20	20								30						50	30	30				50			50	40	50	60	50	50	90	50	50
P. stipitatum													10	10	10	20			20			20														
Stachybotrys atra	10	10	10	10				20	10	20	30		30	30	20	50	40	50		20		40	50	30	50	40	40	30	20	10	20	20	30	10	30	30
Starkeyomyces koorchalomoides				20	10	10		20	30	30	50	30	50	50	60				50	80	90	90	90						100		90	80	50	30	20	50
Stemphylium botryosum																	10																			
Trichoderma viride							10	10		10			20			10	10		30	20	10	50	10		50			80			90			90	50	
Trichothecium roseum										10		10	20	20	20				10	10																
Torula herbarum																			10												20	20	10	20	40	20
Verticillium albo-atrum																40	50	30	50	50	50	40	30		50		40	50			50			50		
White sterile mycelia										10								10				20						10			10	10	20	10	10	10
Black sterile mycelia		10		10	10	10										20	10	10				10	10	20	10			10								
Yellow sterile mycelia																		10										10			10					
Orange sterile mycelia																30	10		30	30		10		10	10			10								

K = Khasaralia variety; N = Ngaba variety; M = Mirikrak variety.

Table 1.7 Showing the Phylloplane fungal population (No.of fungal colonies x 10^3 per gm fresh weight of leaves) of three varieties of Paddy at different growth stages of the plant (Average of two years observations) isolated by Dilution Plate technique.

Fungi	AGE OF THE PLANT IN DAYS																																							
	15 days			30 days			45 days			60 days			75 days			90 days			105days			120 days			135 days			150 days			165 days			180 days					
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M			
PHYCOMYCETES MUCORALES																																								
Absidia glauca										.012												.028																		
Mucor basiliformes																.09	.12		.15	.07																				
M. hiemalis													.02	.02	.05	.03	.051	.02	.13	.16	.02	.2	.18	.05	.25	.25	.05	.46	.46	.22	.26	.24	.15	.15	.14	.00			
Rhizopus nigricans										.2	.21	.15																												
OOMYCETES																																								
Pythium sp.	.01			.02	.01		.21		.04	.24	.11		.21	.15	.12	.52	.21	.11				.15		.12				.18												
ASCOMYCETES PYRENOMYCETES SPHAERIALES																																								
Chaetomium bostrychodes										.16			.2			.21	.18																							
C.globosum											.08																													
Melanospora zamiae							.15																																	
Sordaria macrospora											.09						.21	.12																						
HYPOCOMYCETES SPHAEROPSIDALES																																								
Phoma glomerata							.2			.21										.15	.21							.85	.21	.15										
P.hibernica										.02	.04	.02			.12	.15	.19	.21	.25	.22	.25	.92	1.19	1.25				1.2					.15		.30	.12			
Macrophomina phaseolina																.01																								
MONILIALES																																								
Acrothium persicinum										.04																														
Alternaria alternata							.11									.21	.21								.25			.21			.25	1.25	1.2	.19	.12			
A.solani	.01	.008		.02	.02		.12	.11	.12	.26	.2	.19	.42	.22	.11	.19	.11	.15	.21	.28	.21	.15	.09	.05	.11	.15	.08					.11		.12	.13			
Arthrinium sp.				.08	.02	.01	.18	.09	.16	.25	.12	.1	.8	.73	.72	1.2	.95	.91	1.5	1.2	1.2	1.8	1.1	1.0	1.70	1.72	1.8	1.72	1.5	1.0	1.7	1.2	.91	.2	.95	.22			
Aspergillus clavatus											.04																													
A.candidus														.12																										
A.flavus	.02	.01		.04	.02	.02				.01	.02	.04	.21	.08	.11	.21	.08	.09	.25		.05	.28	.02		.31			.35		.02									
A.fumigatus										.11																														
A.nidulans							.08			.11	.08	.07	.15	.17	.19	.21	.12	.19	.58	.15	.21	.89	.12	.55	.12	.95	.65												
A.niger	.01	.02		.02	.02	.1	.22	.21	.17		.22	.24	.31	.18		.31			.31		.11	.5			.12	.15		.09	.12	.15				.15					
A.Ochraceous																.06																								
A.sydowi													.05	.02																										
A.terreus																					.02																			
A.versicolor																.02																								
Aureobasidium pullulans							.21	.15	.18										.28	.25	.26										7.8	9.9	1.8	1.5	1.8	1.8			
Botrytis cinerea													.15			.18			.21	.15																			
Candida albicans												.04		.06	.08		.15	.12										.06											
Chrysosporium pruinosum										.12			.18	.12	.15						.19														.12				
Cladosporium cladosporoides										.12			.09		.04	.19	.11	.09	.21	.25	.11	.25	.28	.11					.15										
C.herbarum	.02	.01	.02	.02	.03	.03	.21	.17	.18	.35	.24	.21	.34	.26	.3	.38	.31	.02	.35	.21		.39	.3	.02	.4	.32	.11	.82	.45	.12	.75	.2		.6					
C.sphaerospermum														.19																										
Curvularia lunata										.11	.08		.21	.15	.12	.15	.25	.15	.5	.25	.58					1.25								.21	.15	.12			
Drechslera rostrata	.01												.18	.25	.12																								
Epicoccum purpurascens	.02			.12	.09	.13		.09		.02	.11	.12	.02	.05	.11				.08	.11					.09	.22	.11			.15									
H.nigrum										.22			.12			.15						.19	.21		.25	.12	.15							.15						

Table 1.7(Contd...)

Fungi	AGE OF THE PLANT IN DAYS																																
	15 days			30 days			45 days			60 days			75 days			90 days			105 days			120 days			135 days			150 days			165 days			180 days		
	K	E	M	K	E	M	K	E	M	K	E	M	K	E	M	K	E	M	K	E	M	K	E	M	K	E	M	K	E	M	K	E	M	K	E	M
Fusarium moniliforme	.026	.02	.01	.08	.02	.01	.12	.11	.1	.22	.19	.2	.41	.21	.25	.42	.11		.4			.11			.25	.11		.52	.24					.11		
Gliocladium penicilloides											.02			.15			.16	.15										.25	.12	.08						
Helminthosporium oryzae									.01		.02	.02		.16	.18		.45	.58			.12			.11								.81			.92
Humicola grisea										.13	.18	.21																							
Memnoniella echinata		.02								.21	.15	.08																							
Microstroma oryzae			.02							.18	.11	.09		.12		.15	.07	.02	.11	.05								.12			.12	.11		.12	.07	
Papulospora sp.																				.05																
Penicillium funiculosum	.068	.06	.05	.07	.06	.06	.32	.31	.31	.38	.32	.3	.53	.46	.39	.85	.53	.4	1.2	.68	.38	1.3	.62	.3	1.5	.6	.35	1.72	1.5	1.4	1.8	1.5	1.4	1.7	1.55	1.52
P.chrysogenum													.85	.21		.25	.28	.15	1.2	.19					1.2	.9	.15									
P.glaucum																			.02																	
Starkeomyces koorchalomoides					.18					.25	.15	.12	.21	.11	.28							.05	.08													
Stemphyllium botryosum													.15																							
Trichoderma viride				.12			.12			.21	.15	.11	.23	.12	.1	.75	.12	.1	1.2	.19		1.4			1.5	1.25		1.7	1.2		1.2	1.2		1.85	1.2	
Trichothecium roseum													.11		.09	.5	.09	.12				.15	.11	.2							.35	.21	.25			
Torula herbarum																.12	.08	.11																		
Verticillium alboatrum							.12	.06		.18	.1	.09	.12			.15	.12	.15	.25	.15	.25	.12		.11				.28	.21	.15				.29	.15	.196
MELANCONIALES																																				
Pestalotia monochyncha									.08			.12							.15	.09	.12															
White sterile mycelia							.16			.22	.1		.43	.39	.36	.45	.49	.29						.15					.15	.21						
Brown " "										.34		.09	.15	.21	.15	.21	.24	.23				.19	.21	.19												
Yellow " "										.12			.12			.15	.12	.15	.12	.12	.11	.13	.16	.12												
Orange " "										.18	.1		.09	.11	.12	.15	.15	.15	.12	.15	.16	.12	.15	.15												

K - Khonorulle variety
E - Egoba variety
M - Mirikrak variety

Table 1.8: Paired comparisons of average fungal population/gram fresh weight of leaves using student 't' test.

Variety	Observed 't' with different pairs of plant age in days										
	15-30	30-45	45-60	60-75	75-90	90-105	105-120	120-135	135-150	150-165	165-180
K	2.21**	5.01**	.68^{+}	1.62*	0.43^{+}	1.51*	0.41^{+}	1.11*	0.06^{+}	0.92*	0.69*
N	1.45*	3.63**	.87*	2.08**	0.67^{+}	0.61^{+}	1.25*	1.32*	0.19^{+}	1.44**	1.21*
M	0.42^{+}	2.60**	.49^{+}	1.49*	0.33^{+}	1.21*	0.58^{+}	0.84*	0.41^{+}	2.52**	1.19*

K = Khonorullu variety; N = Ngoba variety; M = Mirikrak variety; * Significant at 95% probability
** Significant at 99% probability; + Insignificant.

Table 1.9: Statistical analysis of change in fungal counts at three growth stages of plants using student 't' test.

Variety	Young - Mature stage (15 - 90 days) 't' value	Mature - Senescent stage (90 - 180 days) 't' value	Young - Senescent stage (15 - 180 days) 't' value
K	3.02**	3.32**	2.89**
N	3.16**	2.6**	2.4**
M	1.68*	2.31**	1.23*

* Significant at 95% probability

** Significant at 99% probability

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety.

Table 1.10: Analysis of variations in fungal population within three varieties of paddy using the 'F' test (one way classification) at different growth stages of plant.

Growth stages	Observed F
Young stage (15 days)	2.26+
Mature stage (90 days)	22.02**
Senescent stage (180 days)	3.91+

+ Insignificant; ** Significant at 99% probaility.

Table 1.11: Analysis of variance using the 'F' test (two way classification) among two variables; host variety and growth stage of plant.

Variables	Observed 'F'	Degrees of Freedom
Plant variety	3.20^{+}	(2,4)
Growth stage of plant	41.27^{**}	(2,4)

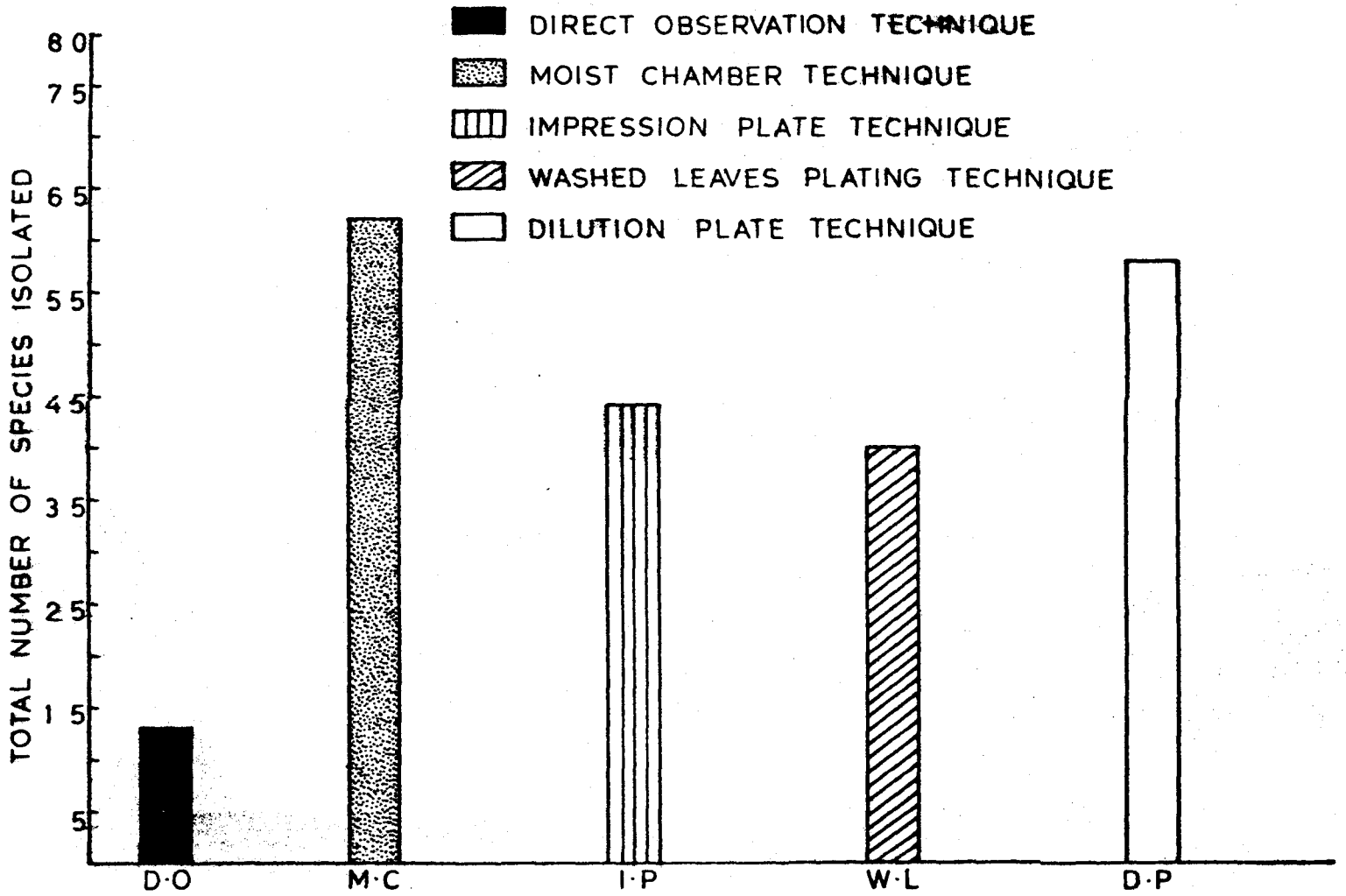
+ insignificant; ** significant at 99% probability.

Table 1.12: Relationship (r values) between fungal population of three varieties of paddy viz., Khonorullo (K), Ngoba (N) and Mirikrak (M) and pH, moisture content of leaves at various growth stages of the plant.

Variety	pH	Moisture content
K	.968**	- .833**
N	.958**	- .92**
M	.959**	- .908**

** Significant at 99% probability.

Fig. 1.2: A comparison of five different techniques used viz., Direct observation, moist chamber, impression plate, washed leaves plating and dilution plate techniques for isolation of maximum number of fungal species.



DIFFERENT TECHNIQUES USED FOR ISOLATION OF PHYLLOPLANE FUNGI

Fig. 1·2

isolation of phylloplane fungi. From Fig. 1.2 and Table 1.2 it is clear that maximum fungal species were recorded in moist chamber followed by dilution plate, impression plate, washed leaves plating and least by using direct observation method.

Maximum number of Ascomycetes were isolated by using moist chamber method, whereas, many species of fungi belonging to Hyphomycetes were isolated by dilution plate, impression plate and washed leaves plating techniques (Table 1.2). Interestingly, the use of cellulose agar medium in dilution plate, impression plate and washed leaves plating techniques as nutrient medium facilitated the isolation of some of the Ascomycetes like Chaetomium spp., Sordaria sp., Melanospora zamae (Plate 1.4; 3,4) which were otherwise restricted to moist chamber technique only. Direct observation technique yielded only those forms which were dominant species on the phylloplane viz., Cladosporium herbarum, Penicillium sp., Alternaria sp. excepting Ascochyta sp. which were exclusive to this method.

Fungal colonization on leaf surface

Based on the observation from the five methods of isolation of fungi (Table 1.3-1.7) the fungal colonizers on leaf surface of paddy varieties belong to 3 main groups viz., Mucorales, Ascomycetes and Hyphomycetes and out of which maximum number of species belonged to Moniliales under the class Hyphomycetes.

Fig. 1.3: Total number of fungi isolated from leaf surface of Khonorullo variety of paddy during different ages of the plant using different techniques of isolation viz., Direct observation, washed leaves plating, moist chamber, impression plate and dilution plate techniques.

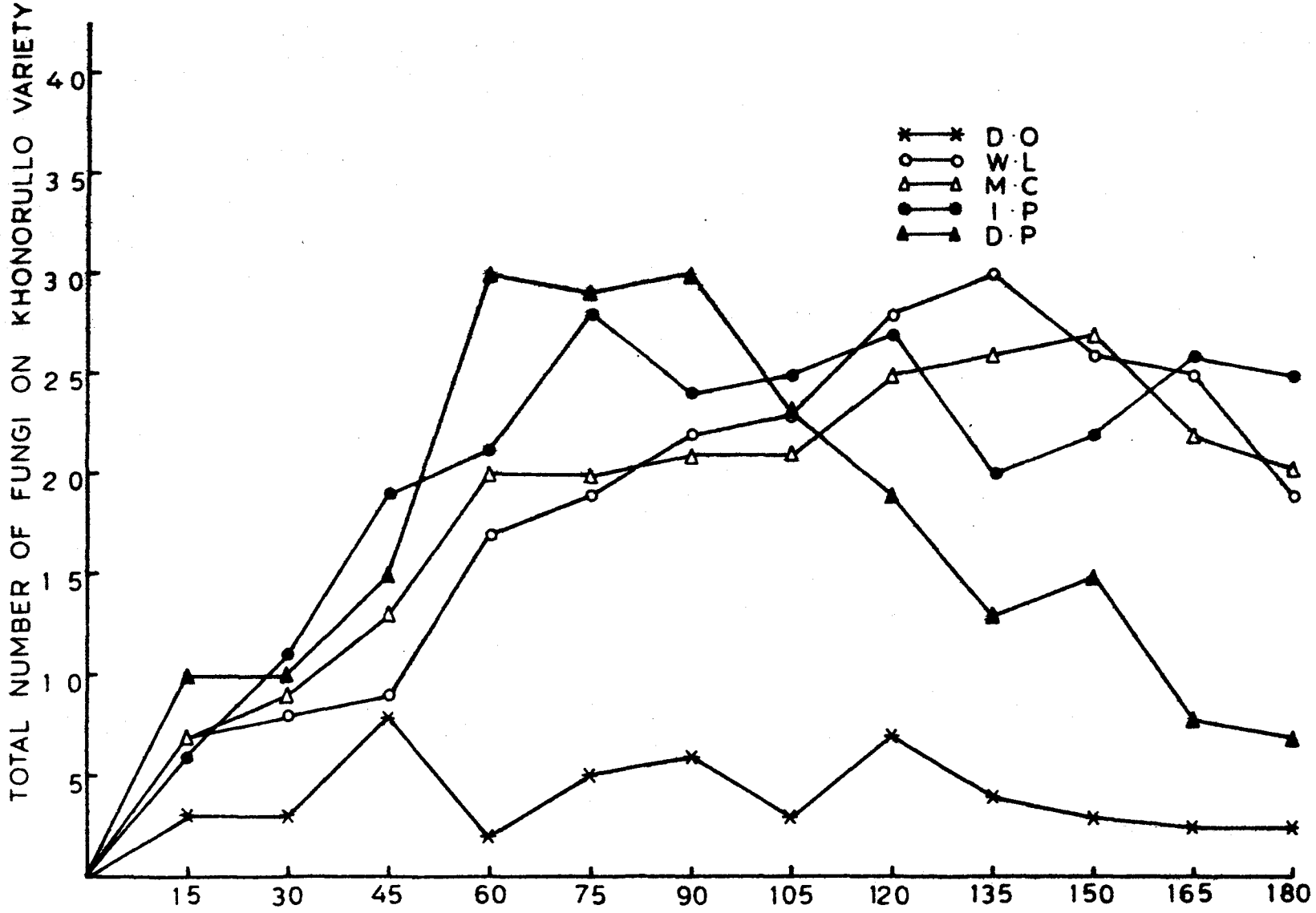

TOTAL NUMBER OF FUNGI ON KHONORULLO VARIETY
40
35
30
25
20
15
10
5
D·O
W·L
M·C
I·P
D·P
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1·3

Fig. 1.4: Total number of fungi isolated from leaf surface of Ngoba variety of paddy during different ages of the plant using different techniques of isolation viz., Direct observation, washed leaves plating, moist chamber, impression plate and dilution plate techniques.

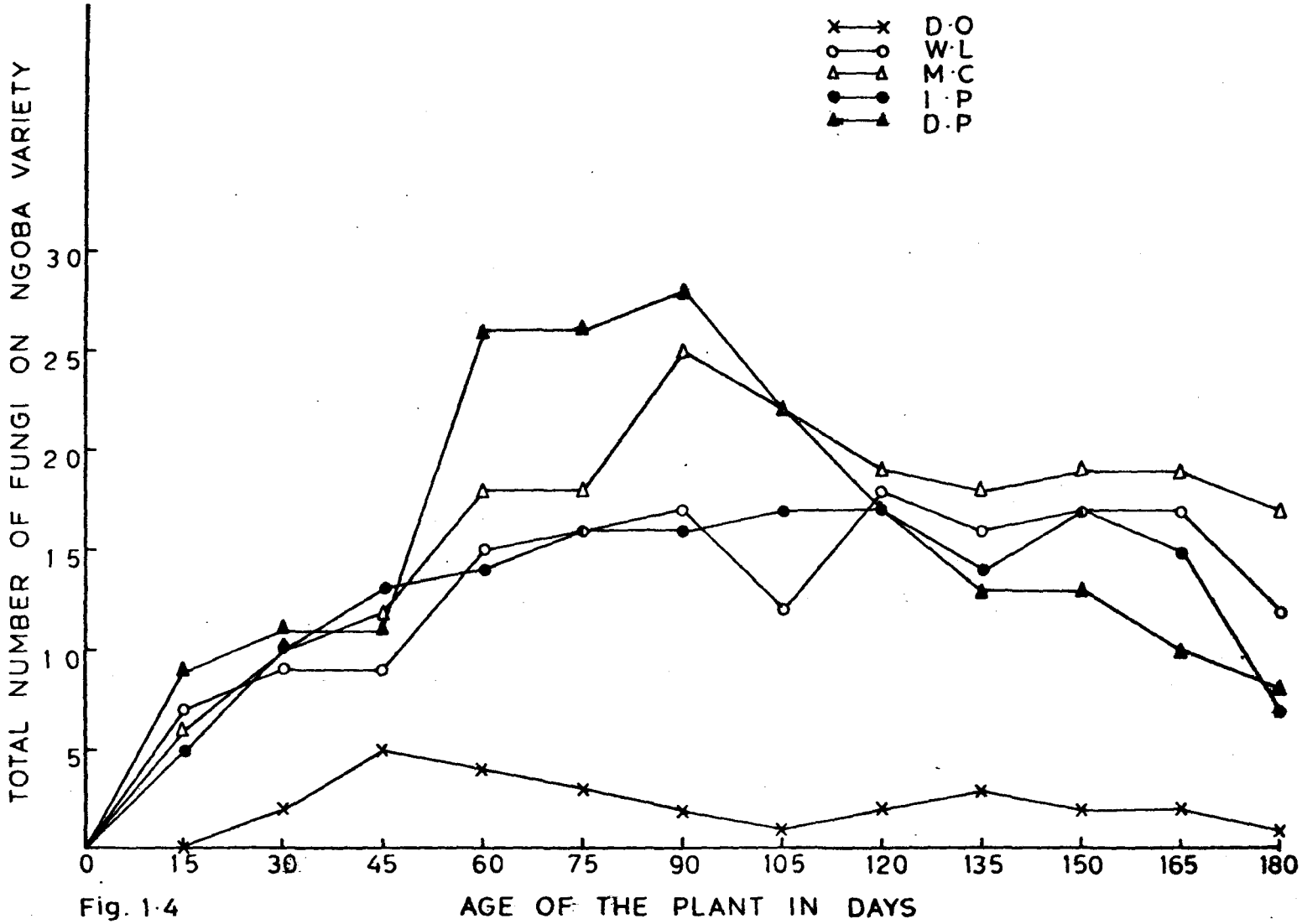

D·O
W·L
M·C
I·P
D·P
TOTAL NUMBER OF FUNGI ON NGOBA VARIETY
30
25
20
15
10
5
0
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1·4

Fig. 1.5: Total number of fungi isolated from leaf surface of Mirikrak variety of paddy during different ages of the plant using different techniques of isolation viz., Direct observation, washed leaves plating, moist chamber, impression plate and dilution plate techniques.

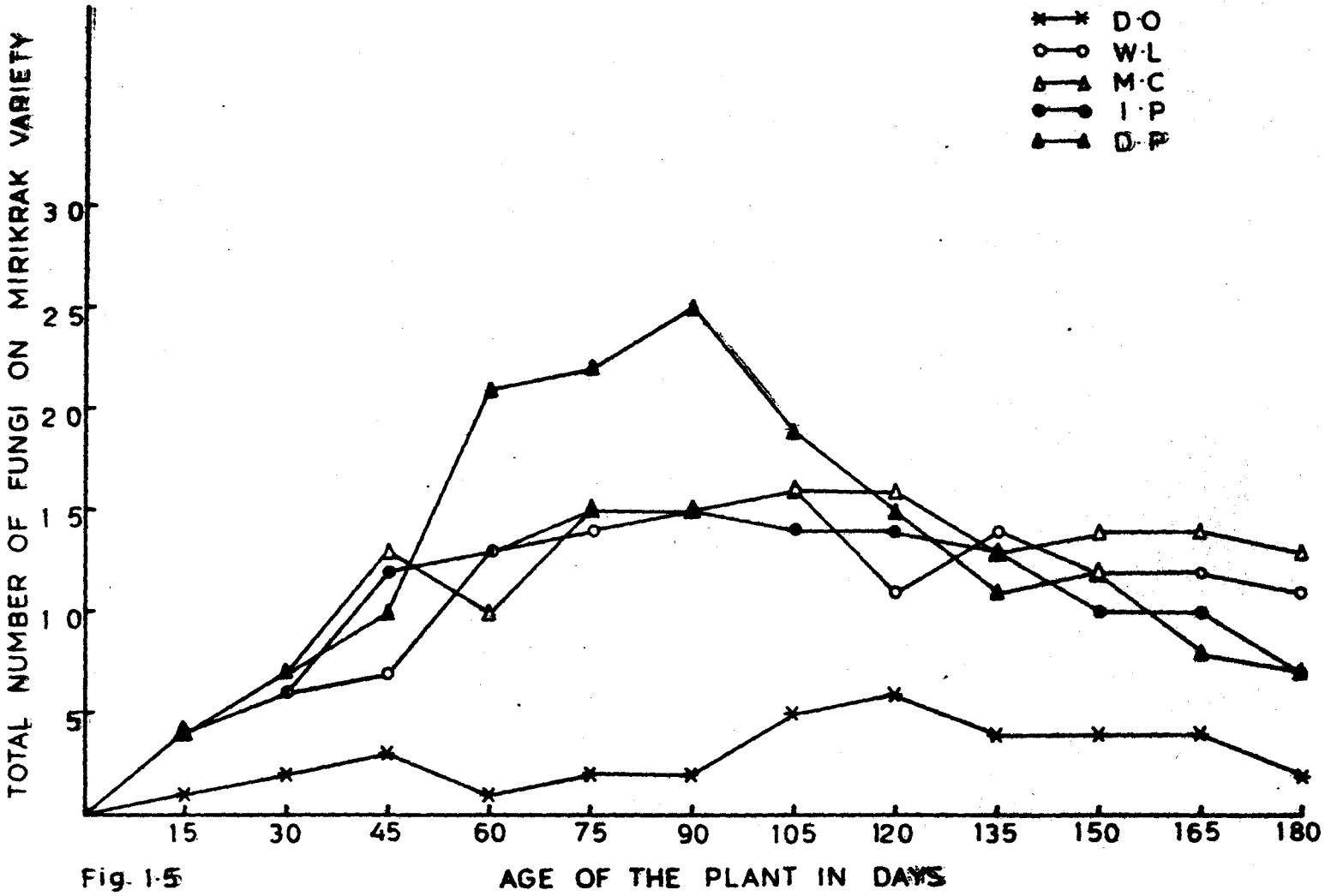

D·O
W·L
M·C
I·P
D·P
TOTAL NUMBER OF FUNGI ON MIRIKRAK VARIETY
30
25
20
15
10
5
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1·5

Qualitative assessment

Tables 1.3-1.5 depict the appearance of various fungal species during different stages of plant growth as observed by direct observation, impression plate and washed leaves plating techniques. The qualitative composition of phylloplane fungal communities was studied during different stages of plant growth. To isolate maximum number of plant species several techniques were used simultaneously.

The change in total number of fungi occurring on the three varieties of paddy viz., Khonorullo, Ngoba and Mirikrak are depicted in Figs. 1.3-1.5 isolated by five different techniques. The total number of fungi showed a gradual increase with plant age with sudden peak at the age of 90 days and later on as the leaves started drying and the plant reached the senescent stage at the age of 180 days the total number of fungi decreased gradually. Further, from Figs. 1.6-1.8 also it is quite clear that there is an increase in total number of fungi with plant age. However, the average percentage frequency of occurrence of fungi showed a gradual increase throughout the growth stages as depicted in Figs. 1.6-1.8 for all the three varieties.

By impression plate method (Table 1.4) only those fungi which were occurring superficially on the leaf surface such as Penicillium spp., Aspergilli, Alternaria alternata, Arthrinium sp., Cladosporium spp. and Fusarium moniliforme were commonly isolated. Other fungi like Mucorales, Ascomycetes and some Hyphomycetes like Aureobasidium pullulans, Gliocladium

Fig. 1.6: Total number of fungi (o——o) and average percentage frequency of occurrence (o----o) on leaf surface of Khonorullo variety of paddy with increasing plant age.

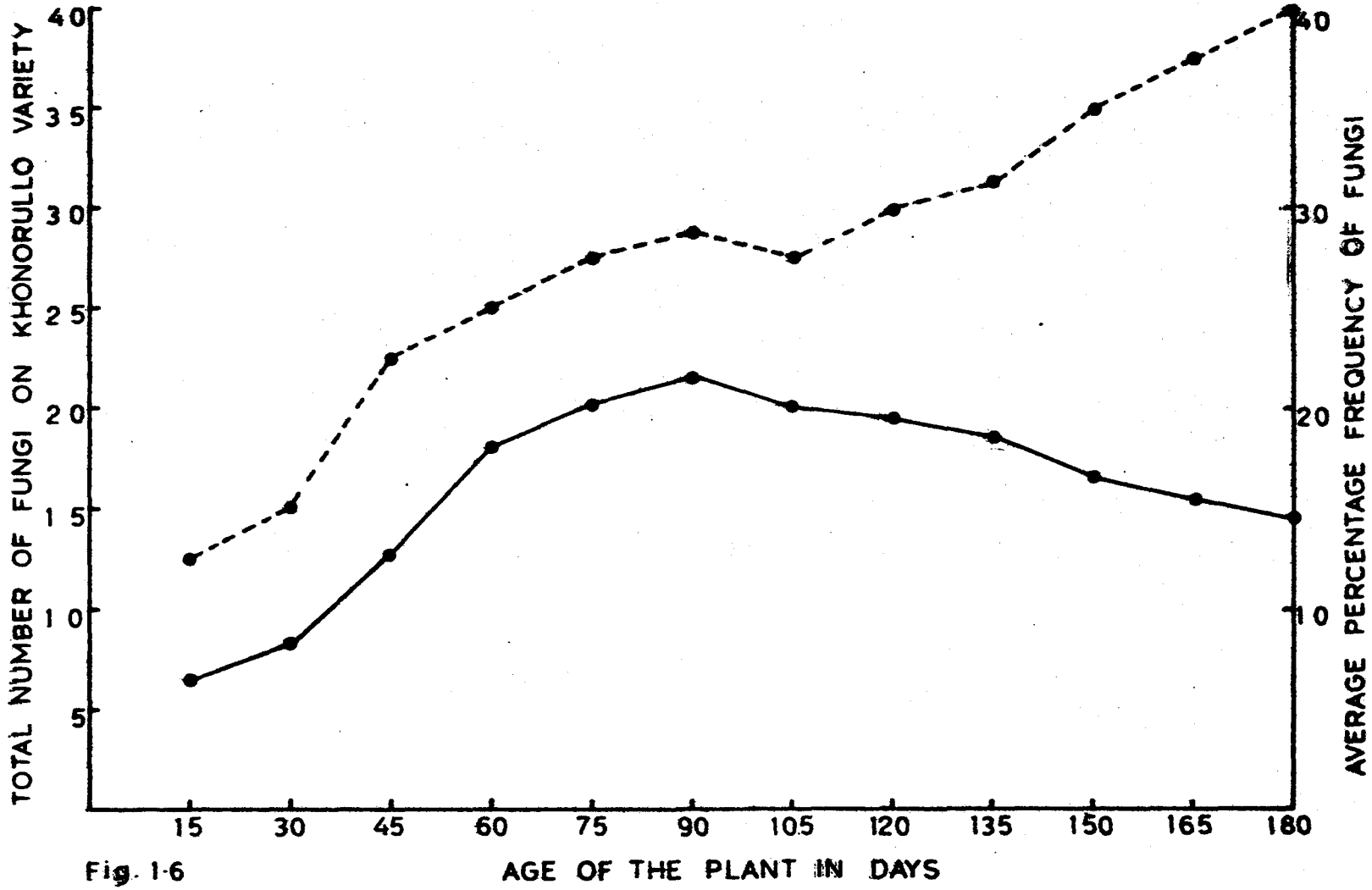

TOTAL NUMBER OF FUNGI ON KHONORULLO VARIETY
AVERAGE PERCENTAGE FREQUENCY OF FUNGI
40
35
30
25
20
15
10
5
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1-6

Fig. 1.7: Total number of fungi (o———o) and average percentage frequency of occurrence (o-----o) on leaf surface of Ngoba variety of paddy with increasing plant age.

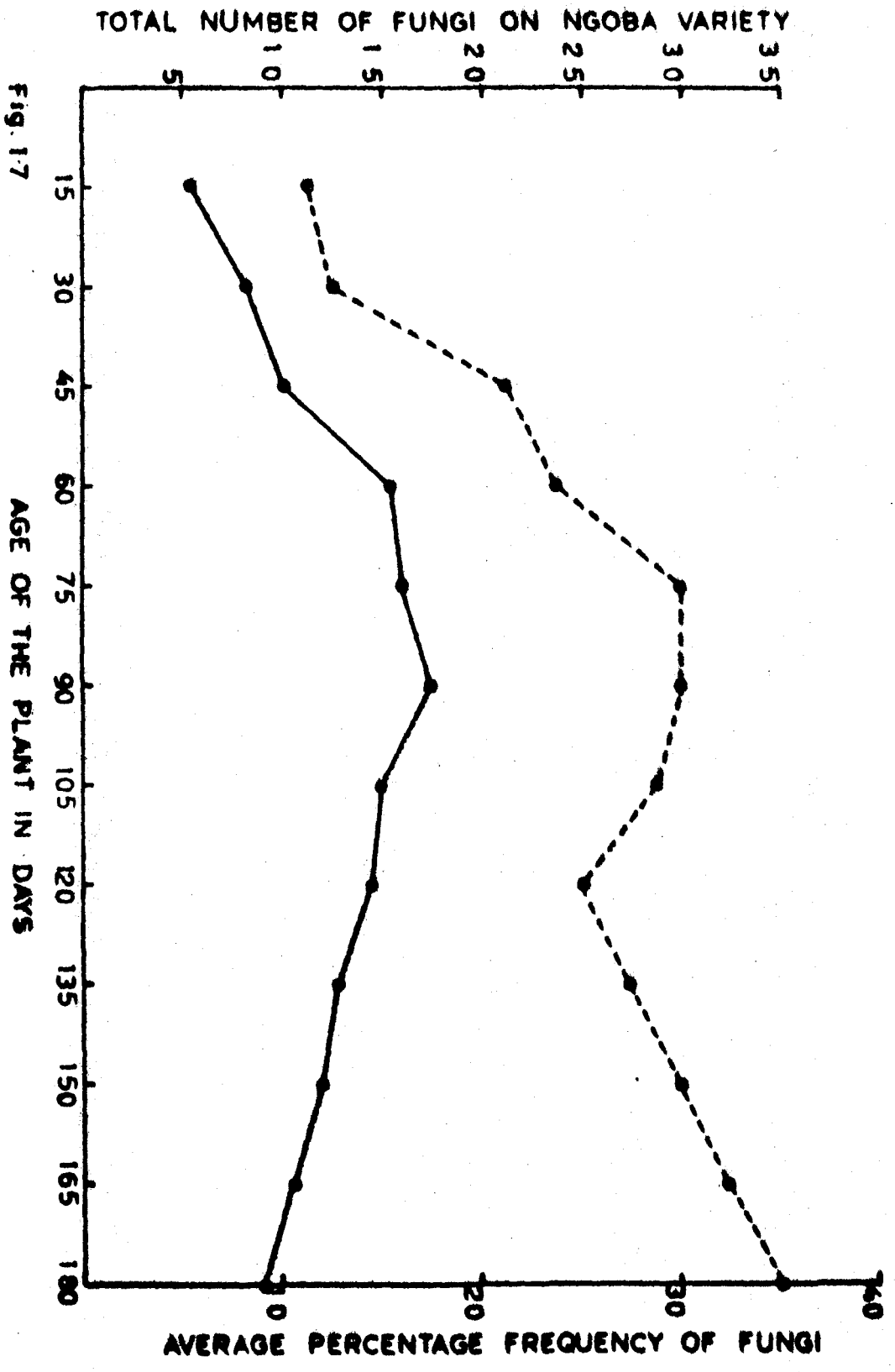

TOTAL NUMBER OF FUNGI ON NGOBA VARIETY
5
10
15
20
25
30
35
AGE OF THE PLANT IN DAYS
15
30
45
60
75
90
105
120
135
150
165
180
0
20
30
40
AVERAGE PERCENTAGE FREQUENCY OF FUNGI

Fig. 1-7

Fig. 1.8: Total number of fungi (o——o) and average percentage frequency of occurrence (o- - - -o) on leaf surface of Mirikrak variety of paddy with increasing plant age.

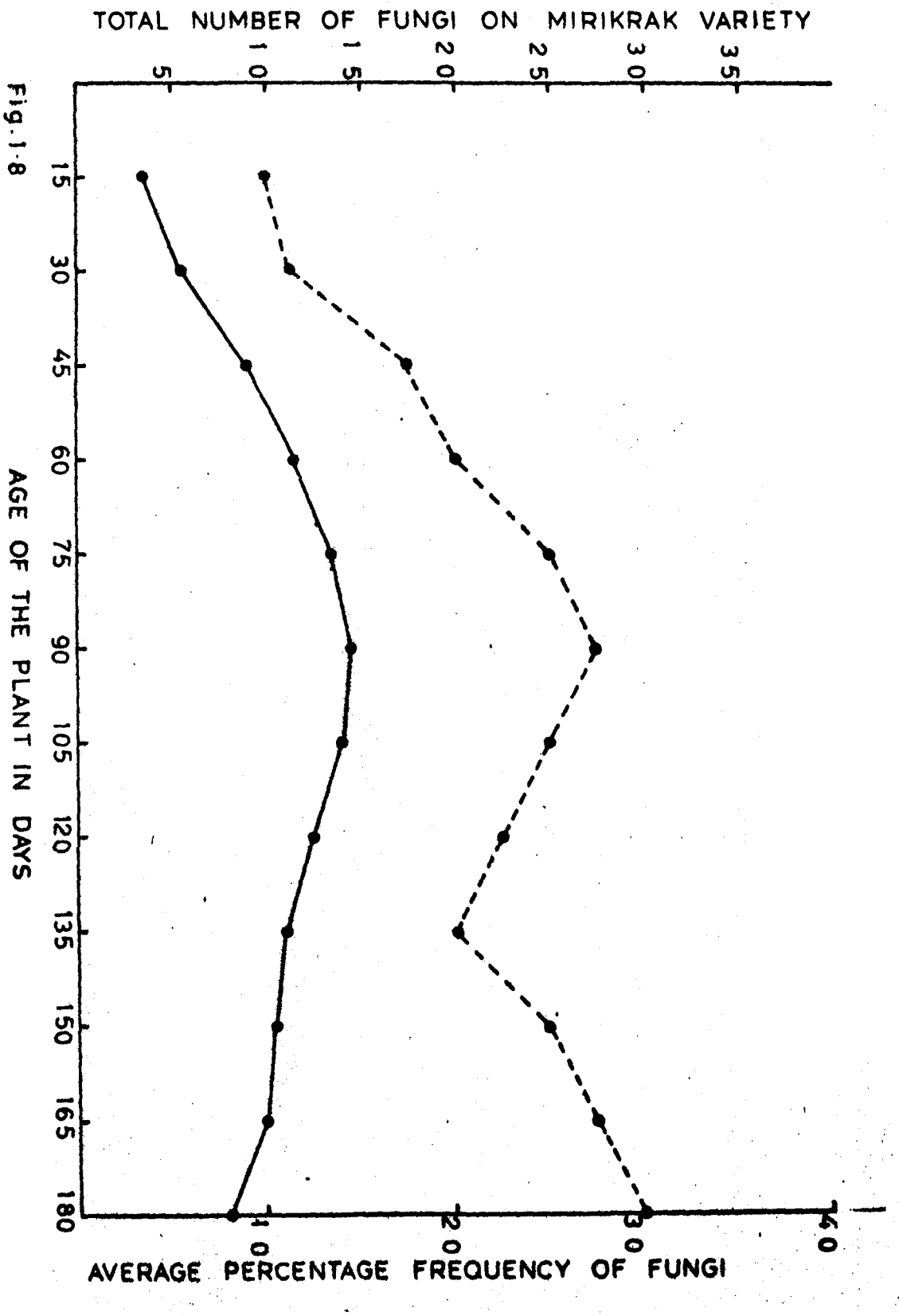

TOTAL NUMBER OF FUNGI ON MIRIKRAK VARIETY
5
10
15
20
25
30
35
AGE OF THE PLANT IN DAYS
15
30
45
60
75
90
105
120
135
150
165
180
AVERAGE PERCENTAGE FREQUENCY OF FUNGI
10
20
30
40

Fig. 1.8

pencilloides, Humicola prisea, Nigrospora oryzae, Pestalotia monorhynca and Stachybotrys atra occurred infrequently.

By plating washed leaves some idea about the actual activity of fungi which had penetrated could be obtained. From Table 1.5 it is clear that fungi like Alternaria solani, Arthrinium sp., Cladosporium herbarum, Fusarium moniliforme, Helminthosporium oryzae, Trichoderma viride and Verticillium albo-atrum were the common colonizers in addition to fungi like Aspergilli and Penicillia which occurred mostly as contaminants. Moreover, some fungi like Candida albicans, Chrysosporium pruinosum, Gliocladium pencilloides, Monilia sitophylla, Nigrospora oryzae and Trichothecium roseum also colonized the washed leaves infrequently. Ascomycetes like Chaetomium bostrychodes, Coniothyrium indicum, Melanospora zamae, Sordaria macrospora and some Hyphomycetes like Didymostilbe ellisii, Pestalotia monorhynca and Starkeomyces koorchalomoides were also isolated by plating washed leaves on cellulose agar medium.

From moist chamber technique it is quite clear that on young leaves Aspergilli and Mucorales and very few Hyphomycetes colonized and on mature leaves dematiaceous Hyphomycetes and Ascomycetes were more dominant and later on as the leaves started senescing very few Ascomycetes like Chaetomium spp., and more Hyphomycetes like Alternaria alternata, Aspergilli, Cladosporium cladosporoides, Curvularia lunata, Fusarium moniliforme, Memnoniella echinata, Penicillium spp., Stachybotrys atra, Starkeomyces koorchalomoides (=Myrothecium verrucaria), Torula herbarum, Trichoderma viride and Verticillium albo-atrum were the common colonizers with high percentage frequency of occurrence.

Further, as depicted in Fig. 1.10 on the basis of moist chamber, impression plate and washed leaves plating techniques the primary saprophytes comprised mainly of Alternaria alternata, Cladosporium cladosporoides, Aureobasidium pullulans and Chaetomium bostrychodes which showed a high percentage frequency of occurrence even at the senescent stage excepting A. pullulans. Other fungi like Epicoccum nigrum, Phoma spp., Macrophomina phaseolina, Dreschlera sp., and Arthrinium sp. also occurred frequently on leaf surfaces of different ages. Some fungi such as Mucorales, Ascomycetes like Melanospora zamae, Cochliobolus miyabeanus, Colletotrichum capsicum, Coniothyrium indicum (Plate 1.4; 2), Gelasinospora tetraspora, Sordaria spp., Triangularia obliqua and Hyphomycetes like Acremonium indicum, Aspergilli, Humicola prisea and Trichothecium roseum showed very low percentage frequency of occurrence at different growth stages of the plant.

Some sterile mycelia were also isolated by the five different techniques and some of them like the white mycelia and orangish coloured mycelia occurred almost at all the growth stages of the plant but they did not show any sporulation even when cultured on different media.

Quantitative assessment

Quantitative estimations as done through dilution plate technique accounts for the fungal propagules present on the leaf surface only and ignores the actual activity of fungi which had penetrated the leaves.

Fig. 1.9: Total fungal population x 10^3 gm fresh weight of leaves of three varieties of paddy viz., Khonorullo, Ngoba and Mirikrak with increasing plant age.

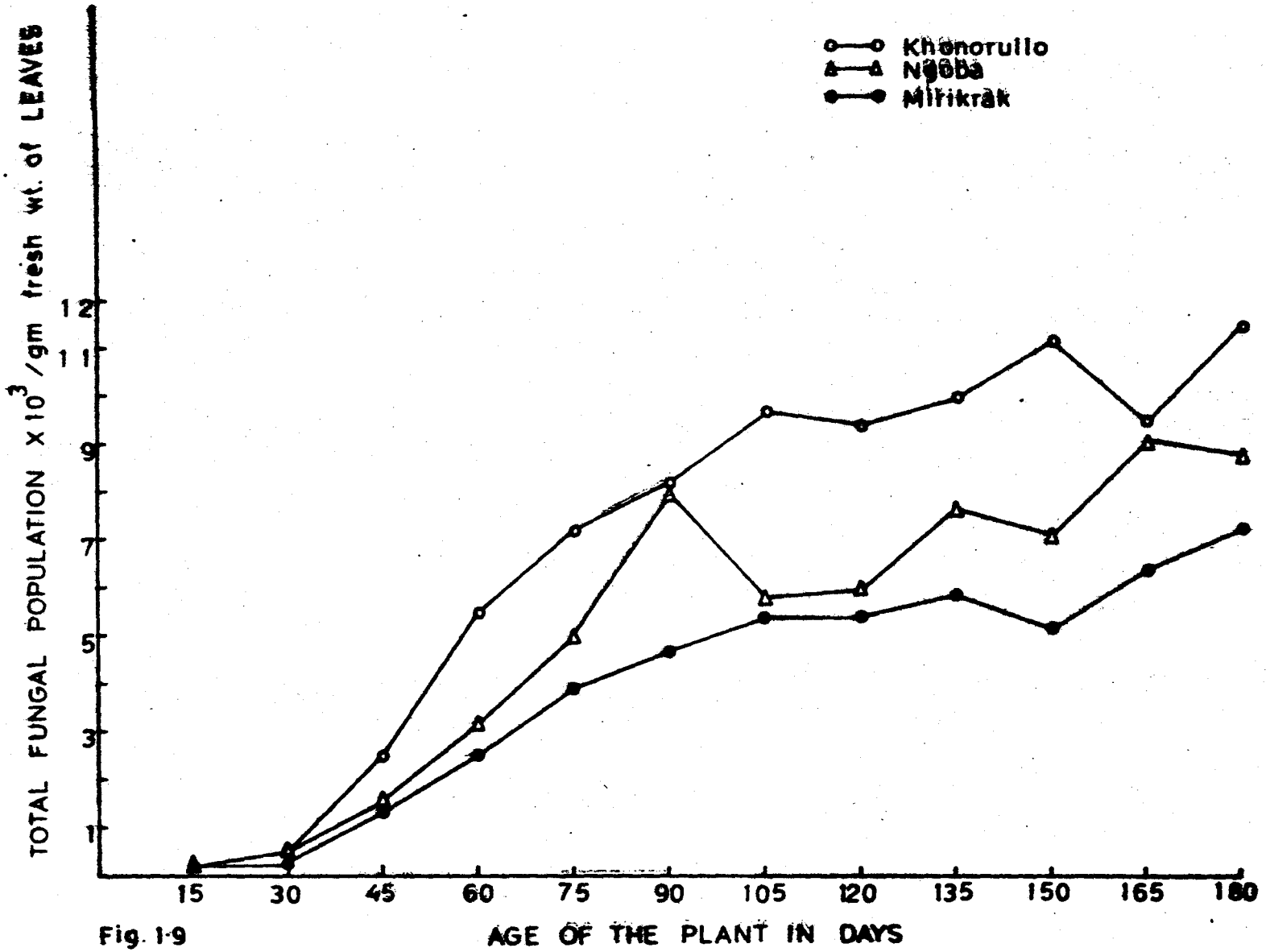

Khonorullo
Ngoba
Mirikrak
TOTAL FUNGAL POPULATION X 10³/gm fresh wt. of LEAVES
12
11
9
7
5
3
1
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1·9

It is clear from Table 1.7 and Fig. 1.9 that total fungal population expressed as number of fungal colonies x 10^3 per gm fresh weight of leaves increased with plant age throughout all the three varieties of paddy studied.

Table 1.7 gives the complete sequential change in fungal populations during different stages of plant growth as observed by dilution plate technique. From this table it is clear that the dominant fungi were Alternaria solani, Arthrinium sp., Aspergillus nidulans, Aureobasidium pullulans, Cladosporium herbarum, Curvularia lunata, Fusarium moniliforme, Helminthosporium oryzae (only in Mirikrak variety), Penicillium funiculosum, Trichoderma viride,(only in Khonorullo variety), Verticillium albo-atrum. It is depicted in Figs. 1.11-1.15 that fungi like Penicillium funiculosum, Cladosporium herbarum, Fusarium moniliforme, Arthrinium sp., Trichoderma viride (in Khonorullo) and Helminthosporium oryzae (only in Mirikrak) dominated the entire phylloplane habitat of the three communities formed by the three varieties of paddy at different growth stages of the plant.

Further, Table 1.8 presents statistical significance of the paired mean comparisons of microfloral counts present at fortnightly intervals of plant growth as estimated by the student 't' test. A significant incfease of mycoflora was observed in 30 days old plants only in Khonorullo and Ngoba varieties, while a similar increase was observed at 45 days and

Fig. 1.10: Percentage frequency of occurrence of primary saprophytes viz., Alternaria alternata, Cladosporium cladosporoides, Aureobasidium pullulans and Chaetomium bostrychodes with increasing plant age.

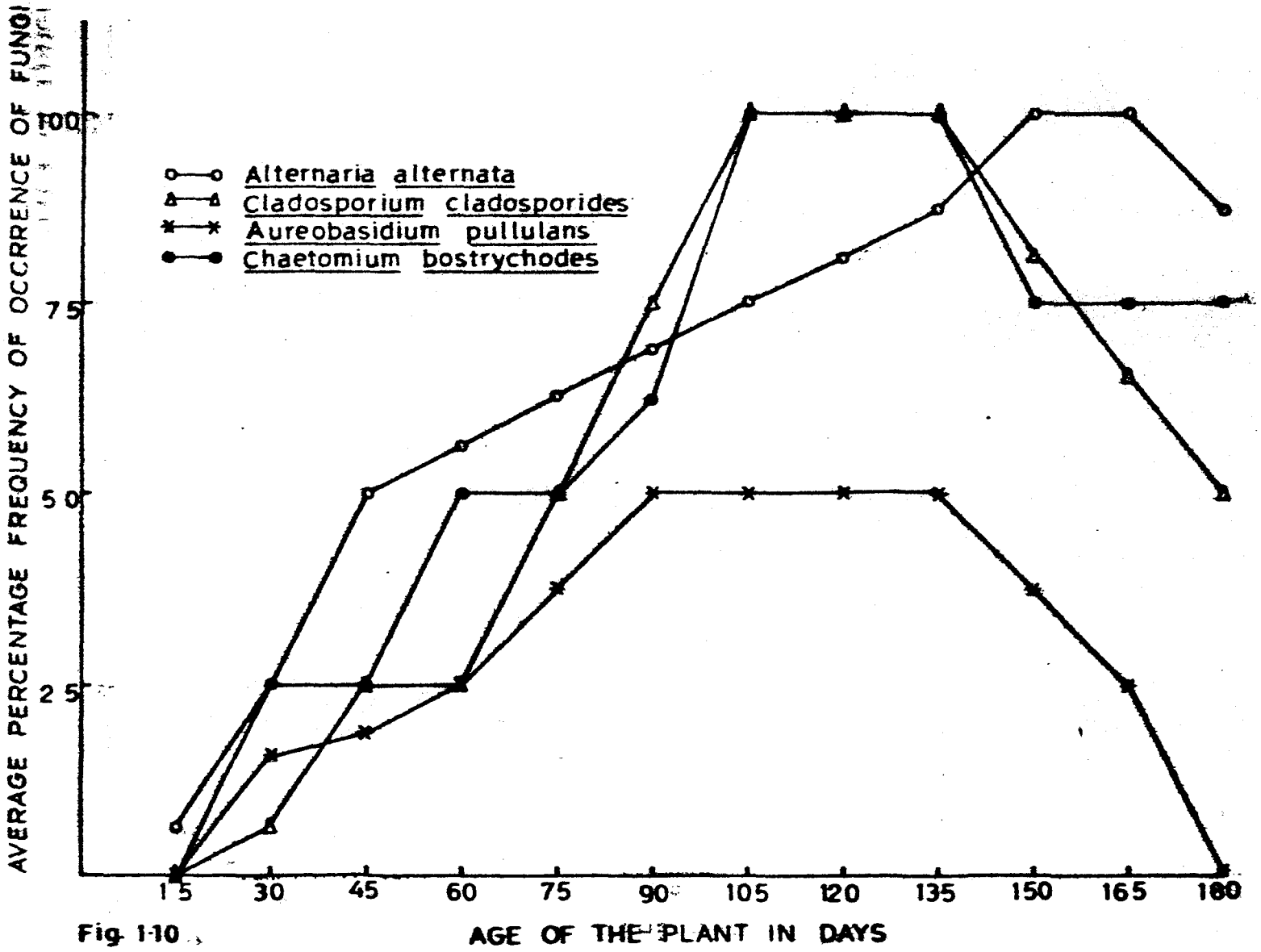

AVERAGE PERCENTAGE FREQUENCY OF OCCRRENCE OF FUNGI
100
75
50
25
Alternaria alternata
Cladosporium cladosporides
Aureobasidium pullulans
Chaetomium bostrychodes
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1-10

Fig. 1.11: Penicillium funiculosum population (x 10^3/gm fresh wt.) occurring on the leaf surface of the three varieties of paddy viz., Khonoru Ho Ngoba and Mirikrak at different ages of the plant.

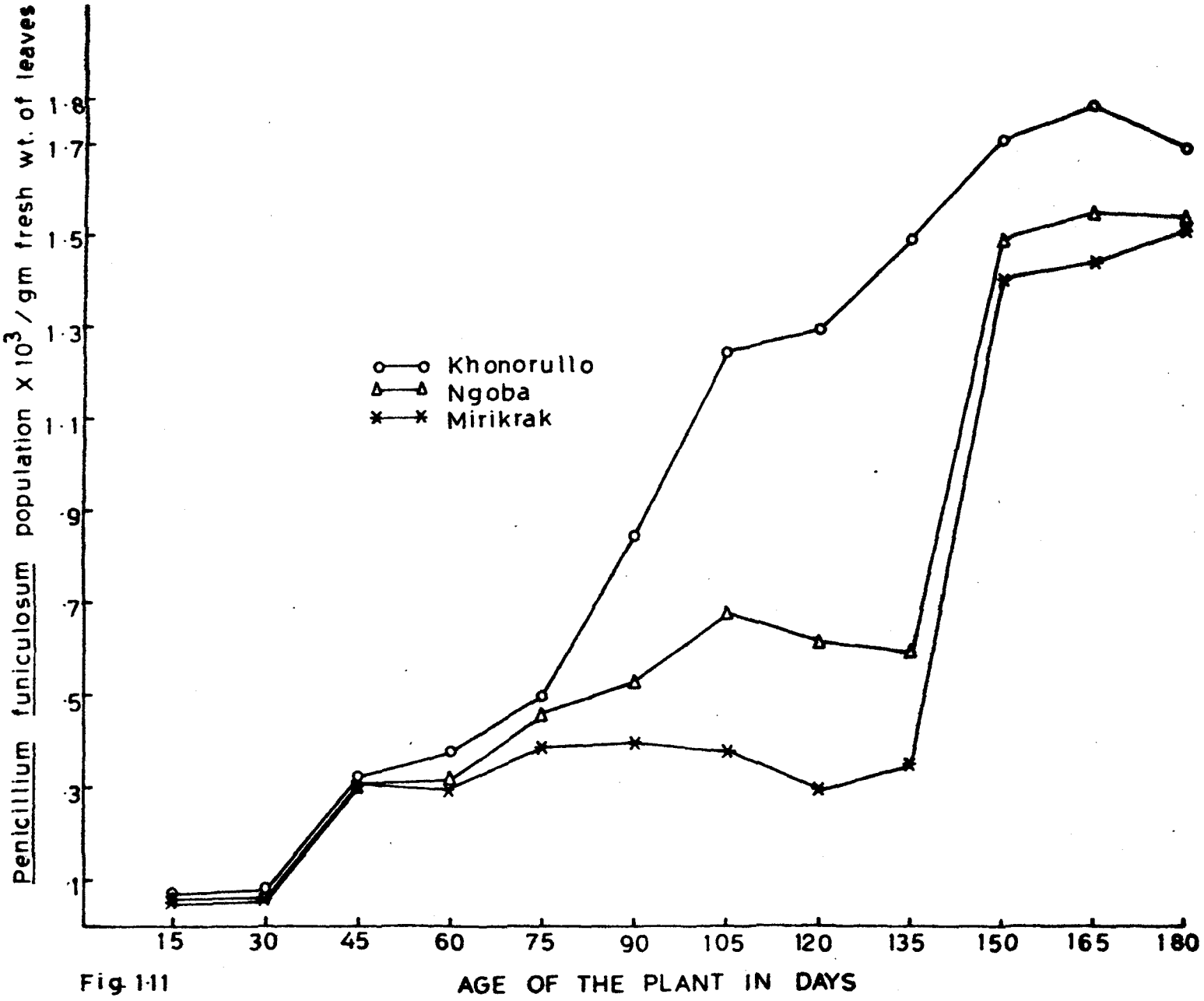

Penicillium funiculosum population X 10³ / gm fresh wt. of leaves
1·8
1·7
1·5
1·3
1·1
·9
·7
·5
·3
·1
Khonorullo
Ngoba
Mirikrak
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig 1.11

Fig. 1.12: *Cladosporium herbarum* population (x 10^3/gm fresh wt.) occurring on the leaf surface of the three varieties of paddy viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

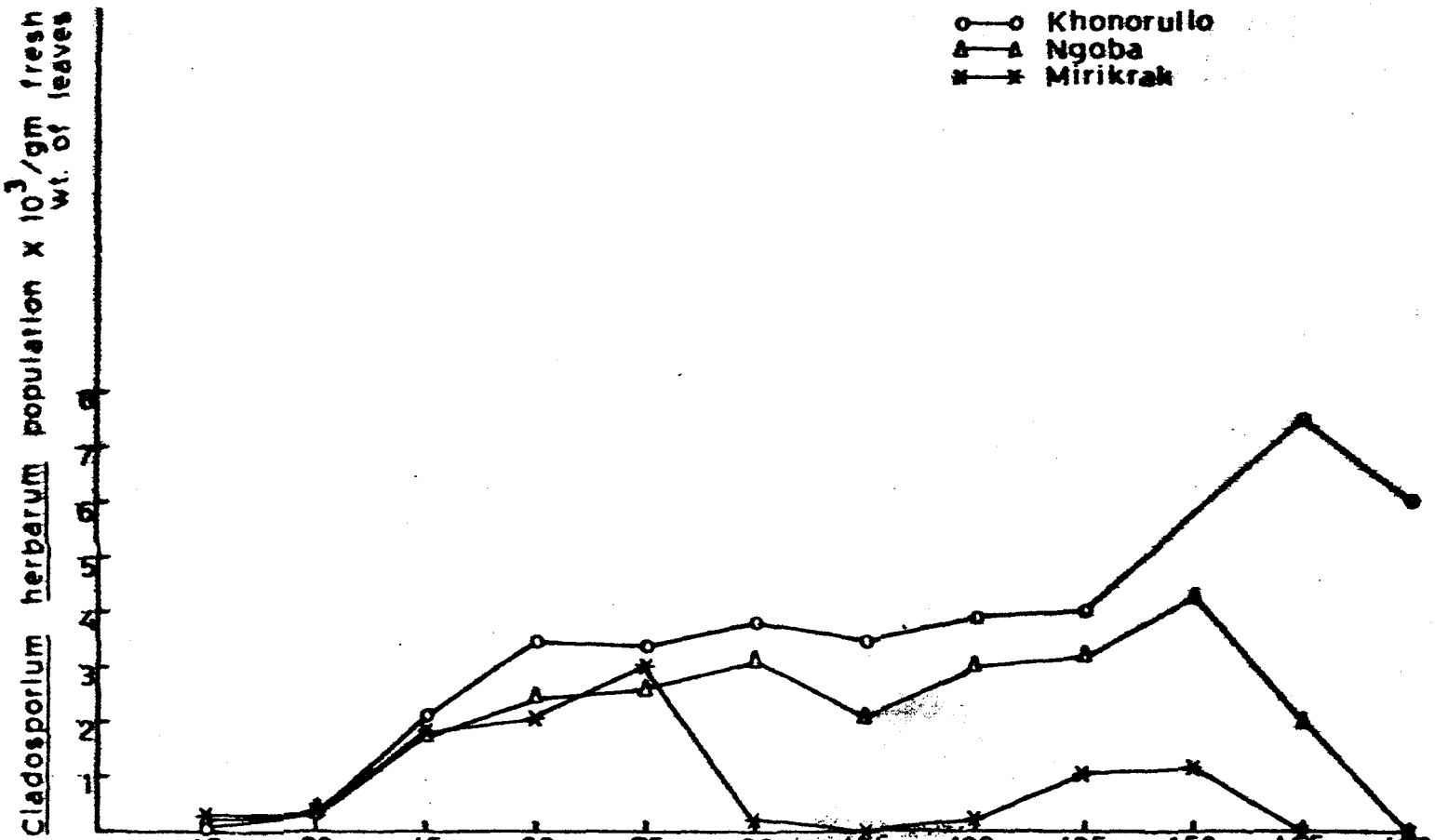

o—o Khonorullo
Δ—Δ Ngoba
— Mirikrak
Cladosporium herbarum population x 10^3/gm fresh wt. of leaves
1
2
3
4
5
6
7
8
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1-12

Fig. 1.13: *Fusarium moniliforme* population (x 10^3/gm fresh wt.) occurring on the leaf surface of the three varieties of paddy viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

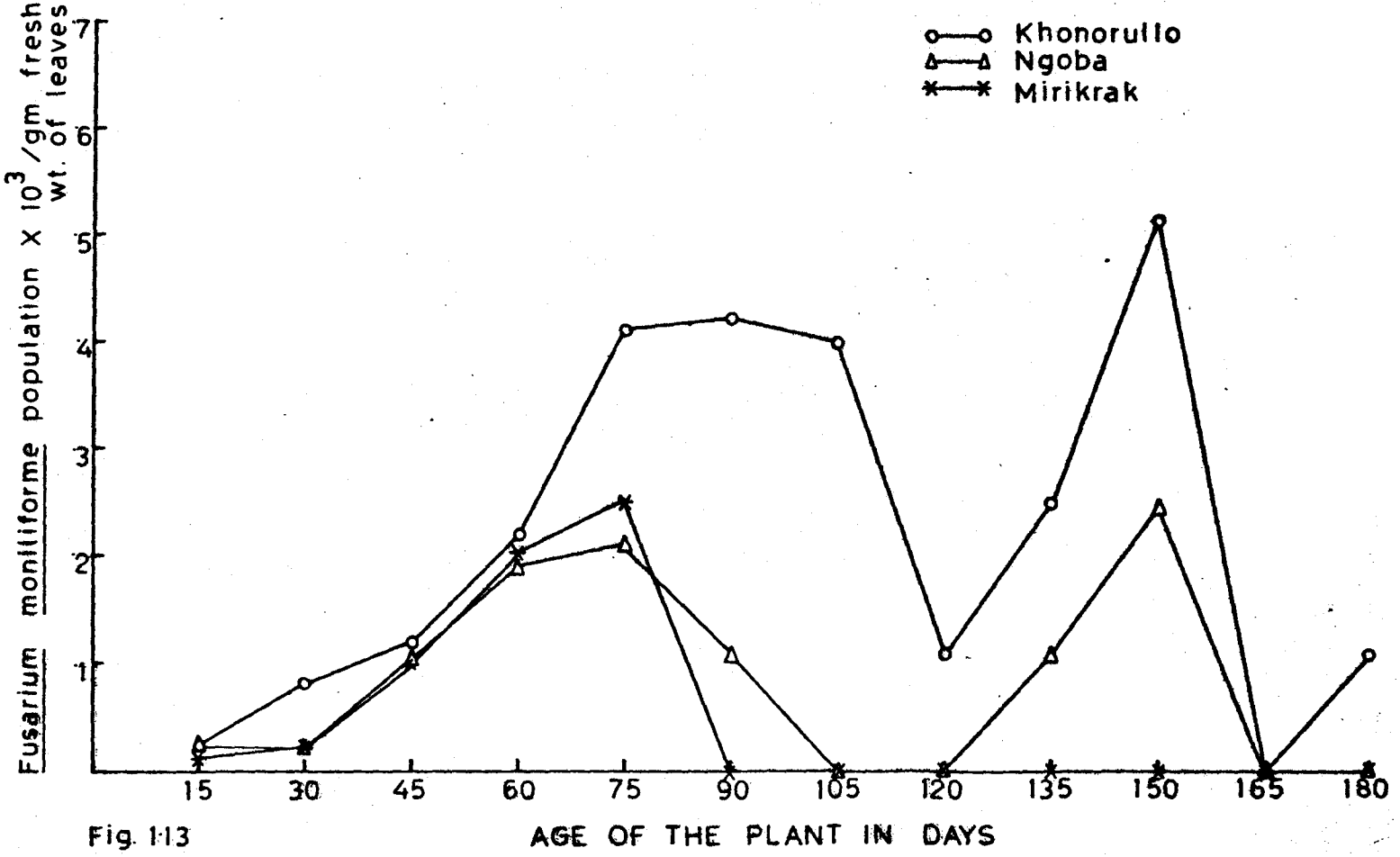

Khonorullo
Ngoba
Mirikrak
Fusarium moniliforme population X 10^3 /gm fresh wt. of leaves
1
2
3
4
5
6
7
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1:13

Fig. 1.14: Arthrinium sp. population (x 10^3/gm fresh wt.) occurring on the leaf surface of the three varieties of paddy, viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

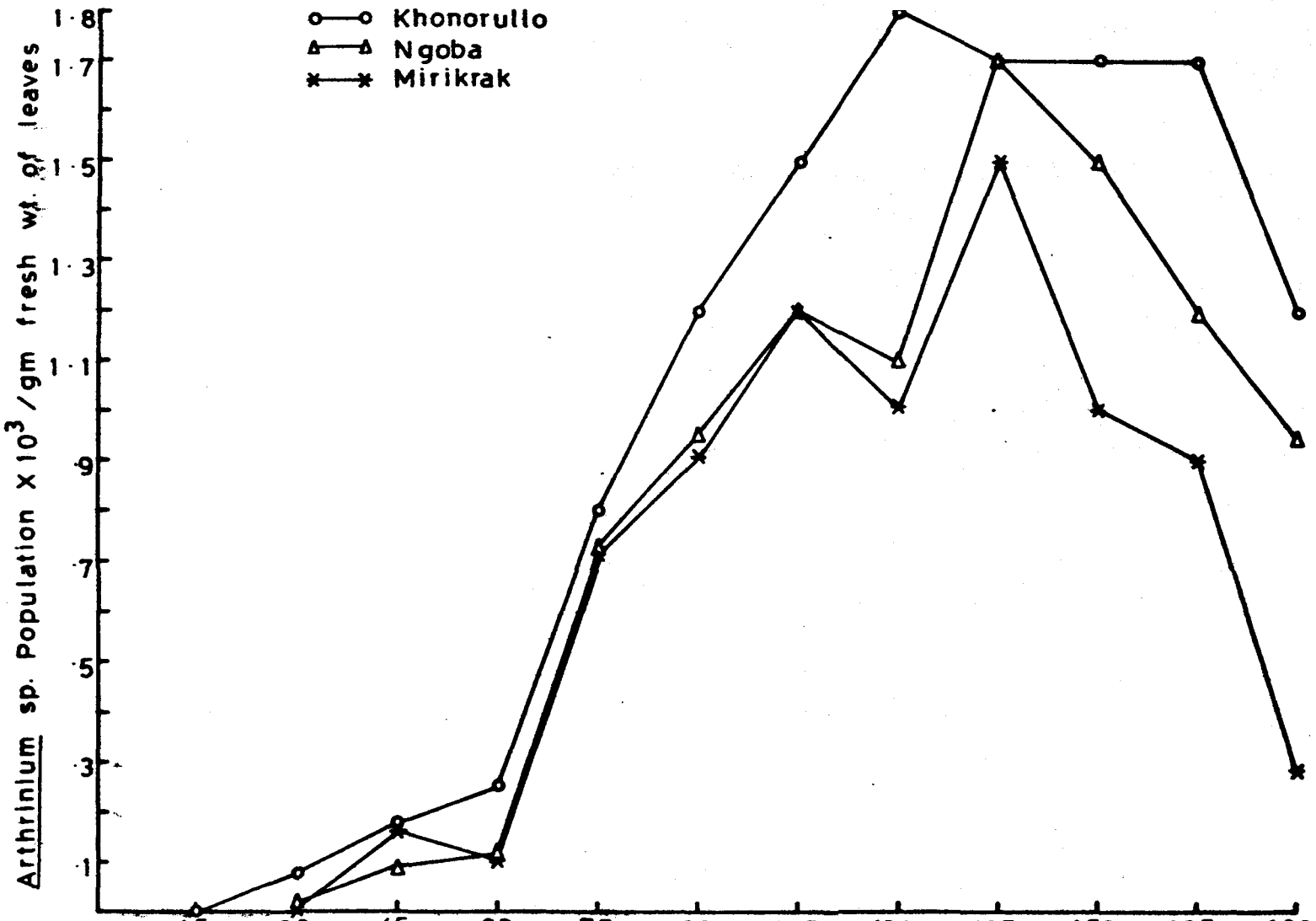

Khonorullo
Ngoba
Mirikrak
Arthrinium sp. Population X 10³/gm fresh wt. of leaves
1·8
1·7
1·5
1·3
1·1
·9
·7
·5
·3
·1
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 114.

75 days irrespective of the variety. Another significant increase was also observed at 165 and 180 days in all the varieties studied. Such a change was insignificant in all the varieties at 90 days.

Moreover, a significant change in fungal population was observed at three main growth stages of the plant viz., young stage (15 days), mature stage (90 days) and senescent stage ((180 days) as given in Table 1.9 independent of the plant variety.

Tables 1.10 and 1.11 give details of analysis of variance by using the 'F' test. The 'F' test (one way classification) was used to find out the variation in mycopopulation among three varieties at different growth stages. A significant variation in fungal population amcng the varieties was found at maturity while it was insignificant at the young and senescent stages (Table 1.10). The 'F' test (two way classification) was also done to find out the extent of the influence of two variables i.e., host variety and growth stages of the plant in changing mycofloral counts. A highly significant variation in fungal population was found with growth stage of the plant while it was insignificant in relation to varieties.

Classification of mycoflora based on their occurrence in different months

The fungi isolated by all the five techniques of isolation from the leaf surface of the three varieties were categorised into three groups based on their occurrence in different months during the growth stages of the plant.

Group I : Fungi found uniformly in all the months.

Group II: Fungi occurred sporadically and not restricted to one season.

Group III: Fungi restricted to one particular season.

Group I : To this group belong fungi like Alternaria spp., Penicillium funiculosum, Aspergillus flavus, A. niger, Cladosporium herbarum, Fusarium moniliforme, Arthrinium sp. (Plate 1.3; 5), Chaetomium globosum and Trichoderma viride.

Group II: To this group belong most of the fungi like Mucorales, Ascomycetes excepting Chaetomium globosum (Table 1.6), Hyphomycetes like Aspergillus nidulans, Aureobasidium pullulans, Botrytis cinerea, Chrysosporium pruinosum, Curvularia lunata, Gliocladium penicilloides, Memnoniella echinata, Nigrospora oryzae, Penicillium chrysogenum, Trichothecium roseum, and Verticillium albo-atrum.

Group III: To this group belong fungi which occur rarely such as Papulospora sp., which occurred at the age of 105 days only on Ngoba variety, Candida albicans only in June and July months in both the crop seasons (1981 and 1982) in the present studies (Table 1.7). Helminthosporium oryzae also occurred from June

and July months till senescence of the plants by various methods of isolation when the temperature was high and there was high atmospheric humidity due to high rainfall and it occurred only in case of Ngoba and Mirikrak varieties. Cladosporium cladosporoides also occurred from July till October and was more predominant at the later stages of plant growth. Macrophomina phaseolina appeared from July-September, Didymostilbe ellisii which is a rare fungus occurring on leaf surface, was isolated on cellulose agar medium by washed leaves plating only once in 1981 in the month of September (Table 1.5). Similarly, Triangularia obliqua, a very interesting ascomycete (Plate 1.3; 1,2) was isolated only once in 1982 in the month of July by moist chamber technique (Table 1.6). Cochliobolus miyabeanus was also observed only in 1982 on moist chamber plates from August-October months (Plate 1.3; 3.4). Pyrenochaeta decipiens (Plate 1.4; 1) and Colletotrichum capsicum also occured only once in August 1982 and July and August 1982 respectively only by moist chamber technique. Some species of Chaetomium viz., C. funiculosum, C. fusiforme, C. succineum occurred rarely in months of July-August (Table 1.6). Some species of Aspergillus such as A. clavatus, A. candidus, A. fumigatus, A. ochraceous, A. sydowi, A. terreus, A. versicolor were also rare and isolated only by dilution plate technique (Table 1.7) in summer months. Penicillium glaucum was also observed only once in August 1981 and Stemphyllium botryosum appeared once in July 1981 only.

Fig. 1.15: Comparison of the occurrence of two fungi viz., Trichoderma viride and Helminthosporium oryzae in terms of their population ($x\ 10^3$/gm fresh wt. of leaves) on the leaf surface of the three varieties of paddy viz., Khonorullo, Ngoba and Mirikrak.

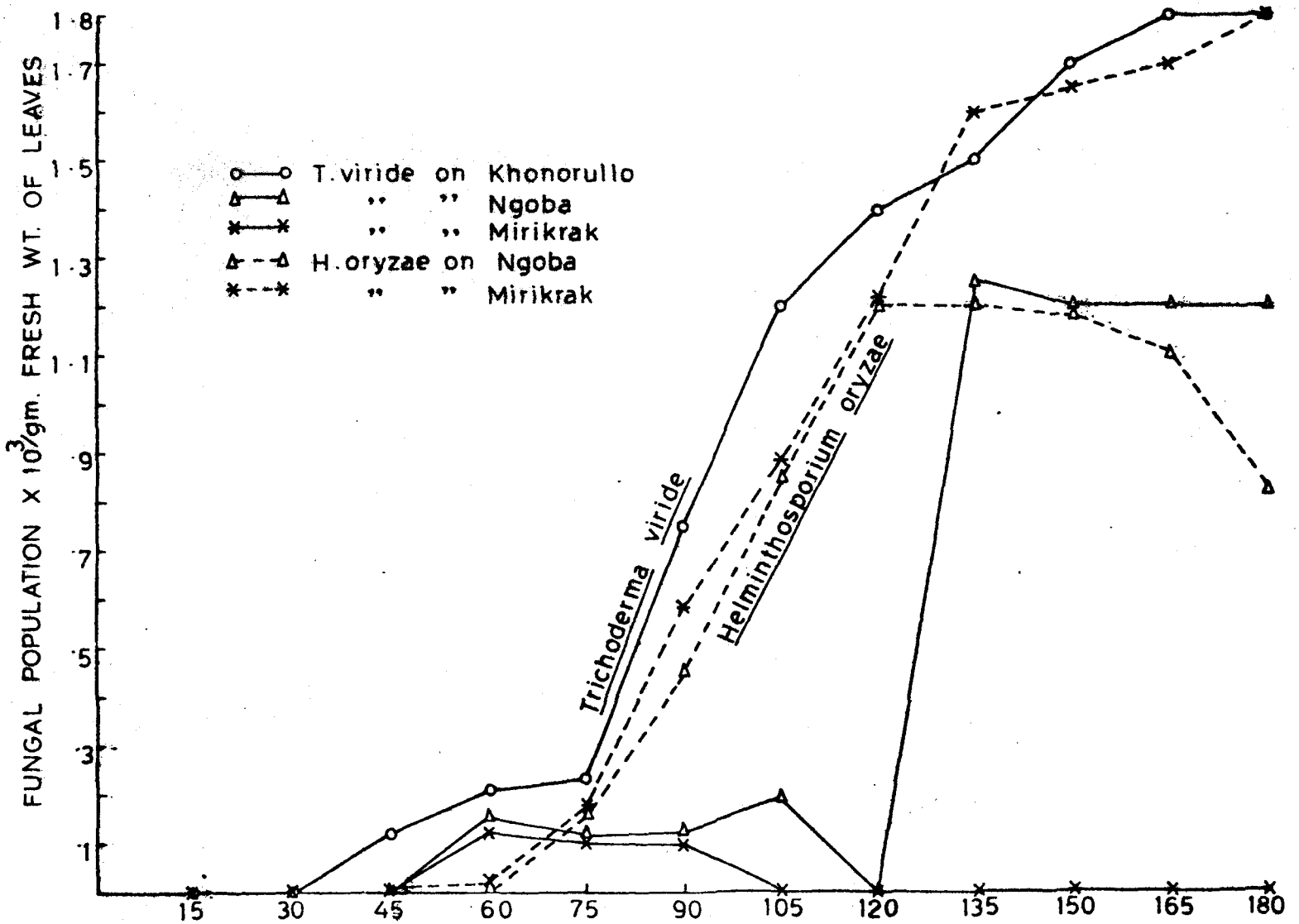

FUNGAL POPULATION X 10^3/gm. FRESH WT. OF LEAVES
1·8
1·7
1·5
1·3
1·1
·9
·7
·5
·3
·1
T.viride on Khonorullo
" " Ngoba
" " Mirikrak
H.oryzae on Ngoba
" " Mirikrak
Trichoderma viride
Helminthosporium oryzae
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1·15

Fig. 1.16: Intercommunity similarities in species composition of three varieties of paddy viz., K-Khonorullo, N-Ngoba and M.Mirikrak with increasing plant age.

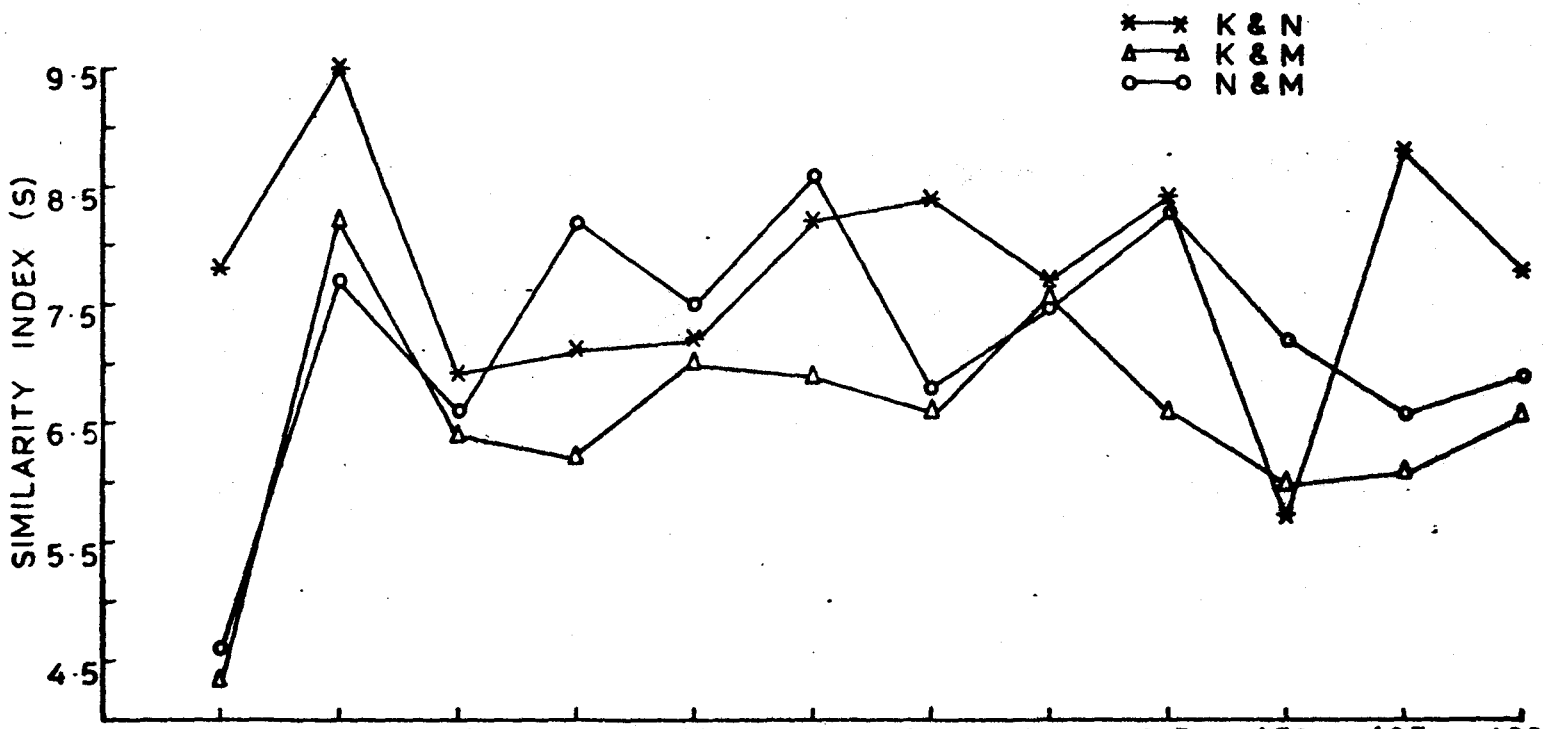

K & N
K & M
N & M
SIMILARITY INDEX (S)
9·5
8·5
7·5
6·5
5·5
4·5
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1·16

Fig. 1.17: Comparison of two factors viz., pH and moisture content (in percentage) of leaves of the three paddy varieties viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

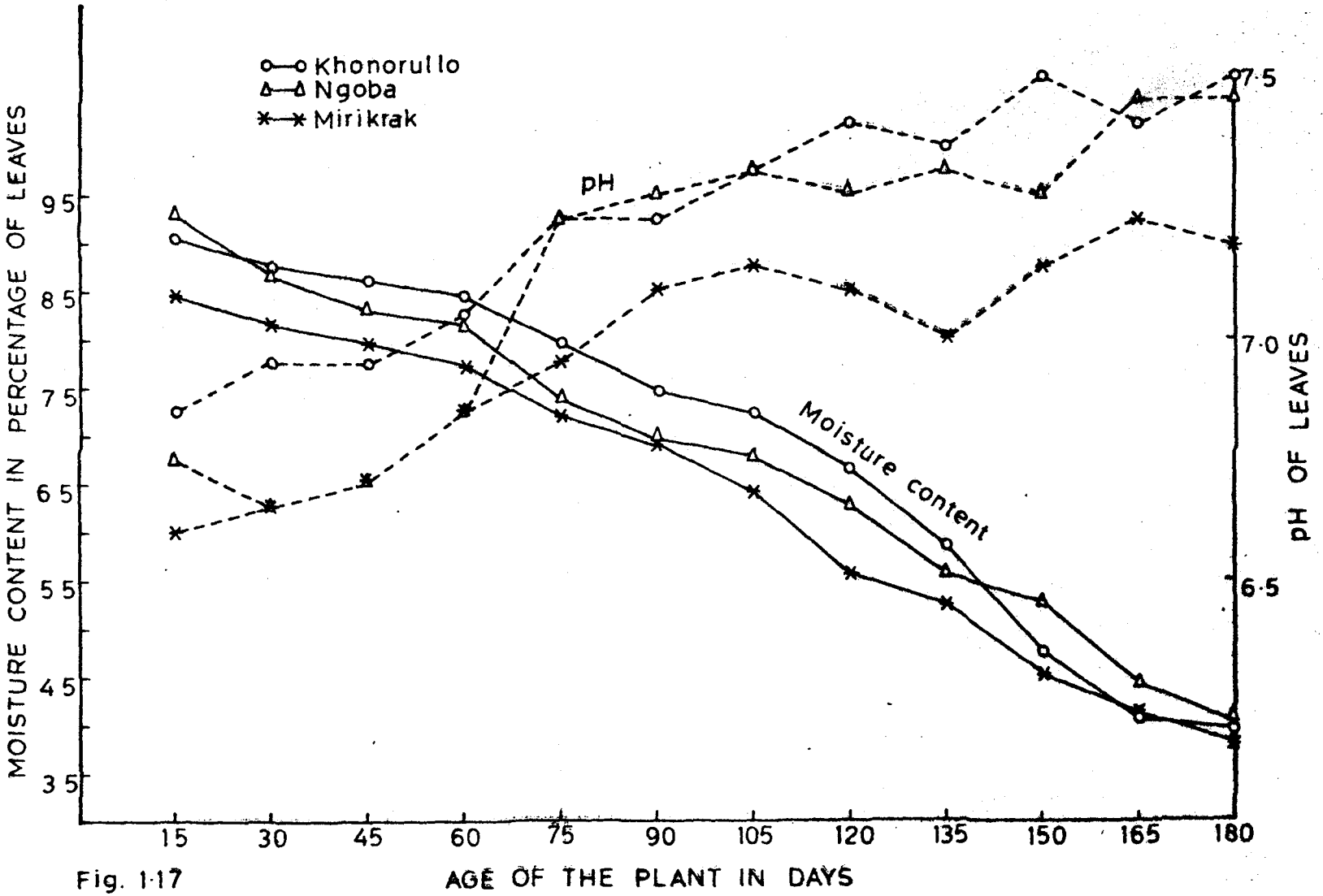

Khonorullo
Ngoba
Mirikrak
pH
Moisture content
MOISTURE CONTENT IN PERCENTAGE OF LEAVES
pH OF LEAVES
95
85
75
65
55
45
35
7·5
7·0
6·5
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 1·17

Neurospora sp. also occurred only once in the present investigation in June 1981 (Table 1.4).

Correlation between pH, moisture content of leaves and fungal population

As seen from Table 1.12 by using pearson product moment correlation, physical factors of leaf namely pH and moisture content, shows positive and negative correlation to the fungal populations at various growth stages of the plant for all the three varieties (Significant at P = 0.01).

The change in pH and moisture content of leaves with plant age is also depicted in Fig. 1.17 which shows that moisture content of leaves decreases with age whereas pH increases in all the three varieties of paddy. This shows that moisture content of leaves apparently does not have much effect on the fungal population.

Interfungal community relationships between three types of paddy varieties

The variations in species diversity during the different growth stages of the plant and the degree of similarity between the three types of plant varieties is illustrated in Fig. 1.16.

From Fig. 1.16 it is clear that there was a distinct change in the similarity index with plant age in all the three

pairs of fungal communities. At 30 days of plant age an increase in similarity was observed among all the communities and then there was a decline in similarity at 45 days of plant age. Later on, from 45-150 days of plant age there was not much difference between the three communities. At 150 days of plant age, a sudden decline in similarity was observed between Khonorullo vs. Ngoba varieties but after 150 days of plant age a distinct increase in similarity was observed.

Moreover, from the Fig. 1.16 it is also clear that fungal communities formed by Khonorullo and Ngoba varieties have the highest similarity index till 45 days of plant age and later on it decreased till 75 days.

The similarity index remained more or less low throughout the growth stages of the plant between Khonorullo and Mirikrak varieties. Whereas, between Ngoba and Mirikrak although initially it had a low similarity index but it became quite high from 45-90 days and later on at the age of 105 days there was a sudden decline but again it increased from 120-150 days. Moreover, at 180 days of plant age an increase in similarity was observed among all the communities excepting the communities between Khonorullo vs Ngoba.

Further, from Tables 1.3-1.7 it is clear that in general, the highest fungal population (Both in total number of species and total fungal colonies isolated) was observed on

Khonorullo variety (disease resistant) followed by Ngoba (moderately susceptible) and was found to be least on the leaves of the susceptible variety, Mirikrak on which the leaf spot disease was quite common.

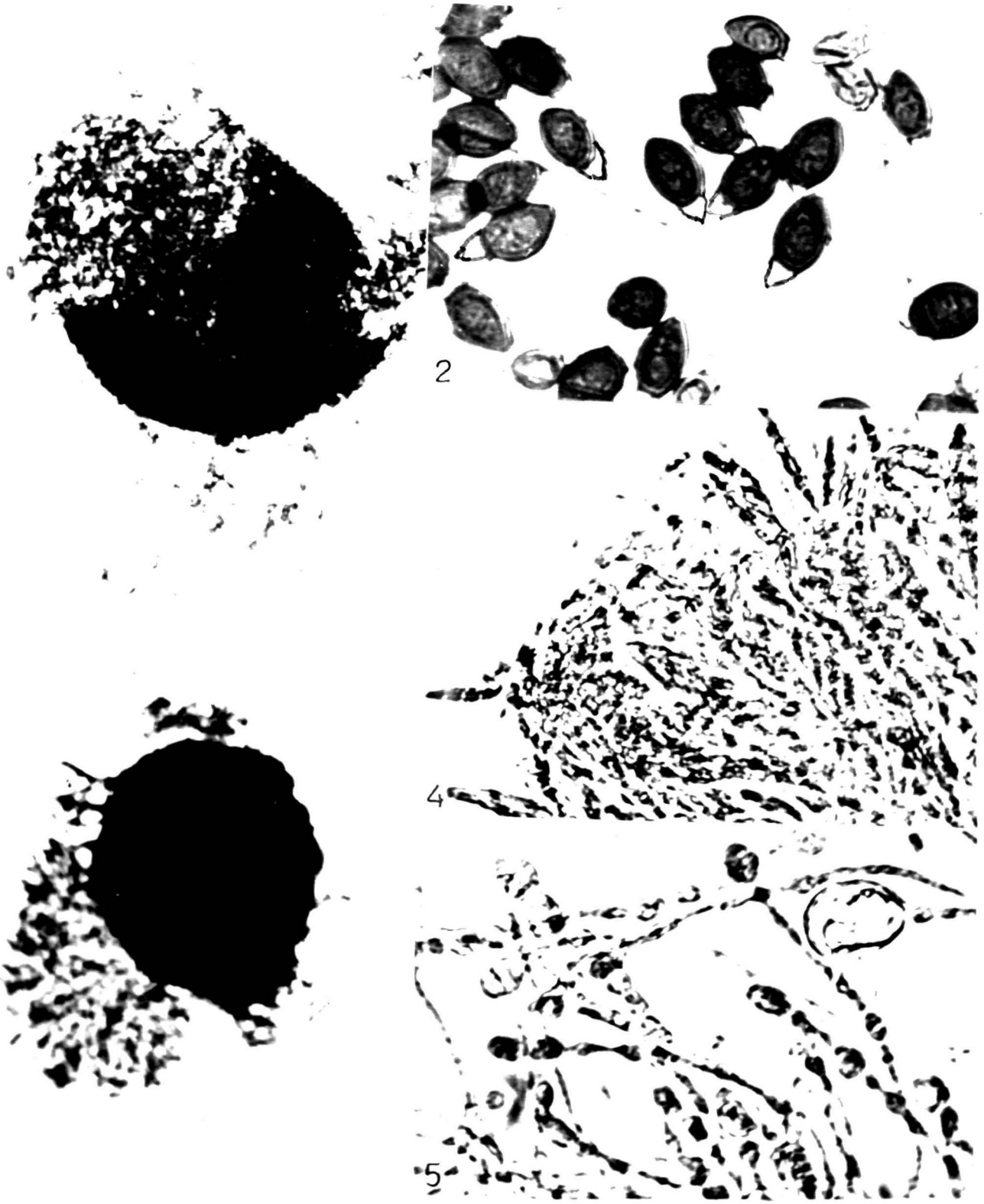
Moreover, it is quite clear from different tables 1.3-1.7 which gives the mycoflora isolated by the different techniques that Helminthosporium oryzae occurred from June to October in susceptible variety (Mirikrak) and also sometimes in moderately susceptible variety (Ngoba). Trichoderma viride population was higher on Khonorullo variety as compared to Ngoba and Mirikrak and from Fig. 1.15 it is quite clear that T. viride population showed sudden increase at the age of 75 days old plant only on Khonorullo variety whereas, in Mirikrak variety the population exhibited a sudden decline and was absent after 105 days when H. oryzae, the pathogen becomes dominant and the disease becomes quite conspicuous. Whereas, in Ngoba variety although at the age of 105 days the population of T. viride goes down and becomes zero but at 135 days it shows a sharp increase and remains on the phylloplane till senescence.

In addition to differences in H. oryzae and T. viride population, other fungi like Cladosporium herbarum, Penicillium funiculosum, Arthrinium sp. Fusarium moniliforme, Nigrospora oryzae, Trichothecium roseum, Verticillium albo-atrum and Aspergillus flavus also showed higher population

PLATE 1.3

1. Cleistothecium of *Triangularia obliqua* x 100
2. Ascospores of *Triangularia obliqua* x 400
3. Cleistothecium of *Cochliobolus miyabeanus* x 100
4. Asci of *Cochliobolus miyabeanus* x 400
5. Conidiophores of *Arthrinium* sp. x 400

PLATE-1·3



2

3

4

5

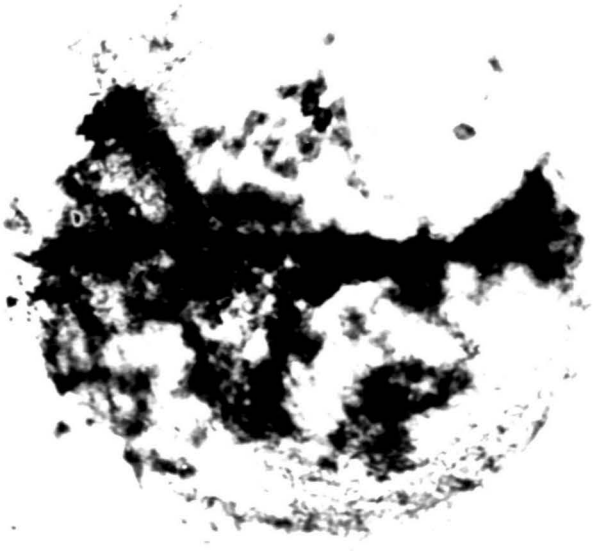
PLATE 1.4

1. Cleistothecium of *Pyrenochaeta decipiens* x 100
2. Cleistothecia of *Coniothyrium indicum* x 100
3. Perithecium of *Melanospora zamae* x 100
4. Perithecium enlarged showing ascospores x 400

PLATE - 1·4

3

2



4

in phylloplane of Khonorullo than on Ngoba and Mirikrak. The perfect stage of H. oryzae, Cochliobolus miyabeanus was also isolated only from Mirikrak variety, the disease susceptible variety from 120 days onwards only by moist chamber technique.

DISCUSSION

Methodology:

The results obtained from different techniques clearly suggest that the study of the leaf surface population needs a combination of various methods for assessing the complete picture of the phylloplane fungal flora. This is in accordance with the view of previous workers (Dickinson, 1971; Sharma et al., 1974) that several techniques of isolation should be used simultaneously to have maximum information about the qualitative and quantitative composition of the communities of the phylloplane fungi, as isolation of fungi largely depends upon the methodology used.

Direct observation techniques (Edward and Hartman, 1952; Masurovsky and Jordan, 1960) were not found to be very useful since very few species were isolated by this method as given in Table 1.3 and also it is quite time consuming. Although this method has been recognised to be useful in giving clues to the activity of fungal flora (Hering, 1965; Hogg and Hudson, 1966; Yadav, 1966; Ruscoe, 1971) the practical difficulty, however,

encountered while dealing with this method was the classification of the active mycelium and various spores observed on the impressions made this technique not much useful in the present studies.

Moist chamber technique (Table 1.6 and Fig. 1.2) was found to be the most suitable for isolation of maximum number of both saprophytic and parasitic fungi present on leaves which may be responsible for future decomposition of senescing leaves. This technique is also reported to be the best for isolation of decomposers (Sharma et al., 1974) from living and senescent leaves before leaf fall.

Cultural isolation techniques gives information about highly sporulating fungi and those which are exclusively surface colonizers. In such cases the nutrient medium used plays an important role in isolation of fungal species (Tsao, 1970). Earlier workers have used media rich in easily assimilable carbohydrates viz., Czapek's Dox agar, potato dextrose agar and oatmeal agar (Saito, 1956; Hogg and Hudson, 1966; Mehrotra and Aneja, 1979; Singh and Rai, 1980). Such media make the technique inefficient in isolating slow growing fungi which is evident from absence of Ascomycetes and prevalence of fast growing Mucorales in records of these workers. Eicker (1976) has also reported that dilution plate technique was less effective and yielded only nine species due to overgrowth of fast growing fungi e.g., Mucor sp.

However, realising this limitation, during the present studies in addition to Czapek's Dox agar, cellulose agar medium (Eggins and Pugh, 1962) was also used which was found to be the best for isolating slow growing Ascomycetes which could not compete with fast growing fungi on Czapek's Dox agar medium. This is evident from the observation that except one isolation of Melanospora zamae no other Ascomycete could be isolated on Czapek's Dox agar when species of Chaetomium and Sordaria were isolated on cellulose agar medium. Cellulose agar not only restricted the growth of fast growing Zygomycetes and Hyphomycetes but also shortened the fruiting time of Ascomycetes.

In general, loosely attached forms with heavily sporulating capacity dominate the nutrient plates (Tables 1.4-1.7). As far as dilution plate and impression plate techniques are concerned, both fail to distinguish a dormant spore from an active mycelium (Dickinson, 1971).

After washing the leaf segments, most of the forms adhering to the leaf surface are detached although few species like Penicillium spp. and Aspergillus spp. occur as contaminants and only few forms which are closely attached to the leaf surface or inside the tissues are isolated by plating washed leaves on nutrient plates. Those fungi which are active in decompostion were obtained through this method and moreover, this technique facilitates isolation of slow growing mycelia present on leaves (Rai, 1976). This technique with dilution

plate method helps in distinguishing surface inhabitants from internal colonizers.

The information obtained from all these methods although gives some picture about the colonization of different fungi but they may entice misleading speculations about successional patterns. As till today, there is no single method which can selectively remove the mycelium directly from the leaf surface for culture purpose, but the information gained from all the different techniques used simultaneously was taken as a measure of fungal activity *in vivo*.

Colonization of fungi on leaf surface

Majority of the forms isolated from the leaf surface of the three varieties of paddy belonged to Deuteromycetes which accounted for approximately 80 percent of the total mycoflora. This is also in accord with the results of other studies (Tewari 1973).

Qualitative assessment

The percentage occurrence of Ascomycetes was higher than Phycomycetes and mycelia sterilia. This is because of use of cellulose agar medium in addition to Czapek's Dox agar medium in various techniques of isolation.

Aspergilli, Alternaria spp., Arthrinium sp., Cladosporium spp. and Penicillium spp. contributed to nearly 75 percent of the total fungal population. Alternaria alternata, A. solani, Arthrinium sp., Cladosporium herbarum, C. cladosporoides, Penicillium funiculosum, Chaetomium globosum and C. bostrychodes were present with varying percentage of frequency of occurrence almost throughout the course of the present investigation (Table 1.4-1.7).

The study on fungal flora reveals that saprophytic flora changes rapidly at senescence. The senescent leaves were found to be colonized by Asperigilli, species of Curvularia, Cladosporium cladosporoides, Stachybotrys atra, Chaetomium spp. Memnoniella echinata, Trichoderma viride, Fusarium moniliforme and Verticillium albo-atrum.

Aspergillus flavus, Epicoccum nigrum and white sterile mycelia were also frequently occurring species with less percentage frequency of occurrence, other forms though considerably high in number were less frequent and were cultured occassionally (Tables 1.4-1.7).

Following the terminology of Leben (1961) Cladosporium herbarum, Alternaria spp., Epicoccum nigrum, Aspergillus flavus and white sterile forms may be categorized under residents and other species such as Aureobasidium pullulans, Aspergillus niger, Dreschlera sp., Phoma sp., Macrophomina

phaseolina and other infrequently occurring forms may be treated as 'casuals' or 'transients' (Lamb and Brown, 1970).

Mishra and Srivastava (1970a) reported that Alternaria alternata, Aspergillus spp., Cladosporium cladosporoides, Fusarium sp., Phoma sp. and Trichoderma viride were found to be dominant on senescent leaves. Similar observations were also obtained during the present studies (Table 1.4-1.7).

The dominance of Cladosporium sp. on the leaf surface has been reported by many previous workers (Kerling, 1958; Dickinson, 1965; Hislop and Cox, 1969; Sinha, 1971; Mishra and Srivastava, 1970a, 1971b, DiMenna, 1971). The fungus is of wide occurrence in colder regions as observed in the present studies and it is a major constituent of the mycoflora of plant surface (Mishra and Srivastava, 1970a),

In addition, Alternaria spp. and Penicillium spp. also appeared to be dominant colonizers in different sere of succession on leaf surface at different stages of the crop.

Cladosporium herbarum, Alternaria alternata, Penicillium sp., Epicoccum nigrum and other resident forms mentioned earlier are fast growing, heavily sporulating and some of them produce deeply pigmented survival structures like chlamydospores, pigmented conidia or pigmented mycelia which account for their successful survival in adverse circumstances

(Tewari, 1973). Hudson (1971) also observed that most of the forms mentioned above as residents were more adapted to adverse conditions and reported them to be the primary colonizer of the senescent leaves.

The dominance of Fusarium sp., Rhizoctonia solani, Trichoderma viride and Verticillium albo-atrum and other fungi in later stages of the plant growth indicates their preparatory role for the ensuing senescent stage.

Surprisingly, the percentage occurrence of Cladosporium sp. increased during the later stages of plant growth possibly due to prevalence of low temperature during October when it proved favourable for the fungus (Fig. 1.12).

It is clear from the present studies that with minor exceptions the dominant member of different successional seres are almost the same in all the three communities. Alternaria alternata, Cladosporium spp., Mucorales and Phoma spp. were found to be the first colonizers of leaves irrespective of plant variety and most of them remained throughout the various growth stages of the plant which is in accordance with the findings of Hogg and Hudson (1966), Pugh and Mulder (1971), Lindsey and Pugh (1976) and Tsuneda and Skoropod (1978). In addition, Candida albicans, an important phylloplane yeast (Sharma and Mukerji, 1974) was also isolated from paddy leaves in the present studies.

In the second sere, the dominant members were Curvularia lunata, Arthrinium sp., Penicillium and the third sere was found to be formed mainly by Fusarium sp., Trichoderma viride and Verticillium albo-atrum. All these fungal species have been reported to colonize senescing plant parts (Lamb and Brown, 1970; Bainbridge and Dickinson, 1972; Ericker, 1976).

In general, the maturity of the leaf was found to be an important factor influencing the composition of microorganisms. Invariably, the numbers of fungi increased with increasing leaf age (Figs. 1.3-1.5). This is in agreement with previous reports and is a common finding of the researchers working with other plant species (Hudson, 1962; Last and Deighton, 1965; Dickinson, 1967; Lamb and Brown, 1970). The increase in fungi could be related to change in the nutritional status of leaves with age because there is often an increase in nutrient containing leachates from aerial plant parts (Webster and Dix, 1960; Tukey, 1971; Sinha, 1971). When leaves senesce there are numerous changes in their morphology, physiology and biochemistry which may account for the upsurge of fungal populations (Narula and Mehrotra, 1981). In addition, increasing trend of total fungal population may be due to weathering of surface waxes (Forester, 1977), decreased production of phytoalexins (Bailey, 1971) and finally the expanding leaves may provide more surface areas to be occupied by the micro-organisms.

Thus the fungal communities are largely formed by wide spread saprophytes such as Alternaria alternata, Aureobasidium pullulans, Cladosporium cladosporoides, Curvularia lunata, Dreschlera graminea, Fusarium moniliforme, Penicillium funiculosum, Trichoderma viride, Verticillium albo-atrum and some restricted fungi like Phoma spp. and Pestalotia monorhynca. It has been known earlier also (Diem, 1971) that hyaline fungi because of their fragility are able to grow and survive to a lesser extent than the dematiaceous forms. The fungi occurring in moist chambers viz., Chaetomium spp., Stachybotrys atra, Memnoniella echinata, Starkeomyces koorchalomoides (= Myrothecium verrucaria), Torula herbarum and Nigrospora oryzae which occurred in higher number on senescent leaves may play an active role in decomposition because they are well known active colonizers of decaying plant parts (Sharma and Mukerji, 1972; Ericker, 1976). Aspergilli and Pencillia although occurred at significant levels on green leaves reflecting their relative abundance in the atmosphere, there is however, a sudden fall in the population of these fungi at senescence and lower percentage frequency of occurrence in moist chambers suggest that they play negligible role in decompostion process (Dickinson, 1976). Moreover, Mucorales too play an unimportant role in the above ground phase of decomposition of paddy leaves as they were quite infrequent in leaf washings and moist chambers.

Seasonal fluctuation in mycoflora

The results of the present studies reveal that the distribution of fungi may greatly depend on weather changes. Meteorological factors such as atmospheric temperature, humidity and rain were important factors (Fig.1.1) influencing the fungi on the leaf surfaces and this is in agreement with the data of Gregory (1950, 1961), Hirst and Stedman (1963), Sinha (1971), and Hayes (1982) who found that such factors determine the periodicity and affect the trapping of spores on leaf surface. The total number of fungi and also the specific composition of the fungal flora was affected by the weather conditions especially during rainy season (Narula and Mehrotra, 1981). The locality where the present studies have been conducted has very high rainfall which in turn may result in high atmospheric moisture and thus the mycoflora is also the highest as compared to the other places where previous similar investigation on phylloplane has been done.

Some species of fungi were present throughout the growing period (Group I as given in the results), whereas others were exclusively associated with a particular set of climatic factors (Group III). But some of the fungi like Pestalotia monorhynca, and Phoma spp. did not appear at any definite time during the course of present studies. This may be because of their colonization behaviour which depends more on the habitat than the inoculum availability.

Quantitative assessment

Quantitative estimations done by dilution plate technique reveal that the fungal population in the beginning when the plants were in seedling and young stages was low, increasing gradually with the age of the plants and reaching its peak at senescence in all the three varieties studied (Fig. 1.9). The population is affected by nutrient level of leaf surface which increases with the release of the substances from the leaf.

The change in total fungal population was statistically significant with increasing plant age with a remarkable increase at maturity and senescence of the plant and was independent of host variety (Table 1.8 and 1.9). The marked increase at maturity might be due to the availability of the maximum surface area, maximum atmospheric moisture (Fig. 1.1) and an increase at senescence can be explained on the basis of increased dead tissue which provides niches to saprohytes which multiply there.

Results of the 'F' test show a significant variation in total fungal population among the three varieties at maturity. The broader leaves of Khonorullo as compared to Ngoba and Mirikrak might be responsible for a significant variation at this stage. Leaf surface morphology is very important as it determines the trapping efficiency of the leaf,

and the suitability of the environment for the germination and persistence of the microflora (Bainbridge and Dickinson, 1972; Baker et al., 1979).

In addition, it was also found through the 'F' test (two way classification) that the growth stages of the plant had a highly significant effect on total fungal population while the influence of host variety was insignificant which is in agreement with findings of Bainbridge and Dickinson (1972); Sharma and Mukerji (1972); McBride and Hayes (1977); Gupta and Mukerji (1982).

Effect of pH, moisture content of leaves on fungal population

From Fig. 1.17 it is clear that moisture content of leaves does not exert much effect on the increase in fungal population. On the other hand, pH showed acidic nature at seedling stage which turned neutral at flowering and post-flowering stages till leaves were green. However, with yellowing of leaves, their pH increased showing their alkaline nature (Fig. 1.17). This is in accordance to previous observations by Tewari (1972) who also got similar results in case of wheat and barley. pH may have some affect on the fungal population in the later stages of plant growth.

By using pearson product moment correlation (Table 1.12) also it was clear that pH and moisture content of leaves were found to be significantly positively and negatively

correlated (at P = 0.01) respectively to fungal population at different growth stages of the plant irrespective of the variety. Many workers have also reported that pH of the leaves may play an important role in the distribution of fungi both quantitatively and qualitatively (Last and Deighton, 1965; Dickinson, 1967; Rao and Manoharachary, 1981).

Interfungal community relationships between the three varieties of paddy

Based upon the observations from Fig. 1.16 dealing with the species diversity of fungi among different communities at various successional stages the following generalizations can be made:

1. Species composition changes with plant age within each community, which is a clear indication of succession. The habitat changes which lead to succession are not only brought about by previous colonizers through their secondary metabolites (Fokkema, 1973) but a major change is controlled by the growth stage of the plant which includes a change in the physiology of the leaf with increasing plant age (Bainbridge and Dickinson, 1972; Gupta and Mukerji, 1982).

2. Initially the disease resitant (Khonorullo) and moderately susceptible (Ngoba) varieties had very high similarity index 'S' values but it decreases with plant age and becomes distinct

with the least 'S' value at the age of 150 days. On the other hand, in Khonorullo (disease resistant) and Mirikrak (disease susceptible) varieties the 'S' value remains relatively low throughout the growth stages. This indicates that the community formed by the disease reistant (Khonorullo) variety resembles more the moderately susceptible variety (Ngoba) than the susceptible variety (Mirikrak) which is quite distinct at early (30 days) and late (150 days) stages of the plant growth.

3. At senescence excepting Khonorullo and Ngoba community relationships the other two relationships between Ngoba and Mirikrak, Khonorullo and Mirikrak show increase in similarities which reflects the role of the habitat characteristics in determining the species compostion of fungal communities.

Further, as observed from the results of the present investigation (Tables 1.3-1.7), the leaf spot disease resistant variety (Khonorullo) supported a significantly higher population of fungi than the moderately susceptible (Ngoba) and susceptible (Mirikrak) varieties. This higher population could play a significant role in imparting resistance to foliar infection by forming a microbial barrier or buffer (Kumar and Balasubramanian, 1981). Fungi may extensively colonize a substrate to the exclusion of other fungi which arrive later (Bruehl and Lai, 1966). Similar results have also been observed by various workers (Balagopal and Oblisami, 1973; Kumar and Balasubramanian, 1981). However, some workers

(Sharma and Sinha, 1972; Kumar and Gupta, 1974; Pace and Campbell, 1974; Tyagi, 1980) have observed higher fungal population in susceptible varieties than resistant ones. The decrease in the fungal population observed on the leaf spot susceptible variety in the present studies could be due to the domination of the fungal pathogen resulting in the reduction of nutrients available on the diseased leaf surface or by eliciting antifungal compounds (Dickinson, 1976). The higher population may also result in the production of phytoalexin like compounds which may contribute to the resistance in resistant cultivar. The presence or attempted penetration of plant surface by organisms normally non-pathogenic on that plant or resident saprophytes is said to promote the production of phytoalexins (Bailey, 1971).

A naturally occurring biological control may be present in the three varieties of paddy, where (Fig. 1.15) it is clear that as Trichoderma viride population increased at the age of 75 days in Khonorullo variety (disease resistant) but in Mirikrak variety (disease susceptible) the population of T. viride declined and Helminthosporium oryzae the leaf spot pathogen became dominant and the disease became quite conspicuous. Whereas, in Ngoba variety (moderately susceptible) T. viride although at 105 days of plant age it decreased but at later stages (at 135 days) it showed a sharp increase and remained till senescence and here H. oryzae population decreased

and the inoculum of H. oryzae although present initially but the disease was not so dominant as compared to the Mirikrak variety. Similar natural biological control was also observed by Fokkema et al. (1975) while studying the buffering capacity of the microflora of rye leaves to artificial infection by Cochliobolus sativus. Akai and Kuramato (1968) tried to control the leaf spot disease of rice biologically. They observed Candida albicans to be antagonistic to Cochliobolus miyabeanus (perfect stage of H. oryzae). In the present investigation also Cochliobolus miyabeanus, the perfect stage of H. oryzae was found only in Mirikrak variety, the disease susceptible one. Sinha and Trivedi (1969) also found fewer spots of Cochliobolus miyabeanus (=H. oryzae) on rice leaves which had previously been inoculated with an avirulent strain of the same pathogen. Their results point to the action of fungitoxic substances formed by the leaves.

Thus from the present studies it is clear that host specificity is another factor known already from the work of Singh and Sinha (1962), Kerling (1964), Sinha (1971), Kumar and Balasubramanian (1981) and this is also established by the present studies.

Thus it can be concluded from the present studies that the distribution of the fungi on leaf surface was dependent mainly on host species, leaf maturity and weather changes. Moreover, in the present studies since the source of inoculum

and climatic conditions were similar for all the three varieties (since they were grown together at the same place) the differences observed in quantitative and qualitative compostion of these communities are mainly as a result of habitat characteristics i.e., leaf morphology and physiology. However, the growth stage of the plant was found to have a major control over the community composition as compared to host variety.

CHAPTER II

SURVEY OF AIR FUNGI AND THEIR IMPACT ON THE LEAF SURFACE MYCOFLORA

INTRODUCTION

It is a well known fact that air borne propagules act as initial source of inocula for the aerial plant parts (Dixit and Gupta, 1980).

The investigation in the aerobiology of India dates back to the researches of Cunningham (1873) who reported changes in atmospheric spore content of Calcutta prison. From that period onwards, the work on aeromycoflora of different localities gained momentum with the researches of many workers (Mishra and Srivastava, 1970, 1971a, 1972; Mishra and Kamal, 1971; Mishra and Tewari, 1976a; Kumar and Gupta, 1976; 1980; Dixit and Gupta, 1980).

The air fungal taxa includes both saprophytes and parasitic fungi and the pathogenic forms of microorganisms among the settlers of airspora are responsible for disease incidence on the leaf surface. Thus an understanding of the nature, periodicity and density of the fungal propagules in the air is much helpful in making a forecast regarding the occurrence of fungal diseases and the quantum of viable pathogenic propagules likely to cause infection.

Under favourable conditions, spores of various fungi produced in tremendous numbers on the leaf surface are released into the air and thus there may exist a close correlation

between the airspora of a locality and the leaf surface fungi of plants growing in the area (Mishra and Tewari, 1976a). Further, Mishra and Srivastava (1971b) also reported that a sort of cyclic phenomenon exists between fungal spores of air, soil and plant surface. Some of the forms, however, maintain their specificity in the specialized environment.

Moreover, the seasonal and diurnal periodicities of microbes existing in the air of a locality is mainly dependent on climatic conditions especially temperature, relative humidity, rain and wind speed.

Many workers (Hudson, 1971; Mishra and Tewari, 1976a; Dixit and Gupta, 1980) have thus suggested that the spores present in the air have a great impact on the leaf surface mycoflora and the leaf surface acts as a landing site for the various microbes present in the air (Pugh and Buckley, 1971; Gregory, 1971; Pady, 1971).

Relatively little work has been done to understand the effect of air fungal population over different fields in relation to the leaf surface fungi (Sinha, 1971; Mishra and Tewari, 1976a; Kumar and Gupta, 1976, 1980; Dixit and Gupta, 1980). Out of several forms of microbes found in the air some may be pathogenic to the plants growing in the locality and thus a comparative study of aeromycoflora and phylloplane fungi may be of suggestive value in the spray schedules against the pathogen (Kumar and Gupta, 1980).

The present work was undertaken to survey the aeromycoflora of the three sites where the three varieties of paddy viz., Khonorullo, disease resistant; Ngoba, moderately susceptible; and Mirikrak, the disease susceptible varieties were grown. Simultaneously the phylloplane studies were also done and the impact of airborne fungi on phylloplane mycoflora was assessed.

MATERIALS AND METHODS

Study area

The aeromycoflora was studied from the sites where the three varieties of paddy were grown. Sampling was done at different growth-stages of the plants during the two crop seasons (1981 and 1982). The plants of paddy were grown in the experimental plots of the Botany Department of the . . .. University.

Sampling

The sampling of the air fungi was done fortnightly on the same sampling dates on which the phylloplane mycofloral studies were made. This was done by exposing nutrient plates containing Czapek's Dox agar medium horizontally in the fields of the three varieties of paddy for 5 minutes at a height of one meter. Ten replicates of each site were maintained. The plates were then incubated for 6 days at 25±1°C and the fungi

appearing in them were identified. The plates were further examined for 20 days for slow growing fungi. The sampling was done from 15 days of plant age till 180 days.

Interpretation of the data

The percentage frequency of occurrence was calculated for each fungus based on the formula given by Tresner et al. (1954).

$$\text{Percentage frequency of occurrence} = \frac{\text{Number of samples of occurrence}}{\text{Total number of samples}} \times 100$$

The results have been expressed as an average of isolations done during two growing seasons of the paddy crop (1981 and 1982).

Further, the fungal population (both total number of fungal species and average number of colonies/plate) isolated from phylloplane of the three varieties of paddy were correlated with fungal population isolated from air of the three varieties at different growth stages of the plant by using Pearson's product moment correlation coefficient (r).

RESULTS

Survey of aeromycoflora from the fields of the three varieties of paddy

As evident from Tables 2.1 and 2.2 a total of 57 species belonging to 36 genera of fungi were isolated from the air of the three varieties of paddy fields viz., Khonorullo, Ngoba and Mirikrak by using culture plate method (Rajan et al., 1951) at different growth stages of the paddy plant. Based on the observations from Table 2.2 the airspora in the fields of the three varieties of paddy belonged to 3 main groups of fungi viz., Mucorales, Ascomycetes and Hyphomycetes out of which maximum number of species belonged to Hyphomycetes.

Cladosporium spp. (particularly C. herbarum), Penicillium spp. and Alternaria spp. dominated the airspora. As evident from Table 2.3, Cladosporium spp. shared 33.12, 30.59 and 25.89 whereas Penicillium spp. shared 26.09, 25.91 and 29.43 while Alternaria spp. contributed 10.22, 10.96 and 11.15 to the average fungal population in the air of three varieties of paddy viz., Khonorullo, Ngoba and Mirikrak respectively. Aspergillus spp. especially A. flavus and A. niger were also quite dominant in the air and were represented by 12.07, 13.02 and 9.58 in the three fields of paddy varieties respectively. Arthrinium spp. followed next which contributed 5.66, 4.84, 5.54 to the average fungal population in the air of the three paddy fields. Trichoderma viride and Helminthosporium oryzae also contributed 1-3% to the average fungal population. Phycomyoetous, Ascomycetous and sterile mycelial forms were poorly represented in the air. In general, Deuteromycetes contributed to about 90 percent of the total fungal population in the air of the paddy crop.

Further, as observed from Fig. 2.3, Cladosporium herbarum occurred almost throughout the growth stages of the paddy crop and its population in terms of average percentage frequency of occurrence varied at different growth stages of the plant being maximum at the later stages of the crop in the air of all the three paddy fields. On the other hand, in case of Penicillium funiculosum the maximum percentage frequency of

occurrence was at the early stages of the crop (Fig. 2.5) and showed a slight decrease at the later stages when Cladosporium herbarum became dominant species in the air of the paddy crop. Fusarium moniliforme was also quite frequent in the air and the percentage frequency of occurrence was maximum at 120-135 days of plant growth. In addition, Alternaria alternata and Arthrinium sp. were also quite frequent in the air and their average percentage frequency of occurrence varied remarkably at different sampling dates (Fig. 2.6 and 2.7).

The pathogen, Helminthosporium oryzae which causes brown spot disease of paddy was also isolated in the air of all the three varieties frequently (as early as in the air of 15 days old plant in the case of Ngoba and Mirikrak varieties and 30 days in the air of Khonorullo variety). The population, however, decreased at 60 days of plant age and later registered a continuous rise from 75 days onwards in the air of the disease susceptible varieties (Ngoba and Mirikrak). In the air of moderately susceptible variety (Ngoba) there was a sharp decline of the pathogen population at 150 days, however, in the air of disease susceptible variety (Mirikrak) the pathogen population registered a continuous rise from 75 days onwards and touched its peak at 105 and 120 days of plant growth and it exhibited high percentage frequency of occurrence throughout. On the other hand, in the air of disease resistant variety, the pathogen although present initially, it decreased later on and disappeared from 135 days onwards (Fig. 2.8).

The population of Trichoderma viride also varied remarkably at the different sampling dates in all the three paddy fields, however, in the air of Khonorullo variety, the percentage frequency of occurrence was quite high as compared to the other two varieties.

As evident from Fig. 2.1 the fungal population in the air increased when the crop matured but the total number of fungal species fluctuated remakably at different sampling dates and showed a decline at 75-90 days of plant age and increased later at 135 days and subsequently decreased with plant age.

Classification of the aeromycoflora based on their occurrence in different months

All the fungi isolated from the air of the three varieties of paddy at different growth stages of the plant were categorised into three groups based on their occurrence in different months during the crop season:

Group I : Fungi found uniformly in all the months.

Group II : Fungi occurred sporadically and not restricted to one season.

Group III : Fungi restricted to one particular season.

Group I : To this group belonged fungi like Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium herbarum, Fusarium moniliforme, Penicilllium funiculosum,

Group II : Most of the fungi such as Mucorales, one Oomycete Pythium sp., Ascomycetes like Chaetomium bostrychodes and many Hyphomycetes like Acremonium indicum, Alternaria solani, Arthrinium sp., Chrysosporium pruinosum, Cladosporium cladosporoides, Curvularia lunata, Dreschlera tetramera, Epicoccum nigrum, Fusarium oxysporum, Nigrospora oryzae, Trichoderma viride, Trichothecium roseum, Verticillium albo-atrum, Pestalotia monorhynca, Sterile mycelia etc. belonged to this group.

Group III : Chaetomium globosum, Melanospora zamae, Sordaria macrospora, Phoma spp., Macrophomina phaseolina, Aspergillus clavatus, A. ochraceous, A. sydowi, A. terreus, Aureobasidium pullulans, Botryotrichum sp., Curvularia pullescens, Dreschlera gramínea, Epicoccum purpurascens, Gliocladium pencilloides, Neurospora sp., Paecilomyces veruta etc. could be placed in this category. Some fungi like H. oryzae showed higher population in the air when the temperature and atmospheric moisture was higher as compared to other months. The fungus was more evident in the air of moderately susceptible and susceptible paddy fields.

Cladosporium herbarum although present throughout in the air but showed a high population in the later stages when the temperature was low. During the rainy season (June-July) most of the fungal forms were washed out and there was a decrease in fungal population excepting few species such as

Penicillia and Aspergilli which were quite common throughout the sampling period.

Correlation between aeromycoflora and phylloplane mycoflora

As evident from Table 2.1 and Fig. 2.2 most of the fungal species isolated were of common occurrence in the two environments viz., Phylloplane and in the air of the three varieties of paddy and only 3 and 34 species appeared exclusively from the air and the phylloplane region respectively. Further, most of the dominant forms in both the environments belonged to the first category. In the air only three infrequently isolated species viz., Botryotrichum sp., Dreschlera tetramera and Penicillium minutum were of specific occurrence.

The general distribution pattern of phylloplane fungal flora at different growth-stages of the plant of the three varieties of paddy was very much similar to that of air fungi. Similarity was also noted in the dominant species and in the average frequency of occurrence of the forms in the two environments i.e., air and phylloplane (Table 2.3).

Further, a perusal of the Table 2.4 indicates that a positive correlation existed between the fungal population of the two environments viz., air and phylloplane which was highly significant (P = 0.01) for Khonorullo variety (in both

total number of fungal species and average number of fungal colonies/plate) and also for Ngoba variety (only in total number of species) but was significant at 95% probability for Ngoba and Mirikrak varieties.

Moreover, from the table 2.3 it is clear that the pathogen Helminthosporium oryzae although common in the air of Khonorullo was absent in the phylloplane. The population of the pathogen, however, was highest in the air and phylloplane of Mirikrak variety and was followed by Ngoba. From Fig. 2.8 it is also apparent that the pathogen, H. oryzae though appeared quite early in the air of three paddy varieties, it could be isolated from the phylloplane as a dominant fungi only at the later stages of the crop.

As far as Trichoderma viride was concerned, although it was present uniformly in the air of all the three varieties of paddy (Fig. 2.9), the population was highest in the phylloplane of the disease reistant variety (Khonorullo) followed by the moderately susceptible variety (Ngoba) and the least on susceptible variety (Mirikrak).

In general, from Table 2.2 it is clear that all the fungi were present almost uniformly in the air of all the three varieties of paddy, the phylloplane fungal population of the three varieties varied being the highest on Khonorullo, the disease resistant one than the other two varieties viz.,

Fig. 2.1: Total number of fungi and fungal population in the air of the three paddy fields viz., Khonorullo, Ngoba and Mirikrak.

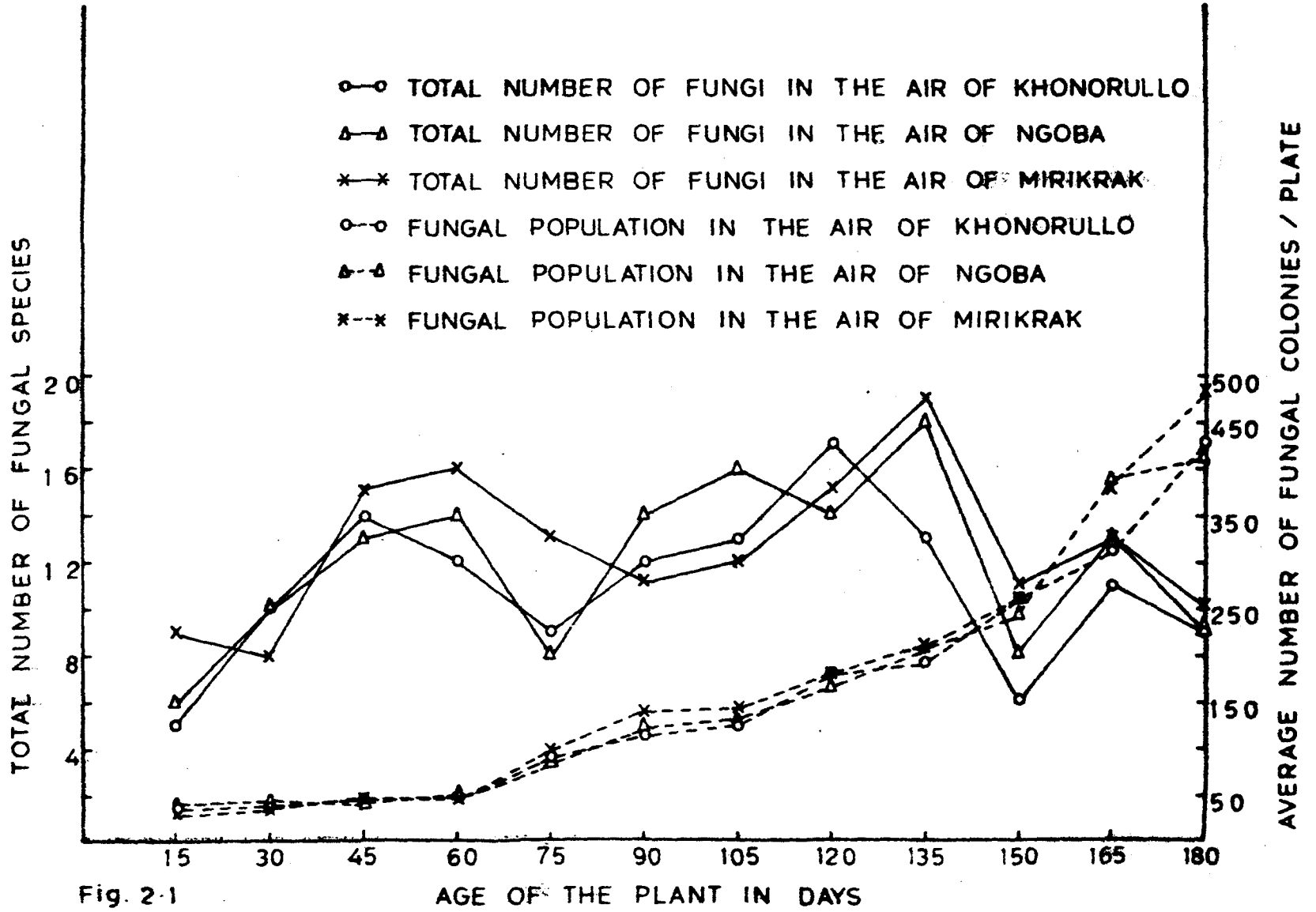

TOTAL NUMBER OF FUNGI IN THE AIR OF KHONORULLO
TOTAL NUMBER OF FUNGI IN THE AIR OF NGOBA
TOTAL NUMBER OF FUNGI IN THE AIR OF MIRIKRAK
FUNGAL POPULATION IN THE AIR OF KHONORULLO
FUNGAL POPULATION IN THE AIR OF NGOBA
FUNGAL POPULATION IN THE AIR OF MIRIKRAK
TOTAL NUMBER OF FUNGAL SPECIES
AVERAGE NUMBER OF FUNGAL COLONIES / PLATE
20
16
12
8
4
500
450
350
250
150
50
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 2·1

Fig. 2.2: Total number of fungal species common to phylloplane and air, only on phylloplane and only in the air of paddy.

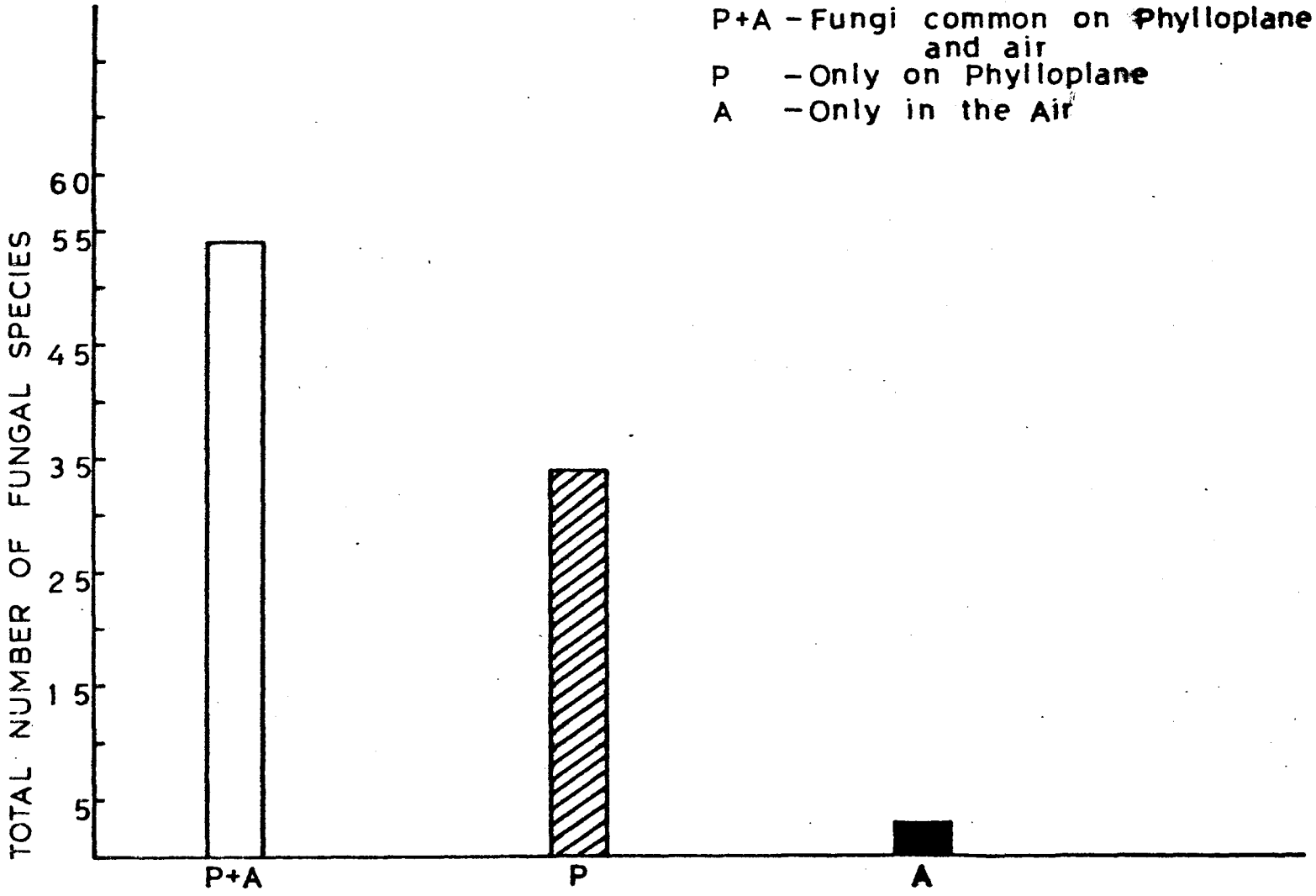

P+A – Fungi common on Phylloplane and air
P – Only on Phylloplane
A – Only in the Air
TOTAL NUMBER OF FUNGAL SPECIES
60
55
45
35
25
15
5
P+A
P
A

Fig. 2·2

Fig. 2.3: Average percentage frequency of occurrence of Cladosporium herbarum in the air of the three paddy fields viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

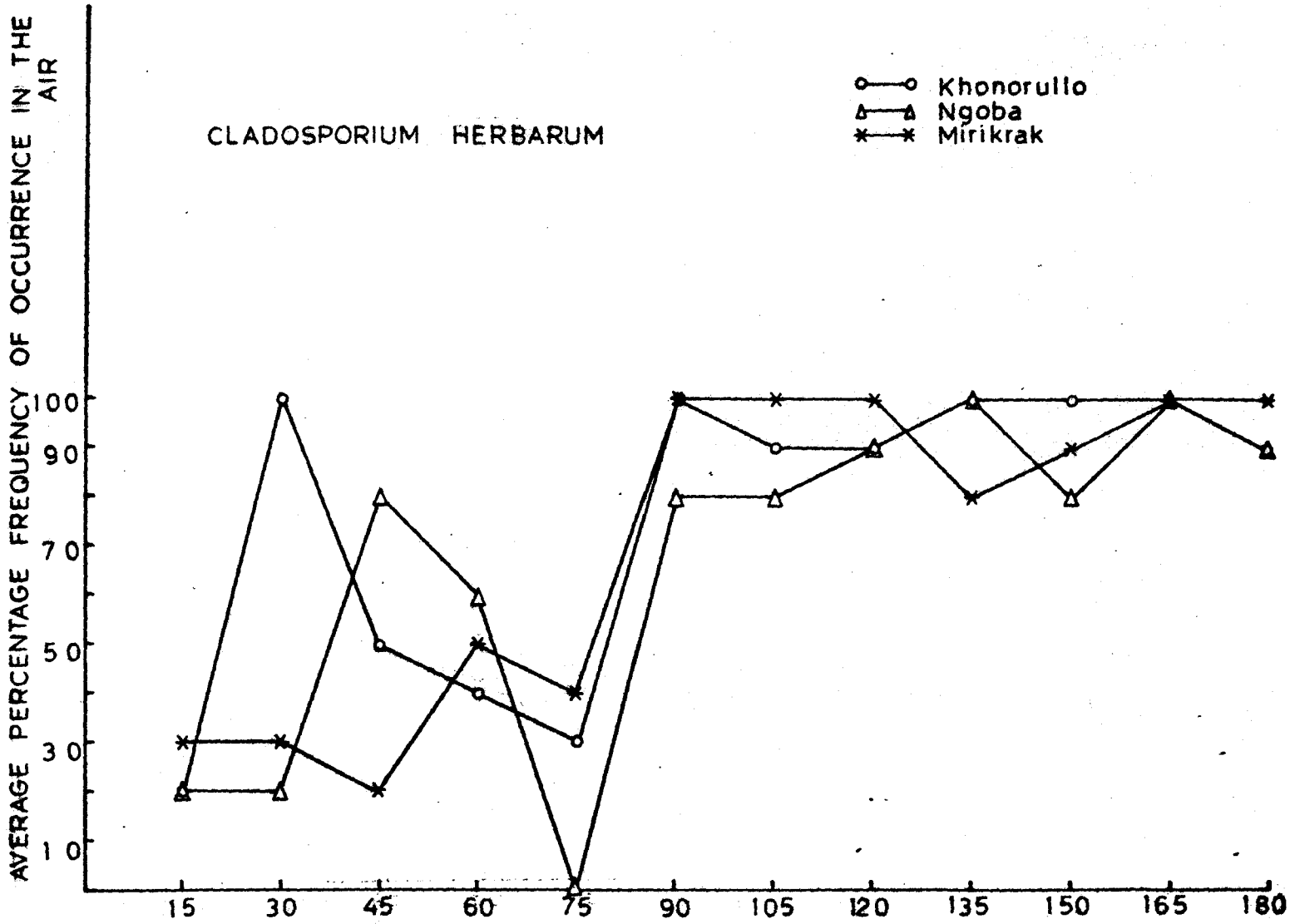

CLADOSPORIUM HERBARUM
Khonorullo
Ngoba
Mirikrak
AVERAGE PERCENTAGE FREQUENCY OF OCCURRENCE IN THE AIR
100
90
70
50
30
10
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 2·3

Fig. 2.4: Average percentage frequency of occurrence of Fusarium moniliforme in the air of the three paddy fields viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

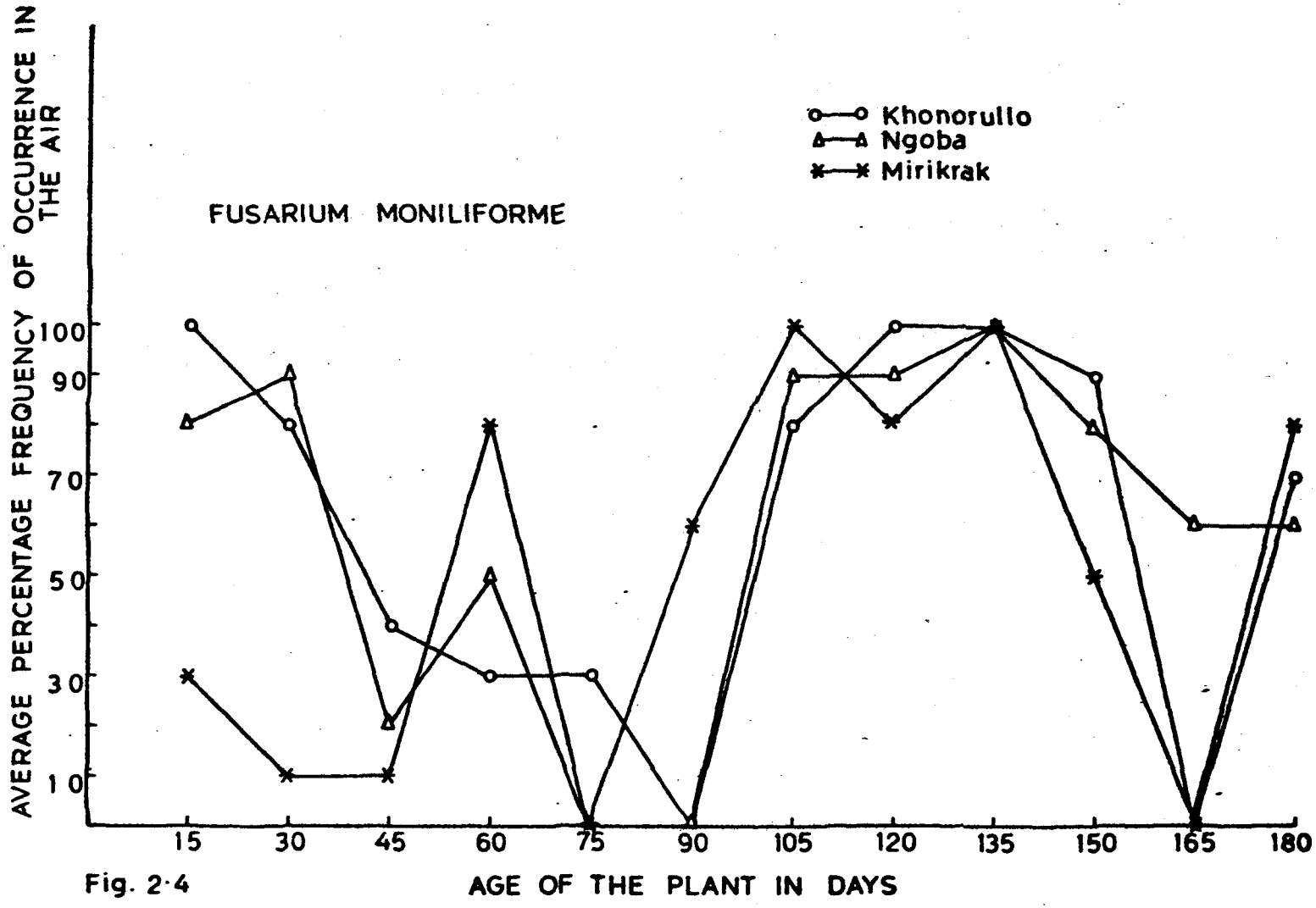

FUSARIUM MONILIFORME
Khonorullo
Ngoba
Mirikrak
AVERAGE PERCENTAGE FREQUENCY OF OCCURRENCE IN THE AIR
100
90
70
50
30
10
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 2·4

Fig. 2.5: Average percentage frequency of occurrence of Penicillium funiculosum in the air of the three paddy fields, viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

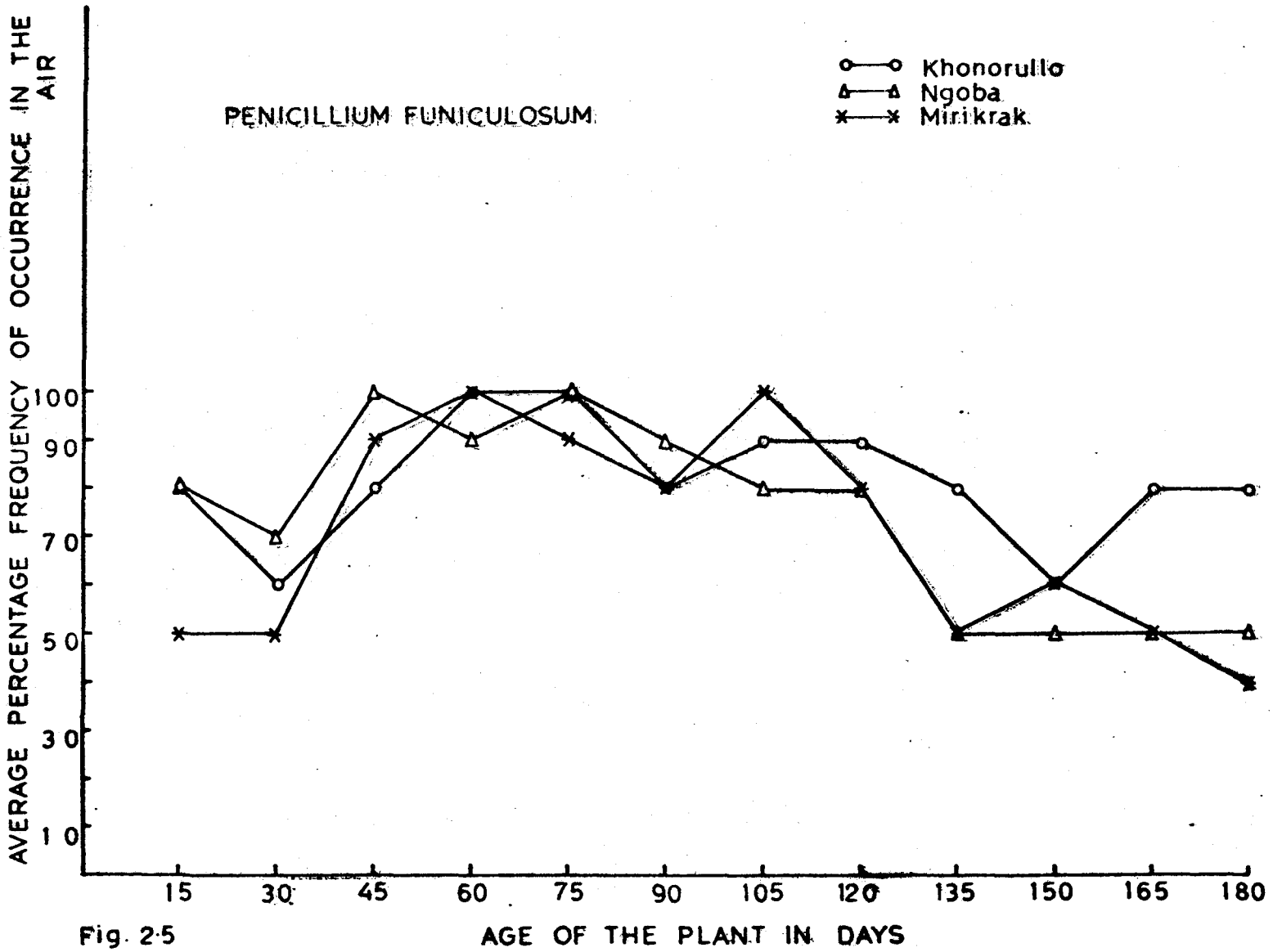

PENICILLIUM FUNICULOSUM
Khonorullo
Ngoba
Mirikrak
AVERAGE PERCENTAGE FREQUENCY OF OCCURRENCE IN THE AIR
100
90
70
50
30
10
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 2·5

Fig. 2.6: Average percentage frequency of occurrence of *Alternaria alternata* in the air of the three paddy fields viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

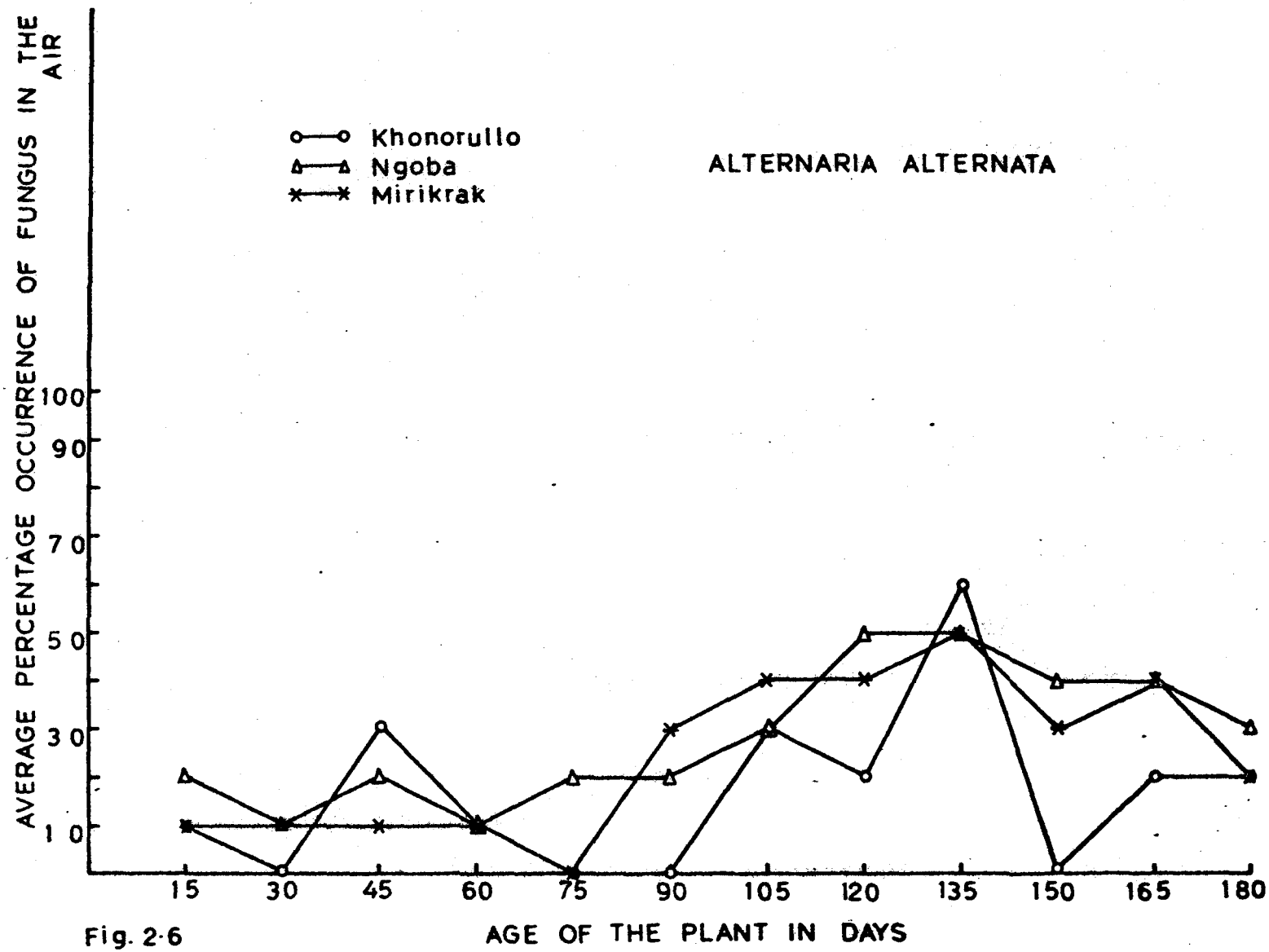

Khonorullo
Ngoba
Mirikrak
ALTERNARIA ALTERNATA
AVERAGE PERCENTAGE OCCURRENCE OF FUNGUS IN THE AIR
100
90
70
50
30
10
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 2·6

Fig. 2.7: Average percentage frequency of occurrence of *Arthrinium* sp. in the air of the three paddy fields viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

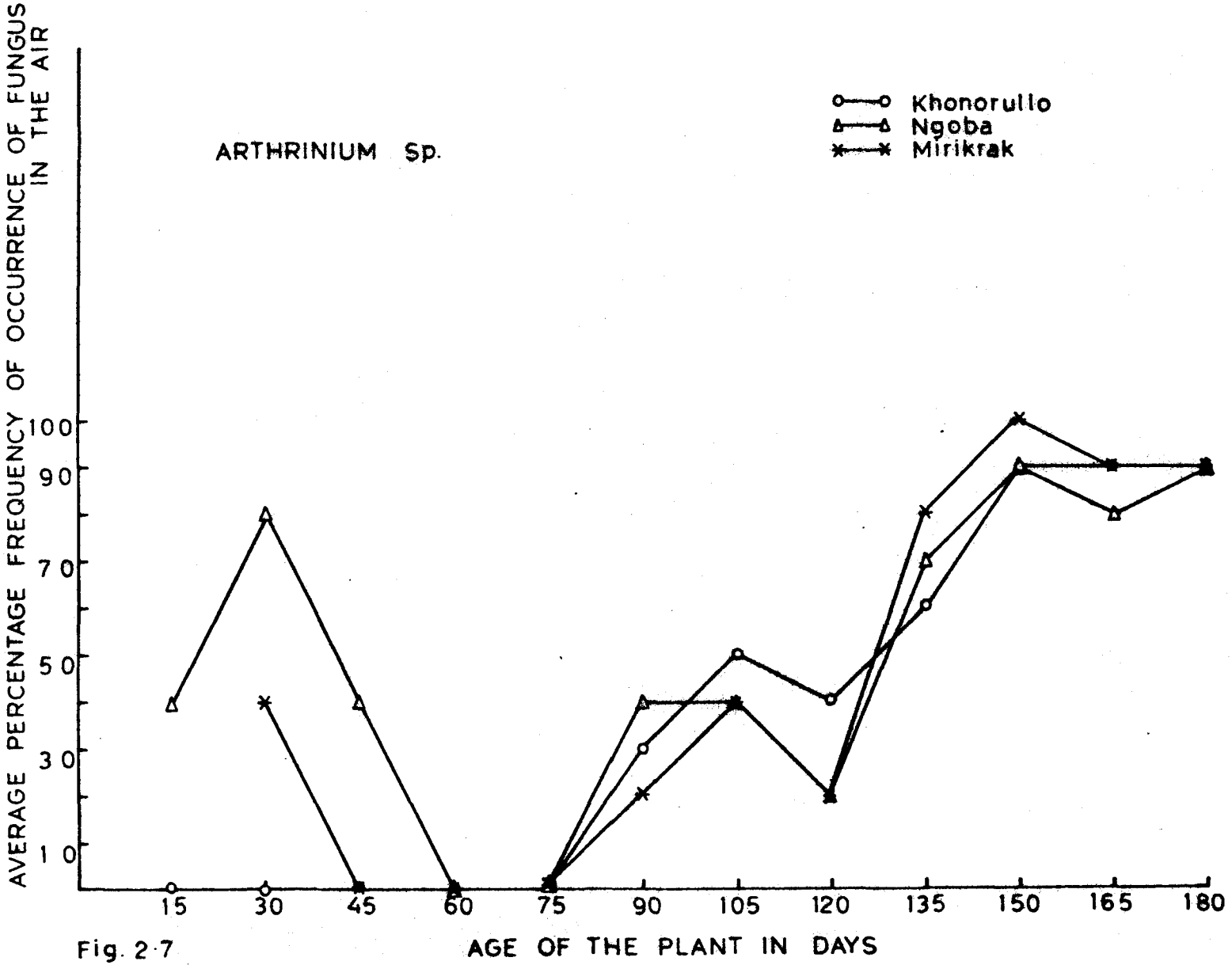

ARTHRINIUM Sp.
Khonorullo
Ngoba
Mirikrak
AVERAGE PERCENTAGE FREQUENCY OF OCCURRENCE OF FUNGUS IN THE AIR
100
90
70
50
30
10
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 2·7

Fig. 2.8: Average percentage frequency of occurrence of Helminthosporium oryzae in the air of the three paddy fields viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

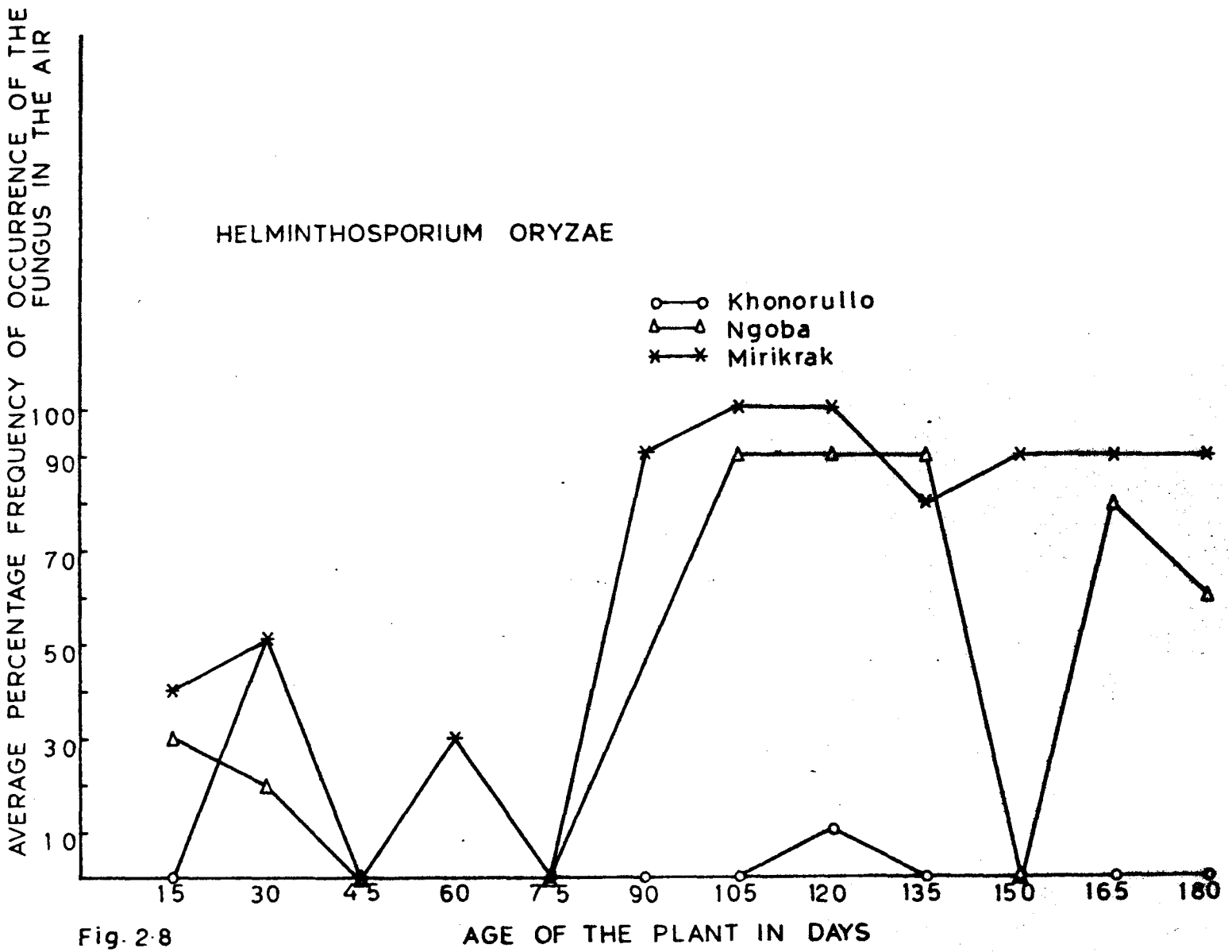

HELMINTHOSPORIUM ORYZAE
Khonorullo
Ngoba
Mirikrak
AVERAGE PERCENTAGE FREQUENCY OF OCCURRENCE OF THE FUNGUS IN THE AIR
100
90
70
50
30
10
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 2·8

Fig. 2.9: Average percentage frequency of occurrence of *Trichoderma viride* in the air of the three paddy fields viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

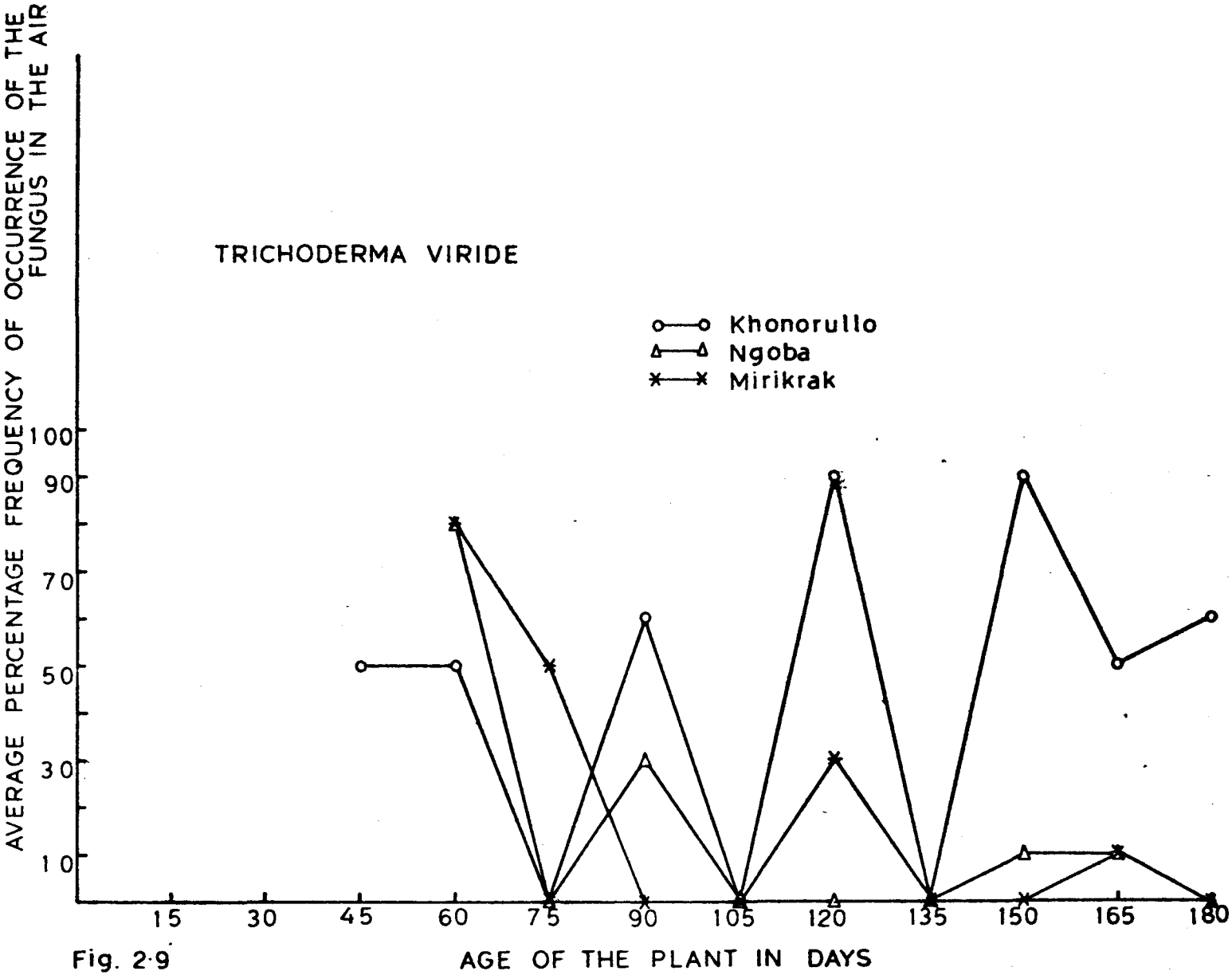

TRICHODERMA VIRIDE
Khonorullo
Ngoba
Mirikrak
AVERAGE PERCENTAGE FREQUENCY OF OCCURRENCE OF THE FUNGUS IN THE AIR
100
90
70
50
30
10
15
30
45
60
75
90
105
120
135
150
165
180
AGE OF THE PLANT IN DAYS

Fig. 2·9

Table 2.1: Record of fungi in phylloplane and air of paddy crop.

Fungi	Phylloplane	Air
PHYCOMYCETES		
MUCORALES		
Absidia glauca	+	-
Cunninghamella echinulata	+	-
Mucor alternans	+	+
M. basiliformes	+	+
M. hiemalis	+	+
Rhizopus nigricans	+	+
OOMYCETES		
Pythium sp.	+	+
ASCOMYCETES		
Ascochyta sp.	+	-
Chaetomium globosum	+	+
C. bostrychodes	+	+
C. funiculosum	+	-
C. fusiforme	+	-
C. succineum	+	-
Cochliobolus miyabeanus	+	-
Colletotrichum capsicum	+	-
Coniothyrium indicum	+	-
Gelasinospora tetraspora	+	-
Masoniella griseum	+	-
Melanospora zamae	+	+
Pyrenochaeta decipiens	+	-
Sordaria humana	+	-
S. macrospora	+	+
Triangularia obliqua	+	-
HYPHOMYCETES		
SPHAEROPSIDALES		
Phoma glomerata	+	+
P. hibernica	+	+
Macrophomina phaseolina	+	+
Rhizoctonia solani	+	-
Absidia sp.	+	-

Fungi	Phylloplane	Air
MONILIALES		
Acremonium indicum	+	+
A. persicinum	+	-
Alternaria alternata	+	+
A. solani	+	+
Arthrinium sp.	+	+
Aspergillus candidus	+	-
A. clavatus	+	+
A. flavus	+	+
A. fumigatus	+	-
A. nidulans	+	+
A. niger	+	+
A. ochraceous	+	+
A. sydowi	+	+
A. terreus	+	+
A. versicolor	+	-
Aureobasidium pullulans	+	+
Botryotrichum sp.	-	+
Botrytis cinerea	+	-
Candida albicans	+	-
Chrysosporium pruinosum	+	+
Cladosporium cladosporoides	+	+
C. herbarum	+	+
C. sphaerospermum	+	-
Curvularia lunata	+	+
C. pullescens	+	+
Dreschlera graminea	+	+
D. rostrata	+	-
D. tetramera	-	+
Epicoccum nigrum	+	+
E. purpurascens	+	+
Fusarium oxysporum	+	+
F. moniliforme	+	+
Gliocladium pencilloides	+	+
Didymostilbe ellisii	+	-
Harphographium fasciculatum	+	-

Fungi	Phylloplane	Air
Helminthosporium oryzae	+	+
Humicola prisea	+	+
Memnoniella echinata	+	-
Monilia stiphylla	+	+
Neurospora sp.	+	+
Nigrospora oryzae	+	+
N. sphaerica	+	-
Oedocephalum glomerulosum	+	-
Papulospora sp.	+	-
Paecilomyces varia	+	+
Penicillium funiculosum	+	+
P. chrysogenum	+	+
P. glaucum	+	-
P. stipitatum	+	-
P. minutum	-	+
Stachybotrys atra	+	+
Sarkomyces koorchaloides	+	+
Stemphylium botryosum	+	+
Torula herbarum	+	+
Trichoderma viride	+	+
Trichothecium roseum	+	+
Verticillium albo-atrum	+	+
MELANCONILES		
Pestalotia monochaeta	+	+
White sterile mycelia	+	+
Brown sterile mycelia	+	+
Orange sterile mycelia	+	-
Yellow sterile mycelia	+	+
Black sterile mycelia	+	-
Total No. of species	88	57

Table 2.2: Showing percentage frequency of occurrence of different fungi in the air of the three varieties of paddy fields at different growth stages of the plant

Fungi	AGE OF THE PLANT IN DAYS: 15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
PHYCOMYCETES																																				
MUCORALES																																				
Mucor alternans				20	20																20				20	10	20			10						10
M. bacilliformes	20		10																					20												
M. hiemalis				30	10					10	10	10	20				20	10							10						10					
Rhizopus nigricans														10	20		10																			
OOMYCETES																																				
Pythium sp.							20		10								10						20													
ASCOMYCETES																																				
Chaetomium bostrychodes														10	20	20	10	10																		
C. globosum															10																					
Melanospora zamae													10																							
Sordaria macrospora												10																								
HYPHOMYCETES																																				
Phoma glomerata				10																																
P. hibernica											10																									
Macrophomina phaseolina																							10													
Acremonium indicum															10										10		10									
Alternaria alternata	10	20	10		10	10	30	20	10	10	10	10		20			20	30	30	30	40	80	30	40	60	50	90		40	30	20	40	10	20	30	20
A. solani			10	10			30				20			10	10				80		10			10				20			20	10	10			
Arthrinium sp.		40			80	40		40								30	40	20	50	40	40	40	50	80	60	70	80	90	90	100	90	80	90	90	90	90
Aspergillus clavatus																	10																			
A. flavus		10		10			20	20			20	30				30		50	20		30	80	30	30	40	20	50	30		20	40	20	30	10	30	20
A. nidulans								20		30						40	50	40		30	30		50	50		60	50					40		60		50
A. niger			40				50	30	40	40	30	50	40			50			30	80	10	90					30									
A. ochraceous																											10									
A. sydowi															10																					
A. terreus																																10				
Aureobasidium pullulans												10																								
Botryotrichum sp.																			10																	
Chrysosporium pruinosum												20										30							10	20						
Cladosporium cladosporioides					10	20	10			50						30			20					50		80	60			100		20			50	80
C. herbarum	20	20	30	100	20	30	50	80	20	40	60	50	30		40	100	80	100	90	80	100	90	90	100	100	100	80	100	80	90	100	100	100	90	90	100
Curvularia lunata						10																50	40			30	10						20			
Drechslera graminea																						30														
D. tetramera				30			40	20	10	30	50	60	20												80	90	100									
Epicoccum nigrum									20			50								80	50	50					50					20				
S. purpurascens																										10										
Fusarium oxysporum								50			10	20							20							10							20			
F. moniliforme	100	80	50	80	90	10	40	20	10	30	50	80	30					80	80	90	100	100	90	80	100	100	100	90	80	50		60		70	80	80
Gliocladium penicilloides																				50																
Helminthosporium oryzae		30	40	90	20	50				30								90		90	100	10	90	100		90	80			90		80	90		60	90
Humicola grisea									20												30	20											10			
Monilia sitophila																				10				10												
Nigrospora oryzae									20	30	50	50	40	10	20					30						10								10		
Paecilomyces varuta																							10													
Penicillium funiculosum	80	80	50	60	70	50	80	100	90	100	90	100	100	100	90	80	90	80	90	80	100	90	80	80	80	50	50	60	30	60	80	50	50	80	50	40
P. chrysogenum							10		30																											
P. minutum																				10																
Stachybotrys atra														10																						
Starkeomyces koorchalomoides																						10														
Stemphylium botryosum																				20																
Torula herbarum																										10					10					
Trichoderma viride							30			50	80	80			50	60	30					90		30				90	10		50	10	10	60		
Trichothecium roseum													30	40	30				30									40	50							
Verticillium alboatrum			30		30			10	20				30		10		30	40	20	10		20	10	20	30	10	20				20			10	20	10
Pestalotia sp.								10			10																									
White sterile mycelia							10	30	50					60	80	90	60			30		40	30	50	60		30		40	80	20		50		10	
Brown sterile mycelia							10		20							30										50	60						20			
Yellow sterile mycelia									50						60		20		30								10									
Neurospora sp.												10																								

K = Khemorulla variety; N = Ngoba variety; M = Mirikruk variety.

Table 2.3: Average frequency occurrence of fungal forms in the air of three varieties of paddy (expressed in percentage).

Fungi	K		N		M	
	A	Ph	A	Ph	A	Ph
Phycomycetous forms	0.80	1.00	0.60	0.90	1.05	0.75
Ascomycetous forms	0.42	3.18	0.16	2.92	0.56	2.09
Aspergillus spp.	12.07	13.12	13.02	12.59	9.58	12.61
Arthrinium sp.	5.66	6.15	4.84	4.95	5.54	5.11
Alternaria spp.	10.22	9.80	10.96	8.93	11.15	8.31
Cladosporium spp.	33.12	31.08	30.59	27.86	25.89	26.90
Helminthosporium oryzae	0.77	-	1.25	8.18	2.51	16.97
Trichoderma viride	3.07	9.18	3.01	5.13	2.90	0.81
Penicillium spp.	26.09	20.01	25.91	20.31	29.43	20.34
Other Deuteromycetes	7.18	7.6	9.54	8.10	10.50	6.05
Mycelia sterilia	0.60	0.05	0.12	0.07	0.89	0.06

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety;
A = Air; Ph = Phylloplane.

Table 2.4: Relationship (r values) between fungal population (both total No. of fungal species and average No. of fungal colonies/plate) isolated from the air and from leaf surface of the three varieties of paddy at different growth stages of the plant.

Variety	Total No. of species	Average No. of fungal colonies/plate
K	+ .848**	+ .796**
N	+ .786**	+ .671*
M	+ .701*	+ .606*

** Significant at 99% probability; * Significant at 95% probability; K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety.

Ngoba and Mirikrak. Moreover, the fungal population in terms of fungal colonies/plate was comparatively higher (number in thousands) on leaf surface environment in all the three varieties of paddy than in the corresponding air (number in hundreds).

DISCUSSION

Survey of aeromycoflora of the three paddy fields:

As evident from Tables 2.1 and 2.2 the majority of the forms isolated from the air belonged to Deuteromycetes which accounted for approximately 90 percent of the total mycoflora. This is also in accordance with the studies of previous workers (Mishra and Tewari, 1976a; Dixit and Gupta, 1980).

Cladosporium spp. and Alternaria spp. were the dominant forms in the air of all the three varieties although Penicillia and Aspergilli also occurred frequently at all the stages of the plant growth. It has been a well known fact that Cladosporium spp. are of wide occurrence in the air of different localities (Pady, 1971; Mishra and Tewari, 1976a). This fungus is known to be a major constituent of the mycoflora of plant surface (Mishra and Srivastava, 1970b) and also of air (Mishra and Kamal, 1971).

Other fungi such as Phycomycetes, Ascomycetes and sterile mycelial forms were poorly represented in the air

probably because of poor survival ability in the air.

Some species belonging to Aspergilli such as A. niger and A. flavus occurred quite frequently from the air of the three paddy fields. Penicillium spp. were also quite dominant and were found in the air of all the three varieties of paddy. Aspergilli and Penicillia are known to be of quite frequent occurrence in the air of different localities (Dickinson, 1967). Arthrinium sp. which was found commonly occurring in the nutrient plates was isolated frequently almost at all the sampling dates of the aerosporal studies. This fungus could be occurring in the air because of the availability of suitable condition for its growth.

The pathogen, H. oryzae was isolated from air from a quite early stage of the crop. The population, however, increased only at the later stages of the growth in Mirikrak variety (disease susceptible) because of release of spores from the leaf surface of the plants to the air. On the other hand, in the disease resistant variety although the inoculum was found in the air its population decreased with age and disappeared completely at the later stages.

As far as the population of T. viride was concerned, although it was present in the air of the three varieties of paddy, the population increased at later stages only in Khonorullo variety, the disease resistant one where the spores

were produced in large number on the leaf surface and they might have got released into the air.

In general, the total air fungal population increased with advancing age of the plants, the total number of species fluctuated at different sampling dates. This is in agreement with the results of previous workers (Mishfa and Srivastava, 1971a; Mishra and Tewari, 1976a; Kumar and Gupta, 1980). The fungal spores produced in large numbers on the leaf surface might be getting released into the air which accounted to the rich airspora of the locality. Most of the air fungal propagules are washed down subsequently because of heavy rains during rainy season (June-July) and only some fungi like Aspergilli and Penicillia occurred throughout the crop season. Later on, after the rains when the atmospheric moisture was high, the fungal population in the air increased. This may be because of the release of spores from the leaf surfaces which were then not subjected to rain wash (Fig. 2.1).

Classification of the aeromycoflora based on their occurrence in different months

The results from the present investigation revealed that the distribution of the fungi in the air depends on weather changes. Meterological factors such as atmospheric temperature, humidity, rain and wind speed were some of the important factors controlling the occurrence of fungal spores in the air and this is in agreement with the data of Mishra

and Tewari (1976a) and Kumar and Gupta (1980) who also found that the seasonal and diurnal periodicities of air fungal spores is mainly on account of climatic conditions. The total number of species and also the specific composition of the mycoflora was also affected by the weather conditions especially during rainy season (Dickinson, 1967).

Fungi belonging to Group I as shown in the results were mostly those which were dominant forms in the air viz., Cladosporium spp., Penicillium sp., Alternaria sp. and Aspergillus spp.

Most of the fungi which belonged to Group II (as given in the results) did not appear at any definite time and were of sporadic occurrence. This may be because of the effect of rain on their occurrence.

The fungi belonging to Group III may be associated with a particular set of climatic factors. Some of the fungi of this group occurred on one particular sampling date because of the presence of the inoculum at that time only. Some of these spores present in the air were carried from great distance due to the action of wind and such spores were infrequent forms found in the air.

Further, from the present studies it is also clear (Fig. 2.3) that Cladosporium herbarum showed the maximum

population at the end of rainy season from September onwards when the winter season sets in and the temperature falls to suit its performance in the air. On the other hand, in summer season, Alternaria sp., Aspergillus spp. and Penicillium spp. were the most commonly occurring fungi. It is commonly known that Cladosporium sp. is of wide occurrence in winter months and it is usually replaced by Alternaria subsequently during warmer days (Sinha, 1971; Dickinson, 1967, 1981).

Further, the maximum incidence of the pathogen H. oryzae in the air of the paddy plants was during the summer months when both the temperature and atmospheric moisture were high (especially after the rains).

Correlation between aeromycoflora and phylloplane mycoflora

The distribution pattern of fungal flora throughout the sampling periods on the leaf surface was very much similar to that of air fungi.

As evident from the present investigation (Table 2.4) a close correlation exists between the airspora of the locality and the leaf surface fungi. The leaf surface release fungal spores which largely contribute to the airspora of the locality. Most of the spores of air were also trapped on the leaf surface (Table 2.1). Mishra and Srivastava (1971b), Mishra and Tewari (1976a) reported that a sort of cyclic phenomenon exists between

fungal spores of air, soil and plant surface. Some of the form forms, however, maintained their specificity in the specialized environment. As evident from the present investigation (Table 2.1 and Fig. 2.2) 71% species were common in the two environments viz., air and phylloplane and only 26% species appeared exclusively from phylloplane and 3% species were of specific occurrence in air. The specificity of forms was due to change in nutritional status, micro-ecology and the other unknown conditions of the different environments. This accounted for the restricted occurrence of certain forms in the phylloplane and air of paddy plants. Similar results have also been obtained by Mishra and Tewari (1976a). Sinha (1971) observed that 12 and 4 fungal species appeared exclusively from the phylloplane and air flora respectively. Further, Lamb and Brown (1970) also reported that Aureobasidium pullulans, Penicillium spinulosum and Oidodendrum sp. isolated from leaves were not detected from the air. Gregory (1950 and 1961), on the other hand, remarked that number of spores deposited on leaf surface was nearly proportional to the number of spores in the air spora. The results of the present investigation are in accord with the observation of Gregory in that the frequency of percentage of the dominant fungi in the air viz., Cladosporium, Alternaria, Aspergilli, Penicillia varied almost on the same pattern as on the leaf surface of the corresponding sampling dates (Table 2.3). The results, however, also agree with the findings of Lamb and Brown (1970), Sinha (1971) and Mishra and

Tewari (1976a).

The dominant forms associated with the leaf surface of the three varieties of paddy at different developmental stages were trapped concurrently with considerably high percentage of occurrence from their air also (Table 2.3). Hudson (1971) suggested that the spores present in the air were impacted on the leaf surface. Pugh and Buckley (1971); Gregory (1971) and Pady (1971) also recorded that leaf acts as a landing site for various microbes present in the air. Once trapped on the leaf surface the microorganisms derive benefit from the substrate and they grow and multiply thereupon in presence of the various nutrients. Sutton (1953) observed that each leaf is surrounded by so called a boundary layer in which the temperature, moisture and wind speed are influenced by the leaf. As evident from the present investigation (Table 2.3) the micro-environment of the boundary layer may not be suitable for all the microorganisms trapped on the leaf surface and most of the forms casually present on the surface do not thrive well. Hudson (1971) also observed that among the various species impacted on the leaf surface relatively few succeed in colonizing leaf surface.

Further, by using Pearson's product moment correlation coefficient (Table 2.4) it is clear that there does exist a positive correlation between the fungal population of the two environments viz., air and phylloplane. This correlation was found to be highly significant for Khonorullo variety (disease

resistant) and also for Ngoba variety (moderately susceptible). In this case it was observed that when the two environments viz., air and phylloplane were compared together there was higher fungal population on the leaf surface of the two varieties (Khonorullo and Ngoba) than in the disease susceptible variety (Mirikrak) although the fungal population was almost similar in the air of all the paddy varieties studied. This is because the micro-environment of the plant surface of Mirikrak variety probably did not prove conducive for their successful colonization because of the domination of the pathogen H. oryzae on the leaf surface. In addition, fungal population was more on leaf surface of the three varieties than in the air. This is because the leaf surface provides nutrients in the form of leaf leachates and the habitat is thus more suitable for the growth of the fungal forms than in the air where the fungi having high saprophytic ability can survive successfully.

Moreover, as evident from Fig. 2.8 and Table 2.3 it is clear that the pathogen, H. oryzae although present in the air of Khonorullo variety, is conspicuously absent from the leaf surface probably because of its disease resistant nature. On the other hand, T. viride although present in almost the same proportions in the air of all the three paddy varieties (Fig. 2.9), was dominant on leaf surface only in disease resistant variety (Khonorullo) and subdominant on the moderately susceptible (Ngoba) variety. This could be an example of the existence

of natural biological control in the Ngoba variety where due to domination of T. viride at the later stages, the infection of the pathogen is eliminated.

Further, it is also clear from Fig. 2.8 that though the spores of H. oryzae occur at an early stage of the crop growth in the air of all the three varieties of paddy, the visible symptom of the brown spot disease were seen only from July-August when the optimum conditions like temperature and moisture were available. The disease developed rapidly and was discernible to its maximum level in the disease susceptible variety (Mirikrak) particularly on old and mature leaves when the plants were mature. The spores of the pathogen which increased tremendously on the leaf surface of the susceptible variety, were also released into the air and thus there was an increase in the population level in the air which is evident from Fig. 2.8. The maximum population of the pathogen in the air was thus registered in the susceptible variety only at later stages of the plant growth which coincided with the maximum disease intensity in the field.

Thus from the present studies it is quite clear that the fungal spores present in the air showed a great impact on the leaf surface of the three varieties of paddy since there was a direct correlation between them. Further, it may also be concluded that the occurrence of the pathogen, H. oryzae in the air at an early stage of the crop growth may be helpful

in making a forecast regarding the occurrence of the brown spot disease of paddy which is quite prevalent in the locality where the present investigation was carried out. Moreover, such aerobiological and phylloplane studies thus may be of suggestive value in the control measures of the pathogen.

CHAPTER III

ISOLATION OF THE PATHOGEN AND THE ROLE OF DIFFERENT FACTORS CONTROLLING ITS OCCURRENCE

INTRODUCTION

The surface colonizers are responsible for manifold activities on the plant parts. Some of them may be simply accidental settlers while others play an important role in leaf infection as well as in the disease syndrome(Sharma and Gupta, 1980).

The fungi are the most important group of plant pathogens because of the number of diseases they cause and financial losses incurred thereby.

Usually, any investigation in pathological studies begins with a search of the pathogen which causes a particular plant disease. In order to establish the disease caused by a particular pathogen, it is necessary to satisfy Koch's postulates which require experimental reproduction of the disease by inoculation with a pure culture of the suspected pathogen. Many organisms have been decisively recognised to be the causal agents of plant diseases by use of Koch's postulates.

An important step in pathogenicity studies is the isolation of the suspected pathogen from the diseased plant in order to establish the organism in pure culture. Disease tissues may often contain more than one organism. In most instances, the primary pathogen enters the host tissue first

and later, secondary organism usually nonpathogens may enter and colonize the tissue previously penetrated by the primary pathogen. Other nonpathogens contaminating the diseased tissue may be removed by surface sterilization and the pathogen can thus be isolated by inoculating the surface sterilized tissue on a nutrient medium.

The rice plant is a warm climate annual which is grown without irrigation in upland areas and with irrigation in lowland. Many diseases are common on paddy. Leaf spot, blast and culm rot maladies are of major importance.

The leaf spot disease of rice is a widespread disease in almost all the rice growing areas of the world. In India the disease is prevalent in all the rice growing areas, especially in heavy monsoon areas in West Bengal, Eastern part of U.P., Assam, Meghalaya, Tamil Nadu and parts of Kerala. The disease is seed borne and is of great importance in several countries and has been reported to cause enormous losses in the leaf spotting phase when it can assume epiphytotic proportions. Aeroscope studies by Padmanabhan et al. (1953) revealed that conidia of the fungus are present over rice fields in the months of April-July, i.e. during the sowing season and sometimes even earlier. The air-borne inoculum from some external source appears to cause the primary infection in a locality. These external sources must be perennial grass hosts and early sown or winter paddy crop.

Two major epidemics have been recorded in India, the most recent being in Bengal in 1942 when loss in yield upto 90% was recorded (Ghose et al., 1960). About two million people died of starvation mainly because of this disease and according to Padmanabhan (1973) nothing as devastating as the Bengal epiphytotic of 1942 has been recorded in plant pathoglogical literature. In 1942, there was excessive rainfall in September, uniformly favourable temperatures of 20-30°C continuously for two months, unusually cloudy weather and rains in November, all contributed to the rapid spread of the disease leading to an epidemic. According to Kawada et al. (1954) annual losses in yield due to this disease are regarded to be 22,000 - 28,000 tons.

Like brown spot, blast and culm rot are also serious diseases occurring, in the humid rice producing areas of the world.

Much emphasis has been placed on different environmental factors affecting overall development of the brown spot disease caused by Helminthosporium oryzae Breda de Haan (Padmanabhan, 1973). It was not until recently however, that the infection process of H. oryzae was considered to be of multicomponent nature (Roy and Sen, 1970). For quantitative studies of host parasite interactions the parasite population should be synchronized (Yadav, 1983) for which, synchrony in

the spore germination is prerequisite. The physiology and pathogenicity of H. oryzae has been studied by a number of workers (Ganguly and Padmanabhan, 1962; Shishiyama et al., 1969; Chattopadhyay et al., 1970) but the influence of various factors on spore germination of H. oryzae has been studied fragmentarily (Nishikado, 1923; Ocfemia, 1924, Roy and Sen, 1970; Purkayastha and Mukhopadhyay, 1974).

The present investigation was aimed to isolate the pathogen, H. oryzae from the diseased leaves of paddy varieties and establish the organism in pure culture and then do pathogenicity test to find out whether H. oryzae was the causal organism. Further, the effects of spore concentration, incubation temperature, culture age, buffer molarity and pH on germination and growth of the fungus were also studied to find out the different factors affecting colonization of paddy leaves by H. oryzae.

MATERIALS AND METHODS

Research materials:

The disease susceptible variety Mirikrak and the moderately susceptible variety Ngoba were used as the plant material from which the pathogen was isolated.

Isolation of the pathogen:

The pathogen was isolated by washing the diseased leaves of Ngoba and Mirikrak varieties first by sterilized distilled water 5 - 6 times and then by 0.1% $Hgcl_2$ for 1 or 2

mins and subsequently washing again with sterilized distilled water 5-10 times. Later on, the leaves were dried on sterilized filter papers, teased into small fragments with the help of sterilized forceps and then plated on nutrient agar media (Potato dextrose agar). The fungus which was growing out of the tissue and sporulating was transferred on to fresh media and pure cultures of the fungus was maintained on slants of potato dextrose agar and Czapek's Dox agar media.

Pathogenicity test

In vitro: Spore suspension of the concentration of approximately 2 x 10^5 spores/ml of the pathogen was made and sprayed on to leaves (with no initial disease symptoms) which were placed in moist chambers. The spraying was repeated 2-3 times. The leaves were then observed for symptoms of the disease after 3 - 5 days. The disease symptoms occurring on the leaves which were kept in moist chambers were compared with the disease occurring in the nature than from the infected leaves kept in moist chambers the pathogen was reisolated by following the same method as described before. The pathogen which was isolated from the leaves kept in moist chambers was compared with the one initially isolated from the diseased leaves occurring in nature.

In vivo: Spore suspension (2 x 10^5 spores/ml concentration)of the pathogen was sprayed on inteact leaves of

paddy plants of both the varieties (Ngoba and Mirikrak) which had no initial symptoms of the disease. These plants were grown in pots and were kept in net house. These plants which were sprayed with the spore suspension of the pathogen were covered with moist polythene bags for 2 - 3 days till visible symptoms of the disease occurred on the leaves. The same procedure was followed for isolating the pathogen as given for vitro.

Effect of different factors on spore germination of the pathogen

I) Effect of Molar concentrations of phosphate buffer on spore gemination and germtube growth of the pathogen.

The spore suspension of the pathogen was made by flooding the culture plates with sterilized distilled water and the concentration of 2×10^5 spores/ml was adjusted with the help of a haemocytometer. Different molar concentrations of phosphate buffer viz., 1, 0.1, 0.01, 0.001 molars were prepared and the spores of the pathogen were germinated in the various molar concentrations by hanging drop method in cavity slides. After 24 hours of incubation at 20-25°C the germination rate and germtube length were measured. A control was also maintained where the spores were germinated in sterilized distilled water.

II) Effect of pH on germination and germtube growth of the pathogen spore.

Different ranges of pH from 5-8 of phosphate buffer 0.01 M were prepared by serial dilutions. The spores of the pathogen were germinated in different pH by hanging drop method in cavity slides. The spore germination rate and germ tube length was measured after 24 hrs of incubation at 20-25°C. A control was also maintained.

III) Effect of spore concentration on germination and germ tube length of the pathogen.

Different spore concentrations of the pathogen (1×10^4 - 24×10^4 spores/ml) were prepared by diluting the spore suspension with sterilized distilled water. The spore concentration of the pathogen was adjusted by haemocytometer counts. The spores were germinated in cavity slides by hanging drop method. After 24 hours of incubation the germination rate and germ tube length was measured.

IV) Effect of age of culture on germination and germtube growth of the pathogen.

The cultures of the pathogen were incubated for different period (5-120 days) and their spore germination and germ tube length were measured by hanging drop method in

Table 3.1: Effect of spraying of spore suspension of the pathogen on leaves kept in moist chamber

Days after inoculation	Length of lesion on Ngoba variety* (in mm)	Length of lesion on Mirikrak variety* (in mm)
4	6	8
10	10.5	11.1
15	13.6	13.9
20	14.8	15.1

* Average of 10 leaves.

Table 3.2: Effect of spraying of spore suspension of the pathogen on intact leaves of the plant.

Days after inoculation	Length of lesion on Ngoba variety* (in mm)	Length of lesion on Mirikrak variety* (in mm)
5	6.5	6.8
10	11.2	11.1
15	14.9	15.2
20	18.9	18.6

* Average of 10 leaves.

Table 3.3: Effect of Molar concentrations of phosphate buffer on spore germination and germtube growth of H. oryzae.

Conc. (M)	Germination* after 24 h (%)	Germtube ** length (µm)
1.0 M	54.59 ± 6.29+	85
0.1 M	68.21 ± 11.89	101
0.01 M	98.03 ± 3.56	256
0.001 M	92.31 ± 10.91	208
Control (distilled water)	98.39 ± 3.07	289

* 500-800 spores counted

** Average length of germ tubes based on 100 spores.

\+ S.D. Temperature 20-25°C.

Table 3.4: Effect of pH on germination and germtube growth.

pH (.01 M phosphate buffer)	Germination* after 24 h (%)	Germtube** length after 24 h (µm)+
5	72.12±4.28+	108
5.6	79.26±3.16	110
5.8	82.12±2.18	102
6.0	86.40±1.57	105
6.6	89.28±2.45	108
6.8	91.82±1.68	112
7.0	92.18±2.43	110
7.6	90.18±1.86	103
7.8	82.22±1.67	102
8.0	80.16±4.32	106
Control (sterilized distilled water)	95.26±0.12	110

* 500-800 spores counted; ** Average length of germtube based on 100 spores; + S.E. Temperature 20-25°C.

Table 3.5: Effect of spore concentration on germination and germtube growth.

Spore concentration (per ml. of water)	Germination* after 24 h (%)	Germtube** length after 24 h (µm)
1×10^4	98.26±1.02+	450
2×10^4	97.08±2.32	420
4×10^4	95.18±1.44	397
8×10^4	81.28±2.39	199
12×10^4	68.02±1.60	105
16×10^4	31.22±2.00	30
20×10^4	12.10±1.12	15
24×10^4	2.23±1.22	Germ pore bulging in some spores but no germination

* 500-800 spores counted; ** Average length of germ tubes based on 100 spores; + S.E. Temperature 20-25°C.

Table 3.6: Effect of age of culture on germination and germ-tube growth.

Age of culture (days)	Germination after 24 hrs (%)*	Germtube length after 24 hrs. (µm)**
5	72.12 ± 1.23+	86
10	85.18 ± 2.08	98
15	93.22 ± 0.60	101
25	94.10 ± 1.02	104
35	95.21 ± 2.16	103
45	95.80 ± 1.11	98
55	81.82 ± 2.62	58
65	70.22 ± 3.12	26
75	51.10 ± 1.26	15
90	33.62 ± 2.58	Germ spores slightly bulging and germ tubes very small
120	0 ± 0.00	0

* 500-800 spores counted

** Average length of germ tubes based on 100 spores.

\+ S.E. Temperature 20-25°C.

Table 3.7: Effect of temperature on germination and germtube growth.

Temperature (°C)	Germination* after 24 h (%)	Germtube length** after 24 h (µm)
10	42.26 ± 1.26+	52
20	85.62 ± 2.01	72
25	90.71 ± 1.05	79
30	92.16 ± 1.07	96
35	96.26 ± 1.32	148
45	82.21 ± 1.04	23
60	0 ± 0.00	0

* 500-800 spores counted; ** Average length of germtubes based on 100 spores; + S.E. Temperature 10-60°C.

cavity slides.

V) Effect of incubation temperature on spore germination and germ tube growth of the pathogen.

Cultures of the pathogen were kept at different incubation temperatures ranging from 10-60°C for 6 days. The spores were taken out from these cultures maintained at different temperatures and by hanging drop method in cavity slides the spores were germinated. After 24 hrs. the percentage germination and germ tube length were measured.

RESULTS

I) Isolation and identification of the pathogen

The pathogen was isolated from the leaves of Ngoba and Mirikrak varieties after surface sterilizing the leaves and plating on potato dextrose agar medium. The pure cultures of the pathogen were maintained on Czapek's Dox agar and potato dextrose agar media.

The fungus grew intra and inter-cellularly within the tissues. Conidiophores arose as lateral branches from the hyphae usually in tufts, and were short, erect, branched only at bases, segmented dark brown to olivaceous at the base and somewhat paler at the growing tip. Conidia were olivaceous brown, slightly curved, tapering towards the rounded ends and varied greatly in shape and septation.

II) Pathogenicity test

I) In vitro: When the spore suspension of the pathogen was sprayed on to leaves kept in moist chamber after 3-4 days, minute brown coloured leaf spots occurred on the leaves. After 10 days of incubation in the moist chambers, the leaf spots spread and coalesced together and became ellipsoidal or oval shape with a reddish brown margin. Table 3.1 clearly shows that with increase in days of incubation the lesion length also increased both on Ngoba and Mirikrak varieties. Further, on Mirikrak variety the lesion length was slightly more than on Ngoba variety.

The lesions occurring on the leaves kept in moist chamber were compared with those occurring on the plants growing in the field. It was clear that the symptoms were almost similar in both the cases.

Further, the pathogen was reisolated from the diseased leaves kept in moist chambers and the pure cultures were compared with the culture obtained from the field samples. It was seen that the cultures obtained from the two sources resembled each other.

II) In vivo: When spore suspension of pathogen was sprayed on intact leaves of paddy plants, it was observed that after 5 days of inoculation the lesions occurred on the leaves

as minute brown spots which with increase in days of inoculation also increased in length. These brown spots also occurred on the coleoptile, leaf blades, leaf sheaths and sometimes even on the culm. They were found scattered all over the leaf surface. After 20 days of inoculation the spots on leaves coalesced with each other thus becoming irregular in shape and later on the entire leaf became brown and started drying. This was quite distinct on leaves of Mirikrak variety where the spots measured as much as 18-19 x 1-3 mm (Table 3.2).

The pathogen was reisolated from the leaves sprayed with the spore suspension and it was found that it was similar to the pathogen isolated initially from the diseased leaves occurring in nature.

III) Effect of different factors on spore germination of the pathogen

I) Effect of molar concentrations of the phosphate buffer on spore germination and germ tube growth of the pathogen.

From Table 3.3 it is clear that when different molar concentrations (1, 0.1, 0.01, 0.001) of phosphate buffer solutions were tested at pH 6, almost normal germination and slight stimulation in germ tube length occurred at 0.01 M, but 1 M, 0.1 M, 0.001 M markedly inhibited elongation of germ tubes and also the germination. As the percentage germination

did not vary significantly between the control and 0.01 M, and the average germ tube length was nearest to that of control, the concentration of 0.01 M was chosen for the pH experiment.

II) Effect of pH on germination and germ tube growth of pathogen spores.

Table 3.4 indicates that the spores germinated at a pH range of 5-8. Germ tube length was maximum at pH 6.8 which was slightly higher than the control. Maximum germination occurred at pH 7.0 which was nearest to the control. The optimum pH was thus around 6.8-7.0.

III) Effect of spore concentration on germination and germ tube growth of the pathogen.

Inhibition of germination and germ tube growth were observed in the concentrated spore suspension. Of the eight concentrations used, the most significant inhibition was at 24×10^4 spores/ml (Table 3.5) where the percentage germination was as low as 2% only, germ pores bulged and no visible germ tube could be observed.

IV) Effect of age of culture on germination and germ tube growth of the pathogen spores.

The average germ tube length and germination rate of spores collected from 10-45 days old cultures were similar but it was slightly lower in 5 day old culture under identical

conditions (Table 3.6). Spores collected after 45 days old cultures showed decrease in germination rate and germ tube length was also shorter. Spores from 90 day old cultures showed shrunken, thinner, shorter and contorted germ tubes and sometimes only the germ pores bulged and the germ tubes were not clear. Spores from 120 day old cultures showed no germination at all.

V) Effect of temperature on germination rate and germtube length of pathogen spore.

Table 3.7 clearly shows that the spores germinated in a wide range of temperature, the optimum temperature for germination and germ tube growth of the pathogen was at 35°C. Complete inhibition of germination was observed at 60°C.

DISCUSSION

The pathogen responsible for the brown spot disease of paddy was found to be Helminthosporium oryzae Brede de Haan (= Cochliobolus miyabeanus Ito and Kuribay Drechsler ex Dastur). This pathogen was isolated from the infected leaves of paddy varieties, viz., Ngoba and Mirikrak occurring in the field. The fungus was cultured on potato dextrose agar medium and pure cultures were maintained on potato dextrose agar and Czapek's Dox agar slants.

The fungus was tested for pathogenicity using Koch's postulates both in vitro and in vivo. In vitro, the pathogens caused distinct brown spots on leaves kept in moist chambers. With increase in days of inoculation, the minute brown spots increased in length and they occurred as large spots with pale yellow colour in the middle and reddish brown margins. Sometimes, the small spots coalesced and formed large irregular shaped markings on the leaves. This was similar to previous observations by other workers (Subramanian, 1971; Padmanabhan, 1973; 1974; Singh, 1973; Mehrotra, 1980; Baruah et al., 1980). The lesion length was more on disease susceptible variety (Mirikrak) than the moderately susceptible one (Ngoba). It may be because the leaves of the susceptible variety provides conducive environment for the pathogen with no resistance from it than the moderately susceptible variety.

In vivo, the brown spots of the pathogen occurred on the leaf sheaths, leaf blades, coleoptiles and even on the culms. The spots increased in length after days of inoculation and the spots sometimes coalesced on the leaves and formed irregular shapes. On leaves of Mirikrak variety, the entire leaf became brown and dried. The length of the lesions was more on the intact leaves than on the leaves placed in moist chamber. This may be because the intact leaves provide nutrients and moisture for the pathogen, whereas, the leaves kept in moist chamber have only little nutrients and the leaves

itself start drying after 10-15 days of incubation.

The most destructive phase of the infection is the leaf spot phase which causes the greatest damage. The infection of the leaf result in reduction of effective leaf surface which in turn causes a marked weakening of the plants especially during seedling stage (Singh, 1973).

The pathogen could be reisolated from the infected leaves (both from vivo and vitro) and when pure cultures were made they were found to be same as the initial inoculum proving thereby that Helminthosporium oryzae was definitely the causal organism of the brown spot disease of paddy.

When the effect of molar concentration of phosphate buffer was studied on germination and germ tube growth of the fungus, almost similar percentage germination and average germ tube length at 0.01 molarity and the control indicated unimpaired germination (Table 3.3). Same molarity has been reported as optimum for H. oryzae by Purkayastha and Mukhopadhyay (1974). Thus the concentration of 0.01 M was chosen for the pH experiment.

Results in Table 3.4 indicate that the spores germinated at a wide pH range between 5-8 with optimum at 6.8. Previous workers (Purkayastha and Mukhopadhyay, 1974) have also reported the optimum pH for the growth of the fungus to

be about 6.5.

Inhibition of germination and germ tube growth were observed in the concentrated spore suspensions (Table 3.5) which was quite distinct at 24 x 10^4 spores/ml where the spores showed very low germination rate and sometimes only the germ pores bulged and very minute germ tubes could be seen. The comparatively low percentage of spore germination in concentrated spore suspension may be ascribed to the limitation of nutrients and oxygen due to the overcrowding of spores (Pukayastha and Mukhopadhyay, 1974).

Poor germination and short germ tubes in too young (5 days) or too old (55-120 days) spores (Table 3.6) could be due to immaturity of spores in young cultures (Gottlieb, 1950; Purkayastha and Mukhopadhyay, 1974; Yadav, 1983) and loss in vigour of old spores because of respiration causing depletion of endogenous substrates (Shirashi et al., 1970; Yadav, 1983).

Although the spores germinated at a wide range of temperatures (Table 3.7) the optimum for both germination and growth of the test strain was at 35°C. Optimum zone for H. oryzae is thus transitional to broad and sharp types described by Cochrane (1960). But complete inhibition was however, noted at 60°C. At higher temperature (60°C) the fungus might have got killed which is indicated by complete inhibition in germination.

From the present studies it is clear that the causal organism for the disease occurring on Ngoba and Mirikrak varieties of paddy was Helminthosporium oryzae which caused brown spot disease on paddy. Further, from the studies it is clear that the optimum temperature for its growth was around 25-35°C. Padmanabhan (1973) in his analysis of the great Bengal famine has hypothesized that the disease had assumed such epiphytotic proportions in 1942 primarily because of the existence of optimum weather conditions during the period. Best development of the disease occurs when there is high temperature (around 35°C) and high moisture along with a cloudy weather which is similar to the results of the present investigation.

The susceptibility to the pathogen, H. oryzae increased with age which is clear from the pathogenicity studies conducted in vitro and in vivo.

In addition, for studies involving host-parasite interaction, if the parasite population has to be taken, then in the case of H. oryzae, spores from 15 day old cultures with a concentration of 2×10^5 spores/ml suspended in 0.01 M phosphate buffer at pH 6 if incubated at 35°C may give good results.

CHAPTER IV

INTERACTION STUDIES IN VITRO AND IN VIVO BETWEEN CERTAIN EPIPHYTIC FUNGI AND THE PATHOGEN

INTRODUCTION

The phylloplane is usually inhabited by a variety of saprophytic and parasitic microorganisms which interact among themselves and also with the living host plant. The effect of nonpathogenic microorganisms on infection of aerial parts of plants by pathogens has attracted much attention lately. The obvious attraction of this type of study is interaction phenomenon related to these saprophytic microorganisms which may provide a form of defense against infection and disease development of the biological control of plant pathogens. This is basically the phenomenon of antagonism. Fokkema (1976) defined antagonism as a relation between organisms in which one organism the antagonist, creates adverse circumstances for the other.

There is an extensive literature on antagonistic interactions between saprophytes and plant pathogens in relation to development of possible biological control methods (Rainecka, 1981). The subject is reviewed by Baker and Cook (1974) who defined biological control from the plant pathologist's standpoint as follows: "Biological control is the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist, or by mass introduction of one or more antagonists".

Recent in-depth explorations of foliar microflora and its impact on disease incidence have stimulated an interest in managing microflora and microorganisms for disease control (Cook, 1971). Successful strategies for biological control of certain insect and weed pests and economic considerations of disease control costs also encourage the use of microorganisms to control foliar diseases.

To visualise biologial control of foliar diseases, it is useful to identify two general steps in the research progression, firstly, fungi and bacteria, the predominant microorganisms in the phylloplane, which are antagonistic to the pathogenic fungi must be isolated and secondly the microorganisms must be applied to plants in time to antagonise pathogen thereby preventing infection. This may be achieved by establishing the fungi or bacteria as residents of the phylloplane at an early stage of plant growth or by timely application of the microbes by some physical method such as spraying (Spurr, 1981). The main purpose of the biological control of a plant disease is to suppress the inoculum load of the target pathogen to below the level that potentially causes an economically significant outbreak of the disease. Its suppressive effect is often not as spectacular as chemical means, but unlike most of the chemicals it does not cause pollution of the environment and attacks only limited target pathogens (Tsuneda and Hiratsuka, 1981).

In recent years the increasing use of potentially hazardous fungicides in agriculture has been the subject of growing concern of both environmentalists and public health authorities. The control of plant diseases by chemicals can be spectacular but this is relatively a short term measure and moreover, the accumulation of harmful chemical residues sometimes causes serious problems. Biological methods, on the other hand can be economical self perpetuating and usually free from residual side effects (Baker and Cook, 1974; Snyder et al., 1976; Wilson and Huffaker, 1976).

A serious problem in implementing biological control measures, however, has been the discrepancy between the results obtained in the laboratory and those achieved in the field. A certain biological system may operate admirably in the laboratory, but it often is completely inoperative or its suppressive effect fluctuates considerably under field conditions. This is due to the fact that these measures make use of living organisms and they are effective only within a relatively narrow range of environmental factors. This appears to be quite reasonable because in the field it is a complex microbial ecosystem. Interactions among the vast and complex sphere of miroorganisms are impossible to duplicate in the laboratory. Further, use of mycoparasites may be difficult for controlling plant diseases because physical contact is necessary for mycoparasites to kill plant pathogens and it is often difficult to ensure delivery of mycoparasites to target

organisms. Also, mycoparasites need time to react with the target organisms, and their action may be too slow for diseases of annual crops. Further, as a result of the environmental restrictions such as temperature, pH, moisture content which limit the use of biological control measures, scientists have proposed for a concept of integrated control which combines several techniques of control including the biological ones (Karve, 1982).

The possibility of controlling plant pathogenic fungi by antagonistic microorganisms added either as a substitute or as an additive to fungicides has been the subject of extensive research and recently many common leaf saprophytes have been explored as a possible biological control agent of different pathogens (Heuvel, 1969, 1970, 1971; Mishra and Tewari, 1976c; Rai and Singh, 1980; Srivastava et al., 1981; Spurr, 1981; Reinecke, 1981; Fokkema, 1981; Chet and Baker, 1981; Gupta and Dixit, 1982; Purkayastha and Bhattacharyya, 1982; Elad et al., 1983).

Among the many potentially antagonistic plant surface inhabitants, members of the genus Trichoderma have gained considerable success (Dennis and Webster, 1971). This is primarily because members of the genus are active both as hyperparasites and as antibiotic producers. (Hadar et al. 1979).

Brown spot, which is caused by the pathogen, Helminthosporium oryzae Breda de Haan, is an economically

important disease occurring on paddy all over the world. In India, it is one of the most destructive fungal disease of rice which may cause almost total destruction of the crop (Pans, 1970) as in the case of the Bengal famine in 1942-1943 causing losses in the yield upto 90%. The control of this disease can be only achieved by spraying fungicides such as Bordeaux mixture at regular intervals. But even with fungicides the air-borne spread of the disease cannot be controlled. Only few examples of biological control is available in literature. Akai and Kuramota (1968) found that a Candida sp. reduced the number of lesions on leaves. They, however, observed that none of the microorganisms isolated from rice leaves inhibited mycelial growth of H. oryzae on agar. Initial experiment (Chapter I) during isolation of phylloplane fungal studies showed that possibly a natural biological control could be occurring on phylloplane of paddy varieties viz., Khonorullo and Ngoba between Trichoderma viride and H. oryzae. It seemed probable, therefore, that an interaction on the leaf surface between T. viride and H. oryzae could prevent or reduce brown spot disease. Thus, laboratory and field experiments were undertaken to do a survey of different probable antagonists of the pathogen. Different epiphytic fungi isolated from leaf surface of the three varieties of paddy viz., Khonorullo, Ngoba and Mirikrak were used for interaction studies in relation to H. oryzae in vitro. The most efficient antagonists in vitro studies were used in vivo for biological control of the brown

spot pathogen of paddy.

MATERIALS AND METHODS

Research Materials:

The three varieties of paddy viz., Khonorullo (disease resistant), Ngoba (moderately susceptible) and Mirikrak (susceptible) were used as the experimental plants.

Fungi:

The following fungi were isolated from leaf surface of paddy and maintained in pure culture for the interaction studies:

Alternaria alternata, Aspergillus clavatus, A. flavus, A. nidulans, A. niger, Acremonium persicinum, Arthrinium sp., Aureobasidium pullulans, Botrytis cinerea, Chrysosporium pruinosum, Cladosporium herbarum, Candida albicans, Curvularia lunata, Chaetomium globosum, C. bostrychodes, Epicoccum nigrum, Fusarium moniliforme, Gliocladium penciloides, Humicola prisea, Melanospora zamae, Memnoniella echinata, Mucor hiemalis, Nigrospora oryzae, Penicillium funiculosum, P. chrysogenum, Pestalotia monorhyca, Phoma glomerata, Rhizoctonia solani, Rhizopus nigricans, Stachybotrys atra, Starkeomyces koorchalomides, Torula herbarum, Trichoderma viride, Trichothecium roseum and Verticillium albo-atrum. The pathogen, Helminthosporium oryzae was also isolated from

infected leaves and pure cultures maintained on Czapek's Dox agar medium at 25 ± 1°C. All the test fungi and the pathogen used were 7-day old which were maintained on Czapek's Dox agar medium at 25 ± 1°C.

Interaction studies in vitro

I) Effects on spore germination:

i) All test fungi were cultured for 7 days at 25 ± 1°C in Petri dishes (9 cm in diameter) containing 12.5 ml Czapek's Dox agar. Agar discs, 8 mm in diameter, punched from periphery of the colonies with the help of a cork borer, were inverted, placed in small empty petri dishes and seeded with a drop of washed H. oryzae spores, with a concentration of about 2 x 10^4 spores/ml. Six discs were punched from a group of 3 colonies of each isolate. As a control, agar discs from uninoculated plates, also kept at 25 ± 1°C for 7 days, were seeded with H. oryzae spores in the same way. After incubation for 5 h at 25±1°C, the germination of 100 H. oryzae spores chosen at random from each series of 6 discs, was determined. By comparing germination percentages on discs with and discs without the test fungi, percentage of inhibition of spore germination was calculated using the following formula:

$$G_i = \frac{G_c - C_t}{G_c} \times 100$$

where G_i = inhibition of spore germination (%)

G_c = spore germination on control discs (%)

G_t = spore germination on discs with test fungi (%).

ii) Spores were harvested from 8-day old cultures of test fungi and the pathogen by flooding Petri dishes with sterile distilled water. They were washed twice with sterile distilled water and centrifuged at low speed (800 rpm) before their germination rate was tested. Spore suspension of 50 - 60 spores/0.01 ml was prepared for each fungal strain.

a) The spore suspension of the test fungi (S, saprophytes) and pathogen (P) were mixed in different proportion S:P 3:1, 3:2, 2:3, 1:3 and placed in a cavity slide and percentage germination of the pathogen was determined after 24 hrs. Control was maintained by germinating the pathogen spores in sterile distilled water.

b) Equal volumes of the spore suspension of pathogen and one of the test fungi was mixed and placed in a cavity slide to assess the percentage of spore germination and rate of germ tube extension of the pathogen at different incubation times. Control data were recorded germinating pathogen spores in sterile distilled water. The same procedure was used for different test fungi.

II) Effects on mycelial growth:

Agar discs 5 mm in diameter were punched from the colonies of test fungi and the pathogen by using cork borer. The discs were inverted and placed on newly prepared plates of Czapek's Dox agar. On each plate one disc each of the test fungus and H. oryzae was placed 30 mm apart, each being 15 mm from the centre of the plate. 5 replicates of each test fungus were maintained.

After incubation for 7 days at 25 ± 1°C, the radial growth of the pathogen colonies was measured along two axes from colony centre to the perimeter remote from and adjoining colonies of the test fungi. The former representing 'normal' and the latter 'influenced' growth. Inhibition of the mycelial growth of H. oryzae was expressed in percentages, calculated from the formula given by Heuvel (1970).

$$M_i = \frac{M_c - M_t}{M_c} \times 100$$

where M_i = inhibition of mycelial growth (%)

M_c = 'normal' mycelial growth (mm) and

M_t = 'influenced' mycelial growth (mm).

M_c and M_t were calculated from the average of five replicates.

The assessment of the interaction was made according to a model presented by Skidmore and Dickinson (1976) for colony interaction which was based on the observation of Porter (1924).

III) Effect of antibiotic activity:

i) The antagonists were grown from an inoculum disc over the surface of a cellophane membrane laid on Czapek Dox's agar medium and the metabolits were allowed to diffuse through the cellophane into the agar. A single sterile sheet of cellophane 50 µm thick was placed aseptically over the agar in each dish and the dishes were left overnight to allow excess moisture to evaporate. Discs 6 mm in diameter were cut from the margin of a 7 day old culture of antagonists isolates and each of the prepared plates was inoculated in a central position. The plates were incubated under a bank of light at 25 ± 1°C for 2 days. After this the cellophane and the adhering fungi were removed. A 6 mm disc of the pathogen was then placed on the medium in the plate at the central position previously occupied by the antagonist. Three replicates were maintained. In control, sterilized cellophane sheet was kept without any test fungi for 2 days and the inoculated by the pathogen after removing the cellophane sheet.

ii) The test fungi which had high antagonistic activity towards the pathogen were cultured on Czapek's Dox

liquid medium for 30 days. Mycelial mats were separated from the culture and cell free culture filtrate of different test fungi was obtained by filtering through microfilters. The culture filtrate was used to determine the antibiotic action by germinating spores of the pathogen, H. oryzae in one drop of the filtrates of various test fungi in cavity slide using hanging drop method. The percentage germination and germ tube length of the pathogen spores was measured in culture filtrates of the different test fungi. Two controls were maintained of which in one case the pathogen spores were germinated in uninoculated Czapek's liquid medium (kept for 30 days) and in the other case, the pathogen spores were germinated in sterilized distilled water.

iii) The test fungi were cultured in Czapek's Dox liquid medium for 6, 8, 15, 20, 25 and 30 days and cell free culture filtrate was obtained by filtering through microfilter. One part of the culture filtrate of each test fungi was autoclaved and the other portion was used as such unautoclaved). The spores of the pathogen were germinated in the different culture filtrate collected at different time intervals in both autoclaved and nonautoclaved ones. After 24 hrs. the percentage germination was determined. Two controls were maintained one in which spores of the pathogen were germinated in uninoculated Czapek's liquid medium kept at different time intervals and in the other case, germinated in sterilized distilled water.

iv) The test fungi which showed high antagonistic activity towards the pathogen, were cultured on Czapek's liquid medium for 15 and 30 days. Culture filtrate was obtained by filtering through microfilters and a part of the filtrate was autoclaved while the remaining was used as such (unautoclaved). The Czapek's Dox agar medium was prepared and poured into Petri plates and H. oryzae, the pathogen was allowed to grow for two days near the periphery of the plate. Four folds of filter paper discs 8 mm diameter tied together and soaked in culture filtrates of various test fungi (both autoclaved and unautoclaved) were placed near the other side of the periphery of the petriplate. The observations were taken after 7 - 10 days. In control, the filter paper discs soaked in uninoculated Czapek's liquid medium were kept. The percentage inhibition in radial growth of the pathogen was calculated by comparing the Petri plates containing filter paper discs soaked in culture filtrates of the antagonist with the control.

v) Different concentrations (5, 25, 50, 75, 100%) of the autoclaved and unautoclaved culture filtrates collected after 15 and 30 days of incubation were made by mixing the required quantity of the autoclaved or unautoclaved Czapek's Dox agar medium and adequately cooled. The incorporated media poured in Petri plates were inoculated with the pathogen. In control uninoculated liquid media were mixed with the Czapek's Dox agar medium. Inhibition in radial growth of the pathogen was measured by comparing the control with the culture filtrate

mixed ones and thus the effect of concentration of the culture filtrate of the antagonist was observed on the inhibition in radial growth of the pathogen.

vi) Growth of the pathogen in the presence of concentrate of the antagonist in culture.

The antagonist was grown in liquid Czapek's Dox medium for 30 days. Culture filtrates from replicate flasks were pooled to give 500 ml of filtrate. This was concentrated to about 100 ml and further dried on a rotary vacuum evaporator at room temperature. The dried extracts were tested for antibiotic activity by placing a small amount of the extract in the centre of a plate containing Czapek's Dox agar medium and then the pathogen was inoculated near it.

In control the growth of the pathogen without the dried extract was seen. Percentage inhibition in radial growth of the pathogen was calculated by comparing the control and the one in which the dried extract of the antagonist was kept.

Interaction studies in vivo

I) Interaction directly on the leaf surface:

Pot experiments were conducted in which Khonorullo and Mirikrak varieties were grown in earthen pots, 15 cm diameter containing unsterilized soil. Two months later, leaves were inoculated with H. oryzae grown on Czapek's Dox agar. The

inoculum of the pathogen (mycelium + spore mat) was placed at the junction of leaf sheath and stem and covered with cotton swab which was watered from time to time. Different treatments of Trichoderma viride, the antagonist was done to see its effect on lesion-development of H. oryzae. The different treatments are as follows:

i) After one day of the inoculation of the pathogen the swabs were removed and sprayed with a spore suspension of T. viride (grown on potato dextrose broth for 8 days) containing $4,80 \times 10^7$ conidia per ml and then the swabs were replaced. The spore suspension was again sprayed one day later but without removing the swabs, i.e., spraying of T. viride was done twice.

ii) Same procedure was followed as given above but after another two days swabs were removed and the plants of the second treatment were sprayed again with T. viride twice i.e., spraying was done four times.

iii) In this case similar procedure was followed as given in (i) but the plants were sprayed with T. viride one day before the inoculation of H. oryzae. Spraying of T. viride was done twice.

iv) Same as given in (3) but spraying was done four times.

v) In this treatment instead of spraying the spore suspension of T. viride, mycelial mat containing spores was inoculated together with the pathogen on the same day and they were put together on the same swab so that contact between them persisted throughout.

vi) In this treatment after inoculation with the pathogen the swabs were removed and 30 day old cell free culture filtrate of T. viride was sprayed and the swabs were kept back.

vii) In this case, before inoculation with H. oryzae the swabs were dipped in 30 day old culture filtrate of T. viride and then the pathogen was inoculated next day.

viii) In this treatment, after inoculation with H. oryzae, benomyl fungicide (50 ppm) was sprayed.

ix) In this treatment, in addition to the spray of benomyl fungicide, T. viride spore suspension was also sprayed after inoculating the plants with the pathogen.

Six pots were kept for each of the treatments and observations were taken after 15 days of inoculation and continued at 15 days interval till senescent stage (180 days old plant).

Control was maintained only with the pathogen without T. viride or the fungicide spray.

Inhibition of lesion-development was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{lesion size on leaves inoculated with pathogen} - \text{lesion size on leaves inoculated with pathogen + T. viride}}{\text{lesion size on leaves inoculated with pathogen}} \times 100$$

II) Interaction in soil

Both T. viride and H. oryzae were grown in 4% maize meal sand medium for 15 days and were mixed with sterilized soil in plastic pots, 11.5 cm diameter, each containing 800 gm of soil and inoculum mixture as shown in table 4.9a.

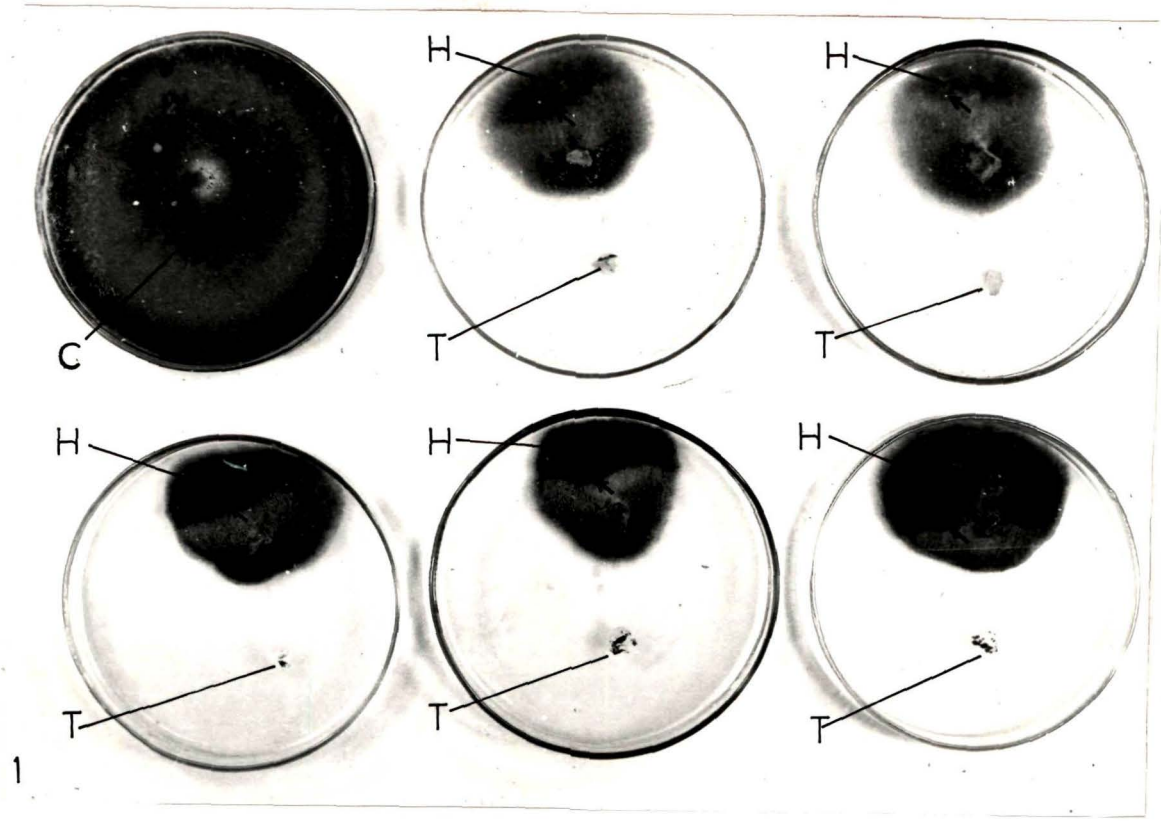
The pots, after mixing, were kept in the laboratory for one month during which light watering was done twice. Five one month old seedlings of Khonorullo, Ngoba and Mirikrak varieties grown in sterilized soil were planted out in each pot and 3 such pots were used for each of the treatments. The number of affected plants was recorded after one month of transplanting and that of leaf sheaths after another one month.

PLATE 4.1

1. Inhibition in radial growth of the pathogen, H. oryzae by T. viride when they were grown side by side:
   C - Control; H - Helminthosporium oryzae, the pathogen; T - Trichoderma viride, the antagonist; arrows indicated the parasitic activity of T. viride on H. oryzae.

2. Enlarged view of the same showing the mycoparasitic growth of T. viride on the pathogen, H. oryzae.
   C - Control; T - T. viride; H - H. oryzae; arrow indicated the parasitic activity of T. viride on H. oryzae.

PLATE - 4·1



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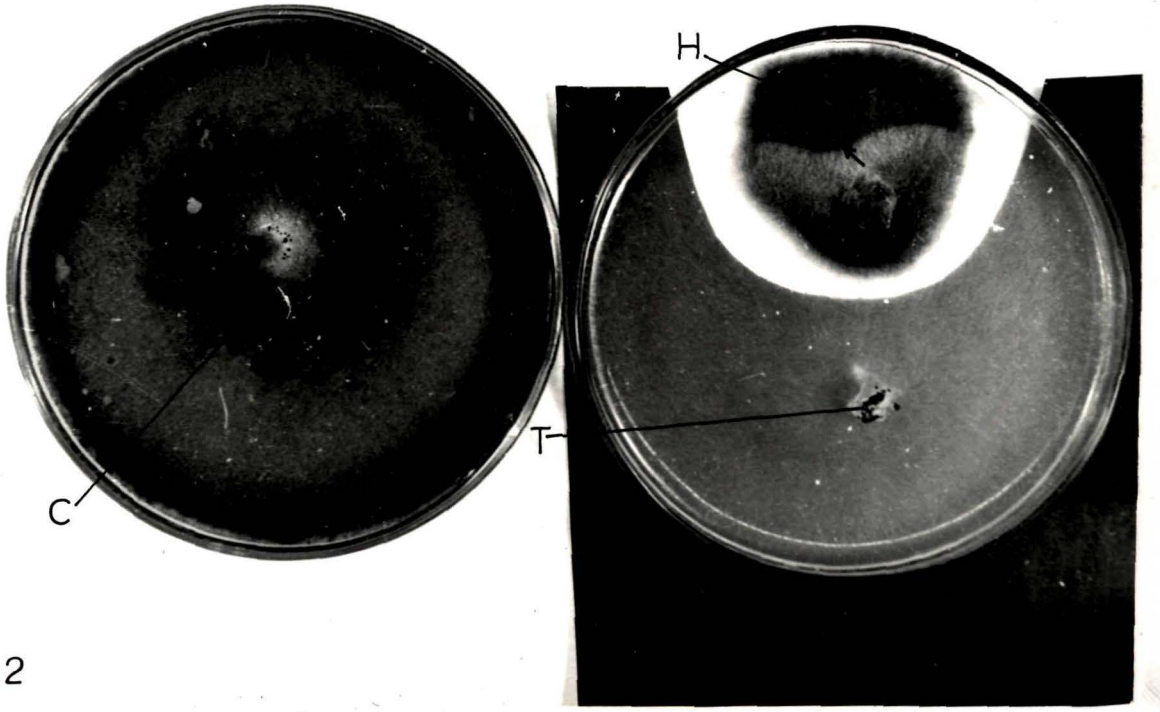


2

PLATE 4.2

1. Interaction of *Arthrinium* sp. and *Helminthosporium oryzae* on agar by dual culture method.
   Ar - *Arthrinium* sp., H - *H. oryzae*.

2. Interaction of *Aspergillus nidulans* and *Helminthosporium oryzae* on agar by dual culture method. An - *A. nidulans*; H - *H. oryzae*.

PLATE - 4·2

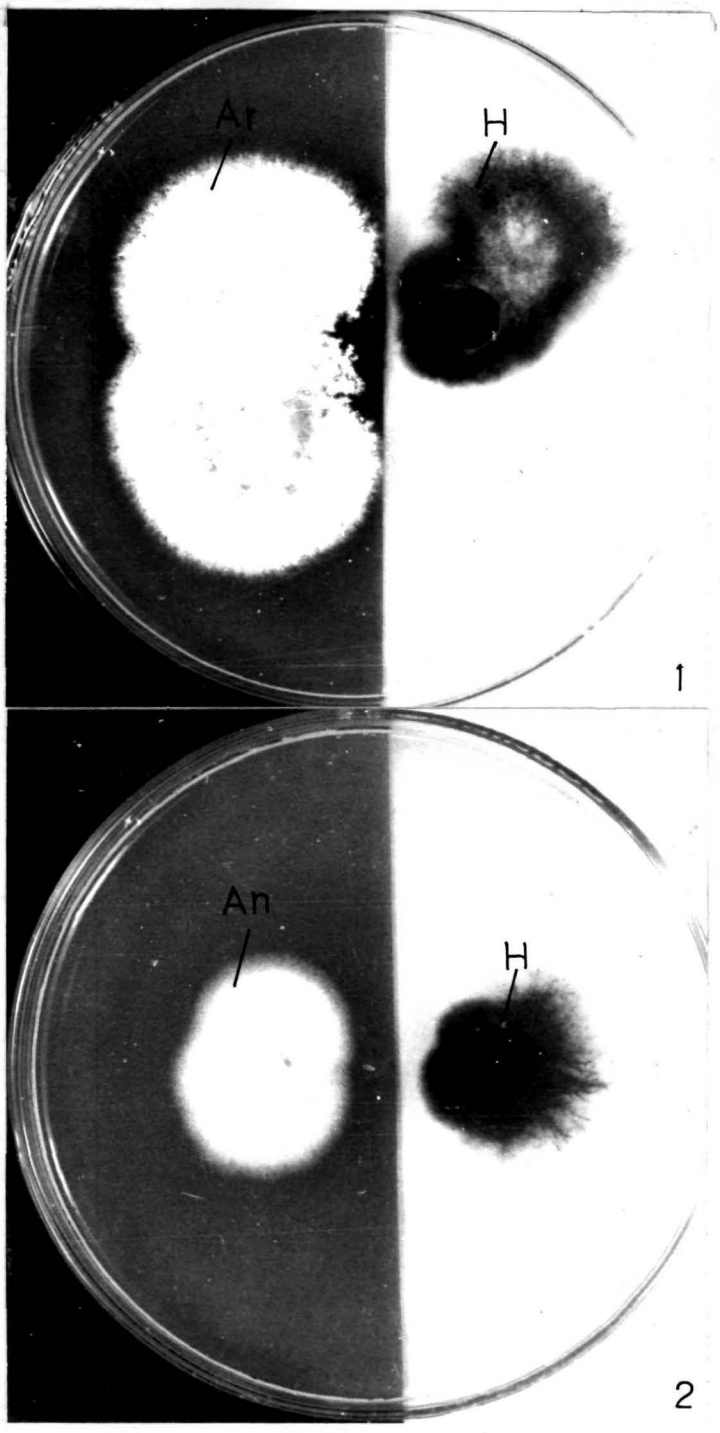
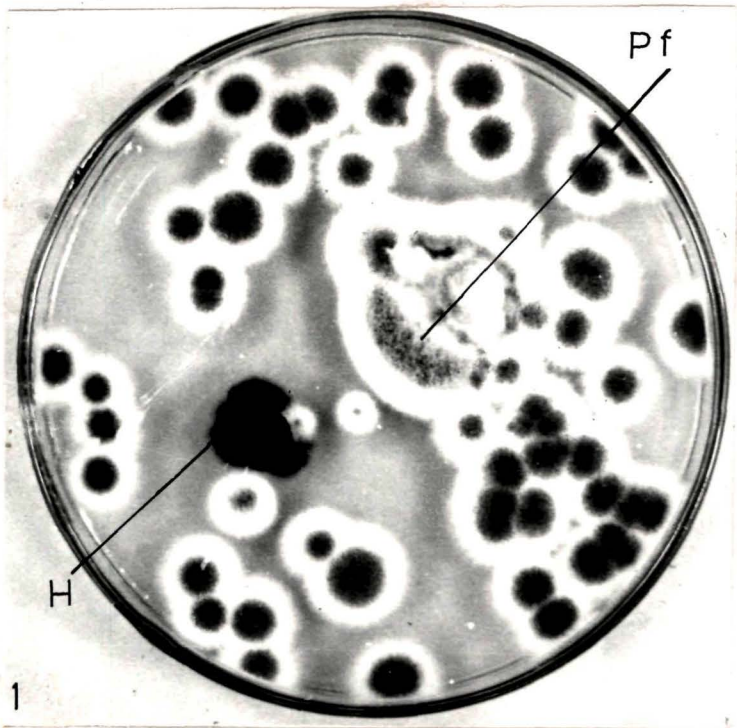


PLATE 4.3

1. Interaction of *Penicillium funiculosum* and *Helminthosporium oryzae* on agar by dual culture method. Pf - *P. funiculosum*; H - *H. oryzae*.

2. Interaction of *Penicillium chrysogenum* and *Helminthosporium oryzae* on agar by dual culture method. Pc - *P. chrysogenum*; H - *H. oryzae*.

PLATE - 4·3



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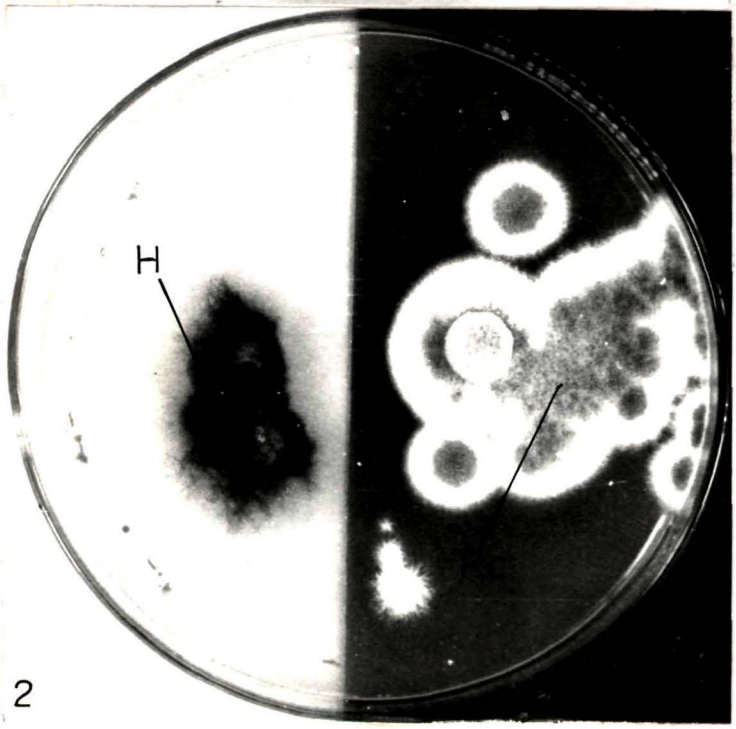
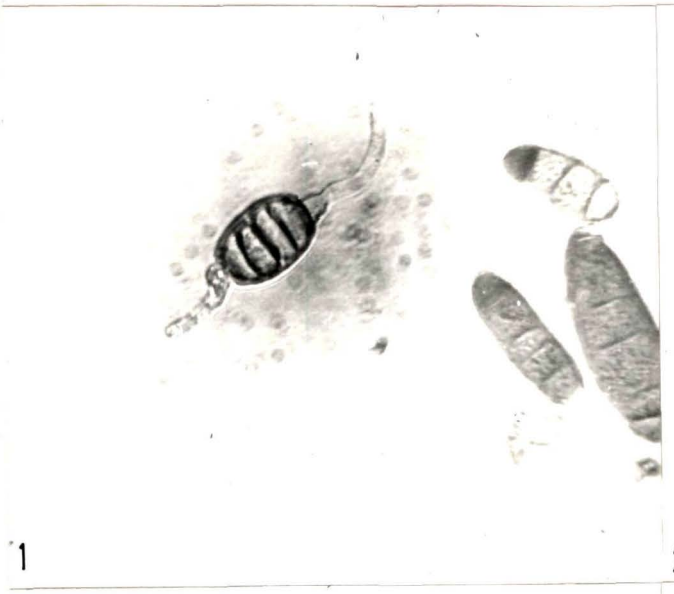


2

PLATE 4.4

1. Normal germination of the pathogen, H. oryzae spores in distilled water.
2. Normal germination of the pathogen, H. oryzae spores showing normal germ pore.
3. Abnormal behaviour of the pathogen, H. oryzae spores when they were grown in 30 day old culture filtrate of T. viride. Arrow showing bulbous outgrowth.
4. Abnormal behaviour of the pathogen, H. oryzae spores when they were grown in 30 day old culture filtrate of T. viride. Arrow showing disintegration of the germpore.

PLATE-4·4



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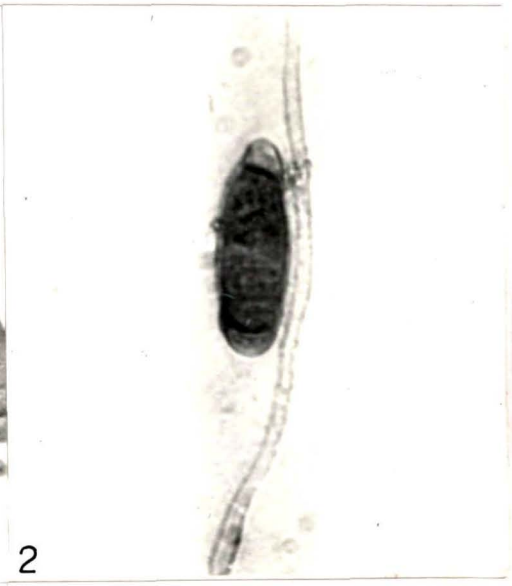

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3

4

Table 4.1: Percentage germination+ of pathogen (H. oryzae) spores when mixed with the spores of different fungi in different proportions after 24 hrs.

Test Fungi	Concentrations			
	I	II	III	IV
Alternaria alternata	22.6	29.2	31.5	38.6
Aspergillus nidulans	38.5	41.9	63.6	88.9
A. clavatus	44.3	45.4	61.5	89.4
A. niger	22.8	31.2	52.3	76.9
Acremonium persicinum	43.1	46.5	69.2	91.1
Arthrinium sp.	21.0	28.5	34.2	41.5
Botrytis cinerea	39.1	42.4	58.9	81.0
Chrysosporium pruinosum	41.2	43.9	65.5	90.4
Cladosporium herbarum	14.6	18.1	19.3	25.4
Curvularia lunata	41.9	46.5	68.1	91.2
Aurebasidium pullulans	39.0	45.6	65.4	90.8
Candida albicans	32.1	44.1	65.6	80.4
Epicoccum nigrum	43.2	48.1	69.1	91.4
Fusarium moniliforme	31.5	42.9	58.7	86.4
Gliocladium pencilloides	28.5	30.1	34.6	40.5
Memnoniella echinata	42.0	48.5	67.5	91.2
Mucor hiemalis	41.5	51.1	76.7	93.6
Nigrospora oryzae	20.9	30.0	41.1	43.5
Penicillium funiculosum	13.5	18.3	18.4	26.3
P. chrysogenum	15.2	20.5	24.4	30.1
Pestalotia monorhynca	31.2	40.5	64.6	90.2
Phoma glomerata	29.5	34.2	67.5	91.6
Rhizoctonia solani	20.5	29.9	42.1	89.5
Rhizopus nigricans	41.2	52.1	72.3	94.4
Stachybotrys atra	42.1	44.0	68.5	90.0
Starkeomyces koorchalomoides	38.2	42.4	65.6	91.7
Torula herbarum	40.5	45.6	62.3	90.6
Trichoderma viride	0	0	11.2	13.1
Trichothecium roseum	20.1	28.2	35.6	41.2
Verticillium albo-atrum	20.0	25.6	38.5	43.4
Aspergillus flavus	36.9	46.4	68.6	89.4

+ Germination based on 500-800 spores counted;

Control - Germination of pathogen spores 96.1%

I S:P 3:1; II S:P 3:2; III S:P 2:3; IV S:P 1:3 ; S = Saprophytes;

P = Pathogen.

Table 4.2: Spore germination, germ tube extension of pathogen (*H. oryzae*) spores mixed with different test fungi in equal proportions at different incubation periods.

Test fungi	Incubation period in hours							
	3 h		6 h		12 h		24 h	
	+% Germn.	Germtube* length	% Germn.	Germtube length	% Germn.	Germtube length	% Germn.	Germtube length
Control germination of pathogen spores	12.9	24	41.3	98	75.4	219	94.5	356
Alternaria alternata	8	10	28.6	36	32.5	129	49.5	291
Arthrinium sp.	9.4	12	22.9	44	33.9	175	52.1	300
Cladosporium herbarum	6	0	22	28	39.5	115	48.1	261
Aspergillus niger	6.5	15	28.5	50	34.5	163	53.2	296
Candida albicans	4.1	12	25.4	45	39.8	152	50.2	265
Gliocladium pencilloides	5.6	15	26.4	42	36.0	171	49.1	286
Nigrospora oryzae	10.8	21	26.1	72	38.5	174	45.9	296
Penicillium funiculosum	6	0	20.9	31	38.1	120	46.4	254
P. chrysogenum	4.8	14	22.1	42	31.2	161	45.6	265
Rhizoctonia solani	15.1	31	24.2	68	35.4	177	56.4	301
Trichoderma viride	2	0	20.1	30	31.1	109	43.6	191
Trichothecium roseum	5.1	13	28.1	45	35	167	49.2	280
Verticillium albo-atrum	3.2	11	20.5	41	32.6	158	48.0	269

+ Germination in percentage based on 500-800 spores counted; * Average germtube length in µm based on 100 spores.

Table 4.3: Showing the percentage inhibition of the pathogen (*H. oryzae*) when the different test fungi were grown on cellophane sheet before inoculating with the pathogen.

Test fungi	% inhibition of the pathogen
Trichoderma viride	60
Cladosporium herbarum	-
Verticillium albo-atrum	-
Gliocladium pencilloides	8
Nigrospora oryzae	-
Penicillium funiculosum	18
Rhizoctonia solani	10
Candida albicans	12

Table 4.4: Germination and germ tube length of the pathogen (*H. oryzae*) spores in cell free culture filtrate of different fungi (filtrate collected at 30 days of incubation)

Fungi	% germination+	Germtube length*(µm)
Arthrinium sp.	32.22 ± 1.82	30
Aureobasidium pullulans	58.22 ± 2.68	56
Botrytis cinerea	46.22 ± 2.12	42
Candida albicans	49.25 ± 2.07	31
Cladosporium herbarum	21.14 ± 1.95	28
Curvularia lunata	86.08 ± 2.22	390
Fusarium moniliforme	52.50 ± 2.95	47
Gliocladium pencilloides	18.26 ± 2.86	19
Humicola prisea	49.26 ± 1.95	45
Melanospora zamae	89.26 ± 2.10	398
Memnoniella echinata	35.6 ± 2.49	32
Nigrospora oryzae	25.62 ± 2.16	22
Penicillium chrysogenum	16.28 ± 2.98	15
P. funiculosum	20.12 ± 1.02	18
Rhizoctonia sp.	59.68 ± 1.22	55
Phoma glomerata	98.20 ± 1.09	420
Stachybotrys atra	39.68 ± 1.44	41
Trichoderma viride	12.13 ± 1.28	12
Trichothecium roseum	82.50 ± 2.92	396
Control (uninoculated Czapek's liquid medium)	98.68 ± 2.05	428
Control (sterilized distilled water)	90.16 ± 1.22	426

+ Germination based on 500-600 spores;

* Average germtube length based on 100 spores.

Table 4.5: Germination percentage of pathogen (*H. oryzae*) spores in cell free culture filtrates (both autoclaved and unautoclaved) of different fungi (filtrate collected at different time intervals).

Fungi	Days of filtrate collection											
	6		8		15		20		25		30	
	A	UA	A	UA	A	UA	A	UA	A	UA	A	UA
Arthrinium sp.	67.5	68.9	65.4	65.1	53.5	54.2	43.2	44.6	41.9	41.88	32.1	35.5
Cladosporium herbarum	45.6	46.1	42.1	40.3	40.6	35.6	30.1	29.7	26.0	25.4	21.5	21.4
Gliocladium pencilloides	54.2	50.6	48.5	40.9	41.2	38.6	32.6	30.1	23.4	22.8	20.1	18.26
Nigrospora oryzae	69.5	60.2	59.9	52.1	46.4	43.2	40.1	38.1	35.4	30.1	28.1	25.62
Penicillium chrysogenum	61.1	58.4	55.2	50.9	40.18	35.2	31.26	28.54	25.6	20.4	20.9	16.28
P. funiculosum	65.2	60.1	58.1	58.2	45.5	42.1	34.2	35.1	30.1	30	24.2	20.12
Trichoderma viride	56.5	34.9	35.6	23.7	32.5	22.1	29.1	18.4	20	16.2	20.5	12.13
Stachybotrys atra	89.5	87.1	80.2	78.5	75.9	70.1	61.2	61.4	52.3	49.1	41.5	39.68
Memnoniella echinata	86.2	86.3	85.5	80.1	54.8	48.1	45.2	45.1	40,2	38.9	36.6	35.6
Control (uninoculated Czapek's liquid medium)	98.68	98.65	96.86	96.8	95.5	95.0	90.5	90	90	90.1	89.9	8.9
Control (sterilized distilled water)	90.6	90.6	90.6	90.6	90.6	90.6	90.6	90.6	90.6	90.6	90.6	90.6

A = autoclaved; UA = unautoclaved.

Table 4.6: Showing the percentage of inhibition of the pathogen (*H. oryzae*) when filter paper discs soaked in culture filtrates of *T. viride* was kept near it.

Filter paper discs soaked in culture filtrates of test fungi	% inhibition in radial growth of the pathogen
1) 15 day old unautoclaved culture filtrate of *Trichoderma viride*	45
2) 15 day old autoclaved culture filtrate of *T. viride*	10
3) 30 day old unautoclaved culture filtrate of *T. viride*	60
4) 30 day old autoclaved culture filtrate of *T. viride*	25

Table 4.7: Effect of different treatments of T. viride on the occurrence of the pathogen (H. oryzae) on Mirikrak variety of paddy

Treatment	DAYS OF INOCULATION														
	After 60 days			After 75 days			After 105 days			After 135 days			After 180 days		
	No. of leaves infected	No. of spots/ leaves	Length of lesion (mm)	No. of leaves infected	No. of spots/ leaves	Length of lesion (mm)	No. of leaves infected	No. of spots/ leaves	Length of lesion (mm)	No. of leaves infected	No. of spots/ leaves	Length of lesion (mm)	No. of ear/ floret	No. of fertile seeds	Weight of 100 seeds (gm)
1. Control (only H)	2	4	29	3	5	35	4	8	49.8	5	12	60.4	1	2	.5
2. After inoculation with H. sprayed T. viride twice	2	3	24.8	2	3	25	3	6	40	4	10	55	2	4	.8
3. After inoculation with H. sprayed T. viride four times	2	2	19.2	2	2	28.4	3	5	35.1	4	9	51	2	5	.9
4. Before inoculation with H. sprayed T. viride twice	2	1	19.9	2	3	22.1	3	4	34.2	4	6	38	2	5	1
5. Before inoculation with H. sprayed T. viride four times	2	1	19.5	2	2	19.8	3	4	32.8	4	7	35.8	2	6	1.2
6. Spore + mycelium of H. and T. viride put together on the same day	1	2	12.3	2	3	13.2	3	6	25.9	4	8	28.8	2	3	.9
7. After inoculation with H. sprayed 30 days old culture filtrate of T. viride	1	2	9.5	2	3	13.5	3	5	25.6	4	6	28.2	2	4	.9
8. Before inoculation with H. sprayed 30 days old culture filtrate of T. viride	1	3	10.5	2	4	13.9	3	5	26.2	4	6	28.2	2	4	1
9. After inoculation with H. sprayed benomyl fungicide	2	2	28	3	5	32.1	3	6	40.5	4	10	60.2	1	2	.7
10. After inoculation with H. sprayed both benomyl and T. viride	2	2	24	3	3	24.9	3	6	39.1	4	9	56.8	2	4	.9

* average of 6 pots each containing 2 plants.

Table 4.8: Effect of different treatments of *T. viride* on the occurrance of the brown spot disease caused by *H. oryzae* on Khonorullo variety of paddy

Treatment	DAYS OF INOCULATION														
	After 60 days			After 75 days			After 105 days			After 135 days			After 180 days		
	*No. of leaves infected	No. of spots/ leaves	Length of lesion (mm)	No. of leaves infected	No. of spots/ leaves	Length of lesion (mm)	No. of leaves infected	No. of spots/ leaves	Length of lesion (mm)	No. of leaves infected	No. of spots/ leaves	Length of lesion (mm)	No. of ear/ floret	No. of fertile seeds	Weight of 100 seeds (gm)
1. Control (only *Helminthosporium*)	2	3	15.8	3	5	29.2	4	8	41.6	5	10	58.9	2	2	.8
2. After inoculation with *H.* sprayed *T. viride* twice	2	3	11.1	3	4	25.3	3	6	36.3	4	8	54.2	2	5	1.1
3. After inoculation with *H.* sprayed *T. viride* four times	2	2	10.3	2	3	18.6	3	5	34.4	4	8	53.9	2	5	1.1
4. Before inoculation with *H.* sprayed *T. viride* twice	2	2	10.1	2	2	18.9	3	4	34.2	4	8	41.1	2	4	.9
5. Before inoculation with *H.* sprayed *T. viride* four times	2	2	8.6	2	2	17.2	3	4	30.5	4	7	36.3	2	5	1.1
6. Spore+ mycelium of *H.* and *T. viride* put together on the same day	1	2	8.1	2	3	11.1	3	5	26.1	4	7	29.6	3	6	1.4
7. After inoculation with *H.* sprayed 30 days old culture filtrate of *T. viride*	1	2	7.5	2	2	10.4	3	5	25.9	4	7	28.3	2	4	.8
8. Before inoculation with *H.* sprayed 30 days old culture filtrate of *T. viride*	1	3	7.3	2	3	11.5	3	5	24.1	4	6	27.6	2	5	1.32
9. After inoculation with *H.* sprayed benomyl fungicide	2	3	14.6	3	5	28.6	4	6	41.3	4	9	57.5	2	3	1.1
10. After inoculation with *H.* sprayed both benomyl fungicide and *T. viride*	2	3	10.2	3	4	18.5	3	7	34.5	4	8	52.1	2	4	.9

* Average of 6 pots each containing 2 plants.

Table 4.9a: Showing a chart in which the inoculum of T. viride, the antagonist and the pathogen, H. oryzae was mixed in different proportions with sterilized soil in pots.

Treatment No:	Concentration	Amount of T. viride inoculum	Amount of H. oryzae inoculum	Amount of soil
1	Control	-	-	800 gm
2	Only T. viride	70 gm	-	800 gm
3	Only H. oryzae	-	70 gm	800 gm
4	T. viride: H. oryzae in the ratio of 2:1	100 gm	50 gm	800 gm
5	T. viride: H. oryzae in the ratio of 1:2	25 gm	50 gm	800 gm
6	T. viride: H. oryzae in the ratio of 1:1	25 gm	25 gm	800 gm

Table 4.9b: Showing the occurrence of brown spot of rice grown on sterilized soil inoculated with H. oryzae and T. viride in different proportions (average of 3 pots each containing 5 plants)

Treatment No.	Number of plants infected			Number of leaf sheaths infected		
	K	N	M	K	N	M
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	13	13	15	3.6	3.7	3.8
4	8	10	11	3.1	3.2	3.2
5	9	10	12	3.2	3.2	3.3
6	11	12	12	3.2	3.3	3.3

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety.

RESULTS

Interaction studies in vitro

1) Effects on spore germination:

I) Germination of pathogen-spores with spores of test fungi on agar: The results of the spore germination tests are summarized in histograms (Fig. 4.1) showing the percentage inhibition of spore germination of H. oryzae on agar due to the effect of different test fungi. 32 test fungi isolated from leaf surface of paddy were used for this experiment.

The inhibitions exerted by the 32 test fungi belonging to various genera are depicted in Fig. 4.1. The degrees of inhibition of germination of H. oryzae spores, exerted by the 32 test fungi ranged from 0 to 90%. Most of them, however, either did not inhibit spore germination at all or the effect was not significant. A few, however, proved strongly inhibitory. Trichoderma viride showed the highest inhibition (90%) of the pathogen followed by Penicillium funiculosum, Cladosporium herbarum, Alternaria alternata, Candida albicans, Verticillium albo-atrum, Arthrinium sp., Aureobasidium pullulans, Gliocladium pencilloides, Nigrospora oryzae and Rhizoctonia solani. Other fungi such as Chaetomium globosum, C. bostrychodes, Melanospora zamae, Pestalotia monorhynca and Starkeomyces koorchalomoides showed no inhibition at all. On the other hand, fungi such as Aspergillus spp., Acremonium

persicinum, Botrytis cinerea, Chrysosporium pruinosum, Curvularia lunata, Epicoccum nigrum, Fusarium moniliforme, Humicola prisea, Memnoniella echinata, Mucor hiemalis, Phoma glomerata, Rhizopus nigricans, Stachybotrys atra, Torula herbarum and Trichothecium roseum inhibited the pathogen to lesser extent.

Some peculiar effect on spore germination was observed microscopically. Some of the fungi like T. viride caused lysis of germ tubes of the pathogen and sometimes short and irregularly shaped germ tubes with swellings and globular structures were observed.

II) Germination of pathogen spores with the spores of test fungi on slides:

a) Spores of thirty one saprophytic (Table 4.1) fungi were used in different proportions (S:P - 3:1, 3:2, 2:3, 1:3) to study their effect on germination of pathogen-spores. Inhibition in spore germination was observed with all the fungi studied when the saprophytic and the pathogenic spores were mixed in 3:1 ratio. The inhibition percentage, however, in most of the cases decreased when the quantity of the pathogen in mixture was increased. The percentage germination of the pathogen was lowest when mixed with T. viride spores and was even zero when S:P ratio was 3:1 and 3:2. However, as the proportion of the pathogen increased in the mixture of the two, the percentage germination of the pathogen also increased and

became 11.2 and 13.1 when S:P ratios were 2:3 and 1:3. Other fungi such as Alternaria alternata, Cladosporium herbarum and Penicillium funiculosum also inhibited the germination of the pathogen spores even when the proportion of the pathogen was more than the saprophyte.

On the other hand, in all other cases the percentage germination of the pathogen was almost near to the control especially when S:P ratio was 1:3. 3:1 ratio of saprophytic and pathogen spores proved most effective where the maximum inhibition was recorded in almost all the fungi and maximum inhibition was observed with T. viride.

b) When equal volumes of the pathogen and saprophyte i.e., S:P = 1:1 was mixed together, Trichoderma viride was found to be the most effective causing more than 50% inhibition of spore germination and 45% of germ tube extension of H. oryzae (Table 4.2). From Table 4.2 it is also clear that with increase in incubation period there was an increase in the spore germination and germ tube extension rate. Other fungi such as Penicillium funiculosum and Cladosporium herbarum also showed distinct inhibition of the pathogen spores.

III) Effects on mycelial growth

The antagonistic effects of the leaf surface fungi on H. oryzae differed according to the species. The nature of colony interactions between H. oryzae and each of 32 test fungi

on Czapek's Dox agar medium and the percentage inhibition of radial growth of the pathogen by each test fungi are depicted by histograms in Fig. 4.3.

According to Porter (1924) and Skidmore and Dickinson (1976) the different types of colony interaction are as depicted in Fig. 4.2. There are four types of inhibition.

1) A - mutual intermingling
2) B - overgrowth by antagonist
3) C - mutual slight inhibition
4) D - growth around
5) E - inhibition at a distance.

Most of the test fungi belonged to C type where there was mutual slight inhibition of both the colonies but approached each other until almost in contact when growth ceases. In these cases, the percentage inhibition of the pathogen by the test fungi were low.

Trichoderma viride showed the highest inhibition of the pathogen being as high as 75% (Plate 4.1, 1,2). Other fungi like Cladosporium herbarum, Verticillium albo-atrum, Gliocladium pencilloides, Nigrospora oryzae, Penicillium funiculosum (Plate 4.3, 1) and Rhizoctonia solani, followed next. All these fungi showed 'B' type of interaction where the test fungi overgrew on the pathogen. T. viride parasitized H. oryzae mycelia and thus inhibited the growth of the pathogen

(plate 4.1, 2). Alternaria alternata also showed high inhibition and it showed 'D' type of interaction which showed growth around the pathogen. Candida albicans showed 'E' type of interaction where it inhibited the pathogen from a distance but later on the pathogen overgrew. Other fungi belonged to 'A' type where there was mutual intermingling and no definite type of interaction was observed.

In addition to the mere stoppage of the growth of the hyphae, as a result of the inhibitory activity exerted by the test fungi some other effects on the development of H. oryzae could also be observed by microcopic examination. Some fungi like T. viride parasitised the mycelia of H. oryzae and others caused lysis of the hyphae of H. oryzae on contact. Penicillium funiculosum showed inhibition of the pathogen from a distance but later overgrew on the pathogen. At the margin of the colony numerous spores of the pathogen were formed. Further, Fig. 4.4 gives a comparison of the degree of inhibition of spore germination with that of mycelial growth exerted by 32 test fungi. This gives a more complete picture of their inhibitory capacities on agar. The percentage inhibition of mycelial growth exerted by each test fungus was plotted against the pertinent percentage inhibition of spore germination exerted by each fungus and is given in point diagrams. From this figure (4.4) it is also clear that T. viride showed maximum inhibition both for spore germination and mycelial growth of the pathogen. Amongst other fungi although some

showed high percentage inhibition of spore germination but mycelial growth was not inhibited much.

IV) Antibiotic activity:

i) When the antagonist was grown from an inoculum disc over the surface of a cellophane membrane laid on agar, T. viride inhibited the pathogen upto 60%. Other fungi which inhibited the pathogen were Gliocladium pencilloides, Penicillium funiculosum, Rhizoctonia solani and Candida albicans.

ii) The data on germination and germtube length of the pathogen, H. oryzae spores when grown in 30 day old cell free culture filtrate of different test fungi are tabulated in Table 4.4. From this table, it is clear that in Trichoderma viride culture filtrate, the percentage germination and germ tube length was the lowest showing more than 75% inhibition. Under microscopic field, the spores of the pathogen showed certain deformations such as lysis of germ tubes and sometimes short and irregular shaped germ tubes with swelling and globular structures were observed (Plate 4,4; 3,4). Culture filtrates of fungi such as Penicillium spp., Cladosporium herbarum, Nigrospora oryzae and Arthrinium sp. also showed higher inhibition rate as compared to other fungi. Some fungi like Phoma glomerata culture filtrate showed almost similar germination rate as the control.

iii) When the pathogen spores were grown in different culture filtrates collected at different time intervals of incubation (6, 8, 15, 25, 30) (both autoclaved and unautoclaved) the germination rate was the lowest in unautoclaved 30 days old culture filtrate of T. viride (Table 4.5). Autoclaved culture filtrate of T. viride showed a slightly lesser inhibition rate of the pathogen spores than the unautoclaved one.

Six days old culture filtrate showed a higher percentage germination than 8 day and the lowest was for 30 day old culture filtrate in all the cases studied. Other fungal culture filtrates such as those of Penicillium spp., Gliocladium pencilloides, Cladosporium herbarum and Nigrospora oryzae also showed quite high inhibition.

iv) When filter paper discs soaked in 15 day old and 30 day old (autoclaved and unautoclaved) culture filterate of T. viride were placed near the periphery of the petriplate and the pathogen was inoculated, highest inhibition (60%) in radial growth of the pathogen was observed in 30 day old unautoclaved culture filtrate (Table 4.6). Whereas, in autoclaved culture filtrate of T. viride there was a decrease in percentage inhibition of radial growth of the pathogen.

v) From Fig. 4.5 it is clear that the highest percentage of inhibition (72%) in radial growth of the pathogen was obtained when 100% of 30 day old unautoclaved culture

filtrate of T. viride was incorporated into the medium. Whereas, with the same concentration of the autoclaved culture filtrate there was decreased in percentage inhibition.

vi) When concentrate dried extract of T. viride was kept in the centre of a petriplate containing medium and when pathogen was inoculated near it, there was 85% inhibition in radial growth of the pathogen.

Interaction studies in vivo

1) Experiments in pot tests with Trichoderma viride showed that (Fig. 4.6 and 4.7) the percentage inhibition of lesion development of the pathogen was highest in the treatment where the 30 day old culture filtrate was directly sprayed on the leaves before inoculation with the pathogen. It was followed by the treatment where the culture filtrate was sprayed after inoculation of the pathogen. Then it was followed by the treatment where the spores and mycelium of both T. viride and the pathogen were put together. Spraying with the spore suspension of T. viride four times before inoculation of H. oryzae was also quite significant for inhibiting the lesion development. There was hardly any inhibition in lesion development when benomyl fungicides was sprayed after inoculating with the pathogen which was almost close to the control. Even when both benomyl fungicide and T. viride spore suspension were sprayed there was no synergistic effect and it showed effect similar to the treatment where only T. viride spore suspension was sprayed twice after the inoculation of the

pathogen. These results were obtained on Khonorullo variety. Whereas in Mirikrak variety the best treatment for inhibition of the pathogen was when 30 day old culture filtrate was sprayed after inoculation with the pathogen followed by the treatment when both the inocula were kept together.

Further, as seen from Figs. 4.6 and 4.7 and Tables 4.7 and 4.8 it is clear that the percentage inhibition of the pathogen decreased with days of inoculation in all the treatments and at 180 days of plant growth (Senescent stage) the pathogen more or less could not be inhibited at all. Due to severe effect of the disease, ears formed were all distorted and very few grains were produced. Only 2-5 seeds/plant were fertile and the rest of the seeds were all sterile and the weight of the seeds was very low ranging from 0.3 - 1.4 gms/ 100 seeds. Thus the treatments with T. viride were not effective at later stages of the growth of the plant in both the cases (Mirikrak and Khonorullo varieties). Usually it was observed that the minute brown spots which were seen initially coalesced together to form irregular brown markings on the leaves with age of the plant and the leaves dried completely. The development of the lesion of the pathogen, H. oryzae could be controlled to some extent (70% inhibition) only at the initial stages of the plant growth and later on the effect was reduced.

Fig. 4.1: Percentage inhibition of the germination of pathogen (Helminthosporium oryzae) spores on agar as exerted by 32 test fungi viz., 1) Alternaria alternata, 2) Aspergillus nidulans, 3) A. clavatus, 4) Acremonium persicinum, 5) Arthrinium sp., 6) Aureobasidium pullulans, 7) Botrytis cinerea, 8) Chrysosporium pruinosum, 9) Cladosporium herbarum, 10) Candida albicans, 11) Curvularia lunata, 12) Chaetomium globosum, 13) C. bostrychodes, 14) Epicoccum nigrum, 15) Fusarium moniliforme, 16) Gliocladium pencilloides, 17) Humicola grisea 18) Melanospora zamae, 19) Memnoniella echinata, 20) Mucor hiemalis, 21) Nigrospora oryzae, 22) Penicillium funiculosum, 23) Pestalotia monorhyca, 24) Phoma glomerata, 25) Rhizoctonia solani, 26) Rhizopus nigricans, 27) Stachybotrys atra, 28) Starkeomyces koorchalomoides, 29) Torula herbarum, 30) Trichoderma viride, 31) Trichothecium roseum and 32) Verticillium albo-atrum.

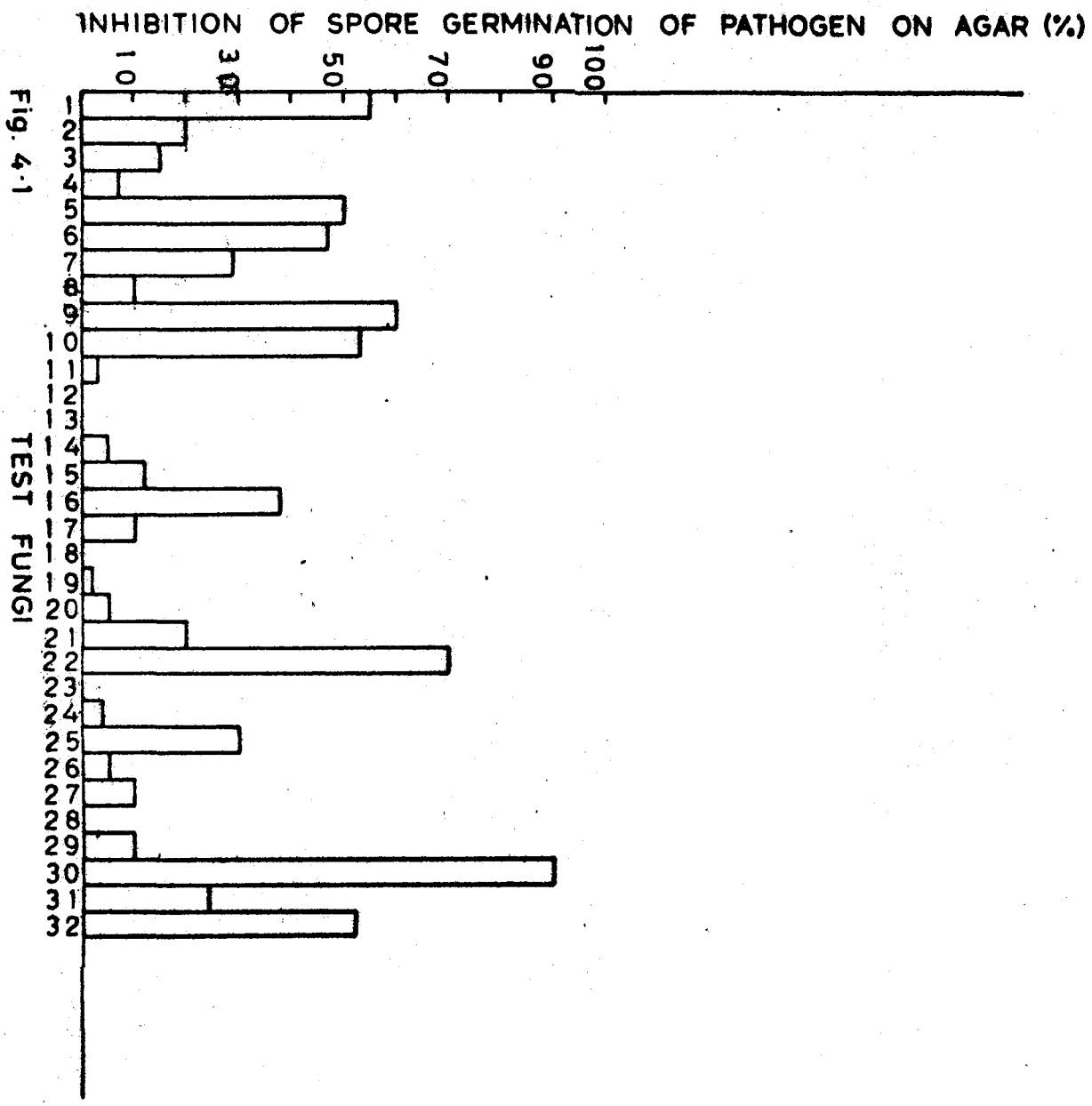
INHIBITION OF SPORE GERMINATION OF PATHOGEN ON AGAR (%)
10
30
50
70
90
100
TEST FUNGI
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

Fig. 4·1

Fig. 4.2: Types of inhibition: A, mutually intermingling, B, Overgrowth by antagonist, C, mutual slight inhibition, D, growth around and E, inhibition at a distance.

TYPES OF INHIBITION

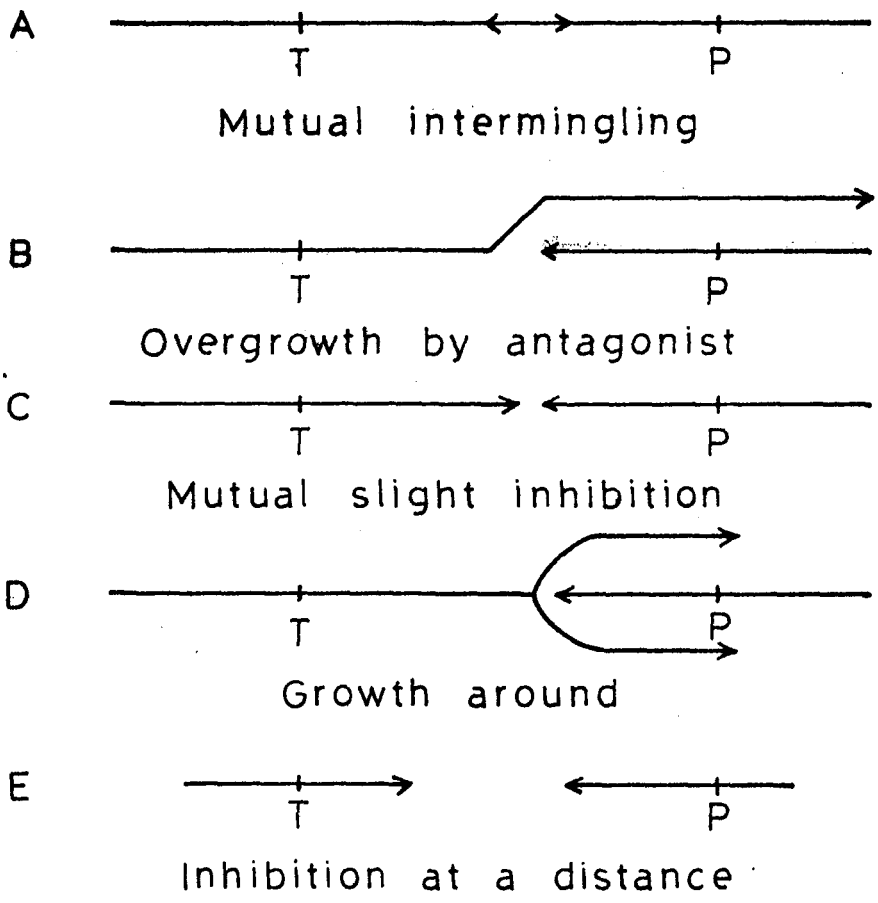

Fig. 4·2

Fig. 4.3: Percentage inhibition of the mycelial growth of the pathogen (Helminthosporium oryzae) on agar, as exerted by 32 test fungi, viz.,

1) Alternaria alternata, 2) Aspergillus nidulans, 3) A. clavatus, 4) Acremonium persicinum, 5) Arthrinium sp., 6) Aureobasidium pullulans, 7) Botrytis cinerea, 8) Chrysosporium pruinosum, 9) Cladosporium herbarum, 10) Candida albicans, 11) Curvularia lunata, 12) Chaetomium globosum, 13) C. bostrychodes, 14) Epicoccum nigrum, 15) Fusarium moniliforme, 16) Gliocladium penicilloides, 17) Humicola prisea, 18) Melanospora zamae, 19) Memnoniella echinata, 20) Mucor hiemalis, 21) Nigrospora oryzae, 22) Penicillium funiculosum, 23) Pestalotia monorhynca, 24) Phoma glomerata, 25) Rhizoctonia solani, 26) Rhizopus nigricans, 27) Stachybotrys atra, 28) Starkeomyces koorchalomoides, 29) Torula herbarum, 30) Trichoderma viride, 31) Trichothecium roseum, 32) Verticillium albo-atrum.

A = mutual intermingling, B = overgrowth by antagonist, C = mutual slight inhibition, D = growth around, E = inhibition at a distance.

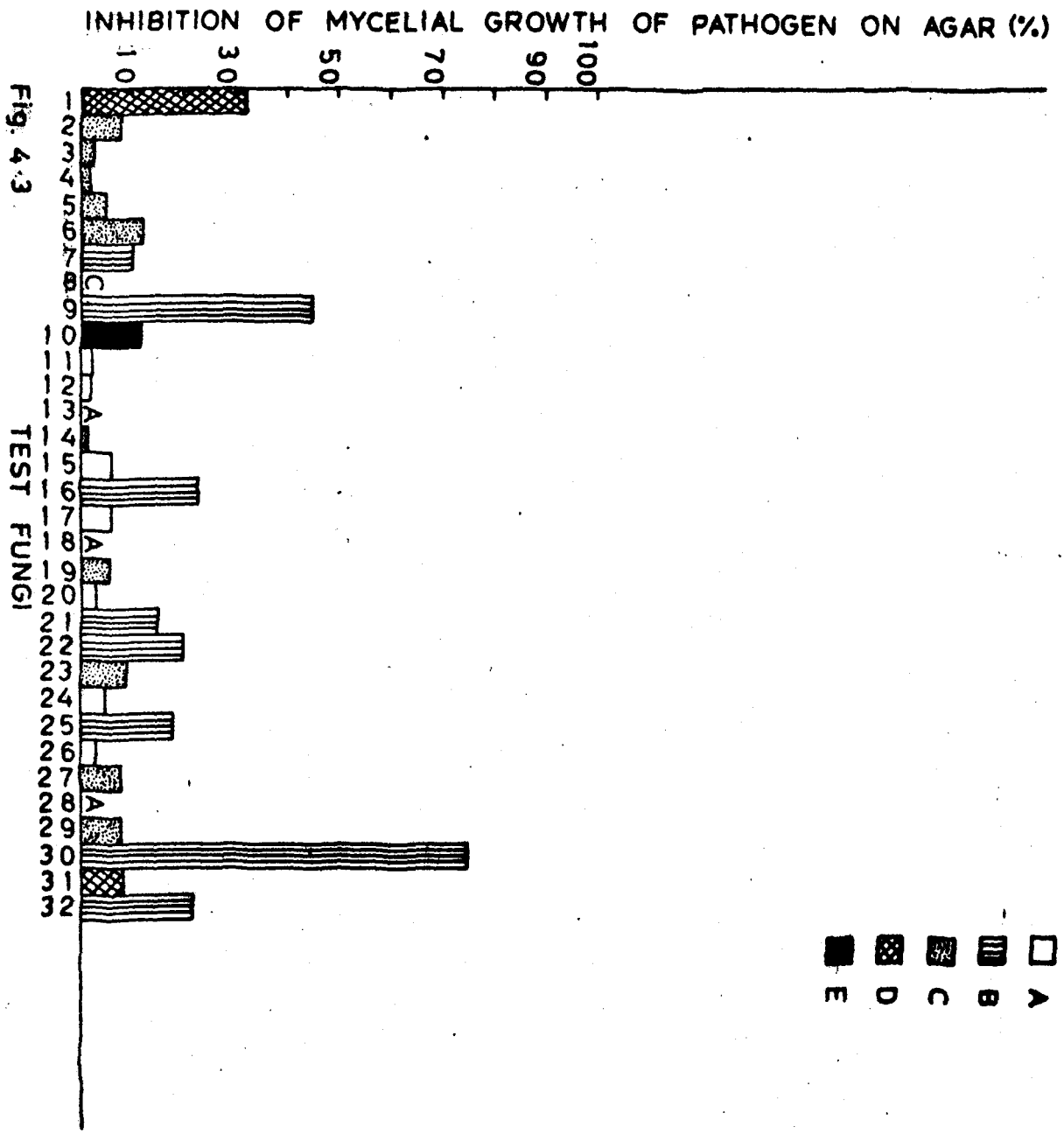
INHIBITION OF MYCELIAL GROWTH OF PATHOGEN ON AGAR (%)
10
30
50
70
90
100
TEST FUNGI
1 2 3 4 5 6 7 C 9 10 11 12 A 14 15 16 17 A 19 20 21 22 23 24 25 26 27 A 29 30 31 32
E D C B A

Fig. 4.3

Fig. 4.4: Relation between percentages of inhibition of mycelial growth and those of spore germination of *Helminthosporium oryzae* on agar, as exerted by 32 test fungi viz., 1) *Alternaria alternata*, 2) *Aspergillus nidulans*, 3) *A. clavatus*, 4) *Acremonium persicinum*, 5) *Arthrinium* sp. 6) *Aureobasidium pullulans*, 7) *Botrytis cinerea*, 8) *Chrysosporium pruinosum*, 9) *Cladosporium herbarum*, 10) *Candida albicans*, 11) *Curvularia lunata*, 12) *Chaetomium globosum*, 13) *C. bostrychodes*, 14) *Epicoccum nigrum*, 15) *Fusarium moniliforme*, 16) *Gliocladium penicilloides*, 17) *Humicola prisea*, 18) *Melanospora zamae*, 19) *Memnoniella echinata*, 20) *Mucor hiemalis*, 21) *Nigrospora oryzae*, 22) *Penicillium funiculosum*, 23) *Pestalotia monorhynca*, 24) *Phoma glomerata*, 25) *Rhizoctonia solani*, 26) *Rhizopus nigricans*, 27) *Stachybotrys atra*, 28) *Starkeomyces koorchalomoides*, 29) *Torula herbarum*, 30) *Trichoderma viride*, 31) *Trichothecium roseum* and 32) *Verticillium albo-atrum*.

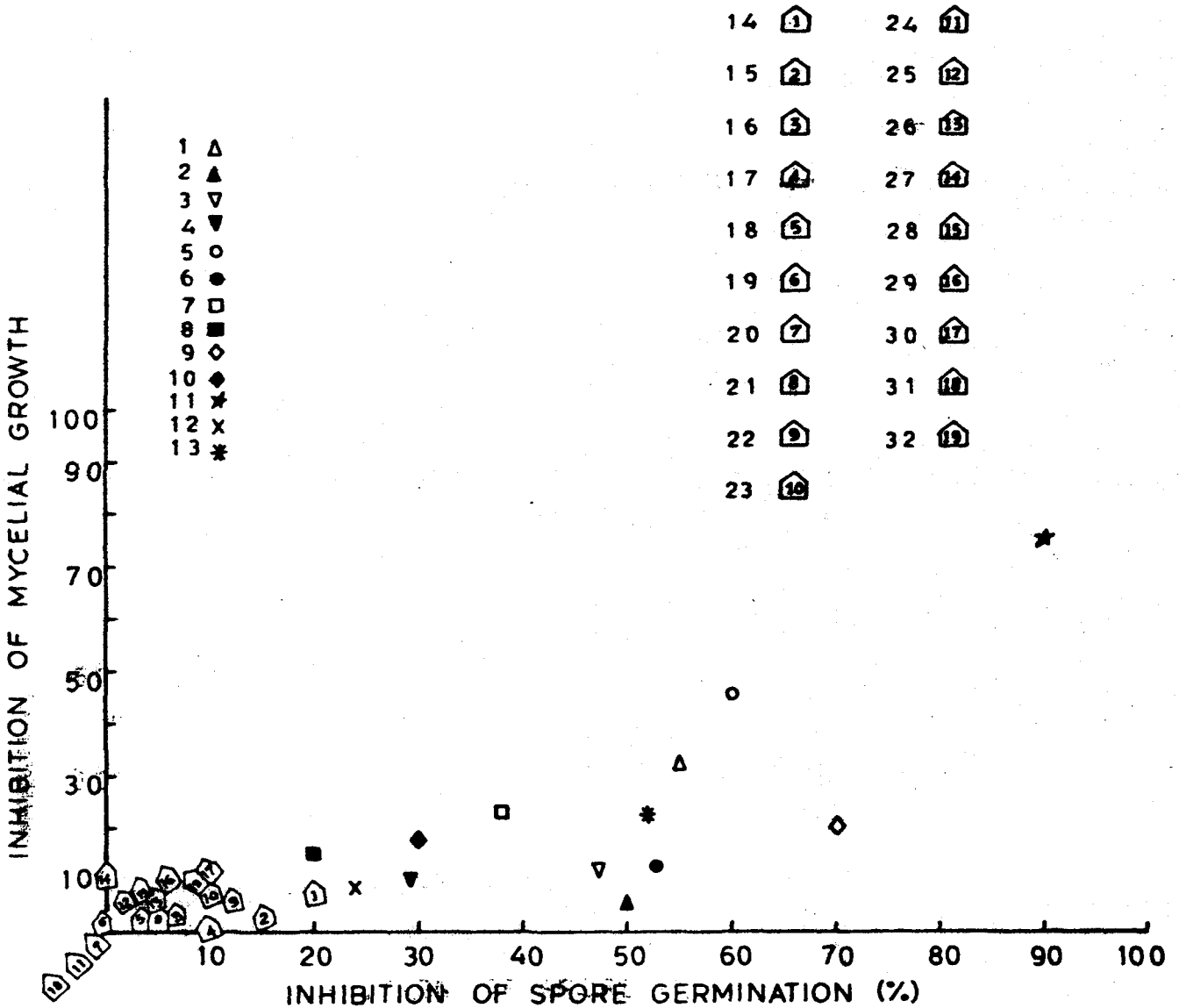
INHIBITION OF MYCELIAL GROWTH
INHIBITION OF SPORE GERMINATION (%)
10
20
30
40
50
60
70
80
90
100
1 Δ
2 ▲
3 ▽
4 ▼
5 ○
6 ●
7 □
8 ■
9 ◇
10 ◆
11 ★
12 X
13 ✱
14 1
15 2
16 3
17 4
18 5
19 6
20 7
21 8
22 9
23 10
24 11
25 12
26 13
27 14
28 15
29 16
30 17
31 18
32 19

Fig 4-4

Fig. 4.5: Effect of different concentrations of autoclaved and unautoclaved 15 day old and 30 day old culture filtrates of Trichoderma viride on the percentage inhibition of radial growth of the pathogen, Helminthosporium oryzae.

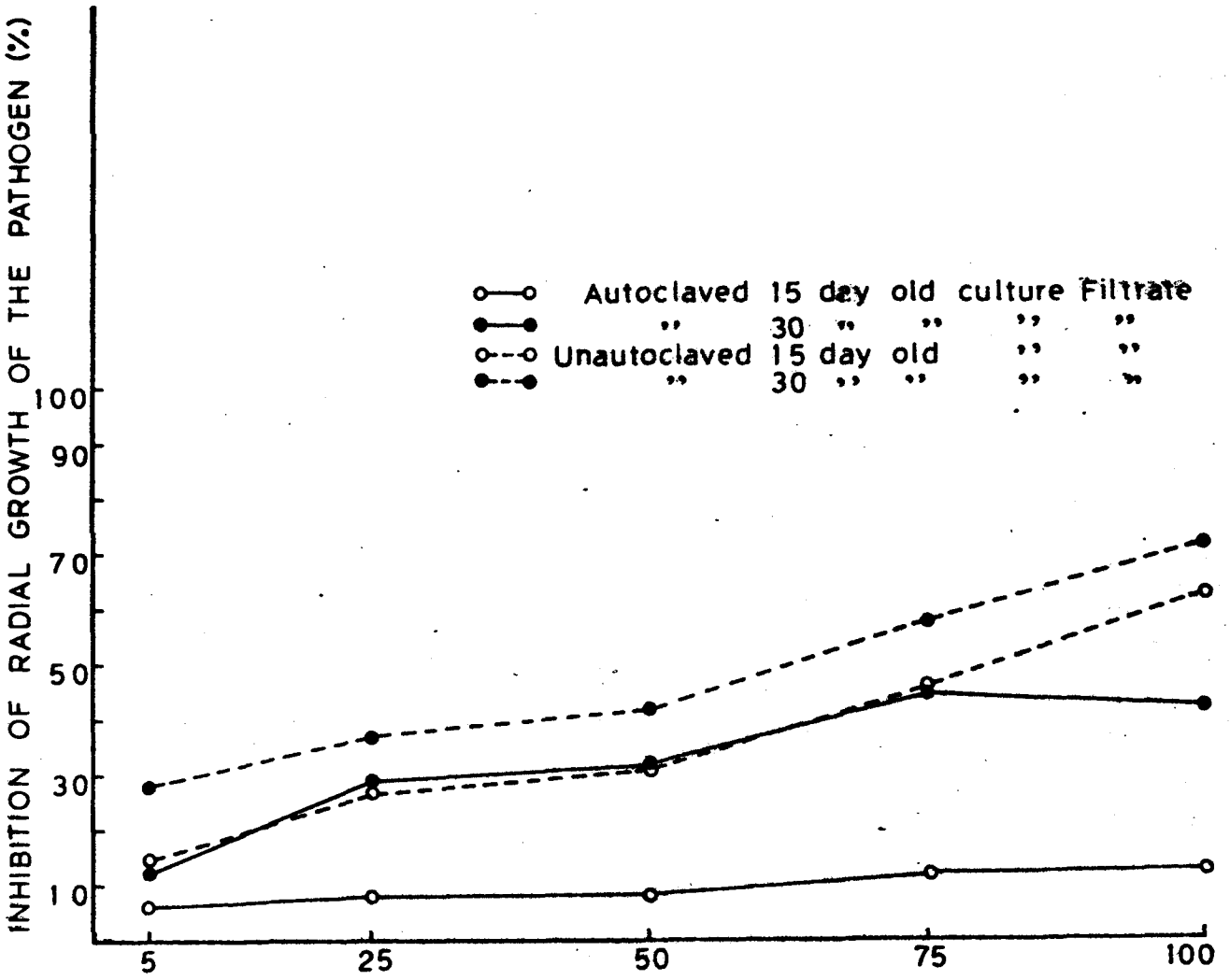
Autoclaved 15 day old culture Filtrate
" 30 " " " "
Unautoclaved 15 day old " "
" 30 " " " "
INHIBITION OF RADIAL GROWTH OF THE PATHOGEN (%)
100
90
70
50
30
10
5
25
50
75
100
CONCENTRATION OF CULTURE FILTRATE OF T. viridae (%)

Fig. 4·5

Fig. 4.6: Effect of different treatments of Trichoderma viride viz., 1) after inoculation with pathogen sprayed spore suspension of T. viride twice, 2) after inoculation with pathogen sprayed spore suspension of T. viride four times, 3) Before inoculation with the pathogen sprayed T. viride spore suspension twice, 4) Before inoculation with the pathogen sprayed T. viride spore suspension four times, 5) Mycelial mat containing spores of T. viride inoculated together with the pathogen, 6) After inoculation with the pathogen 30 days old cell free culture filtrate of T. viride sprayed, 7) Before inoculation with the pathogen sprayed 30 days old cell free culture filtrate of T. viride, 8) After inoculation with the pathogen sprayed benomyl fungicide, 9) After inoculation with the pathogen sprayed both benomyl fungicide and percentrage inhibition of lesion development on leaves of Khonorullo variety of paddy at different days of inoculation.

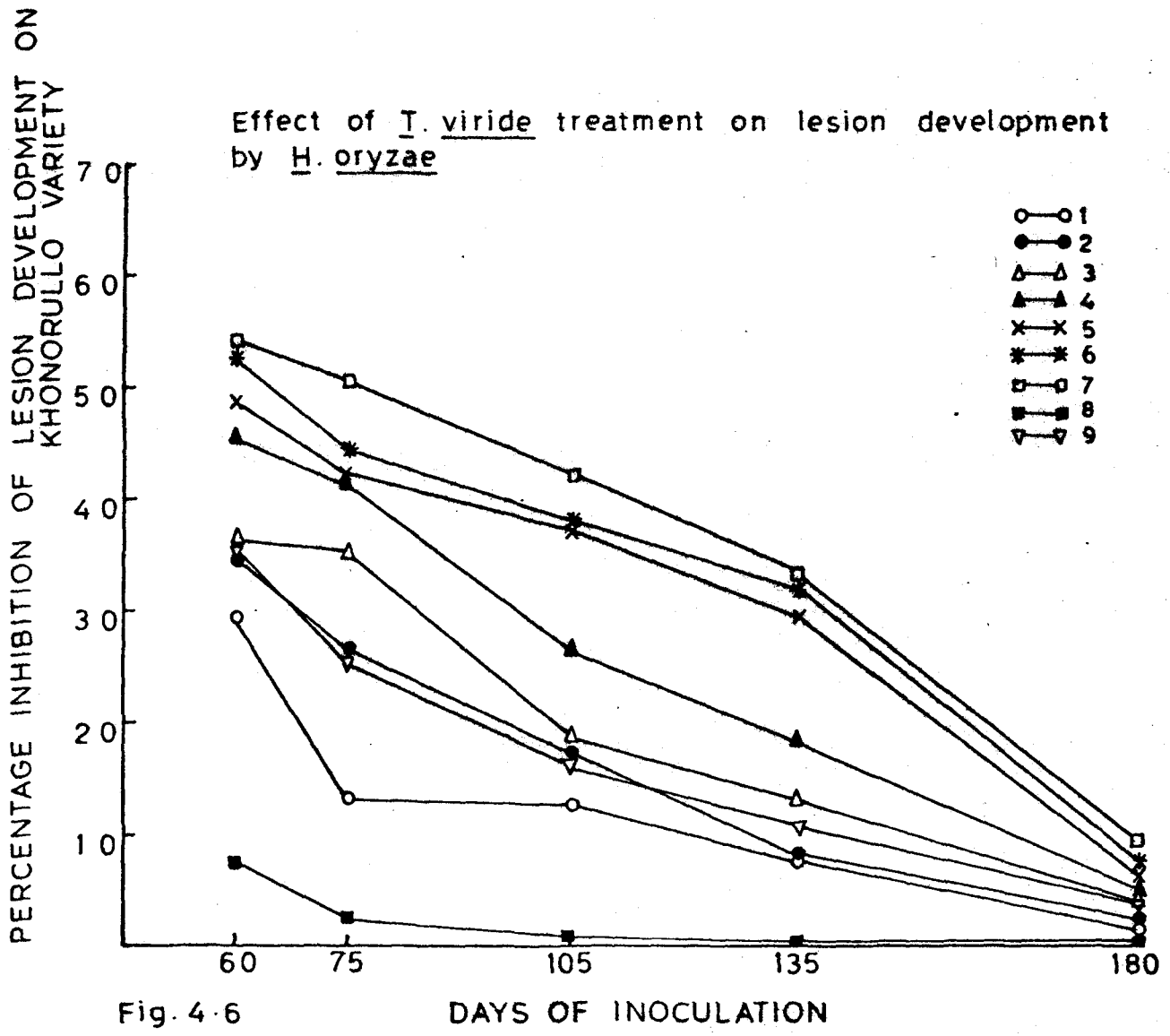
Effect of T. viride treatment on lesion development by H. oryzae
PERCENTAGE INHIBITION OF LESION DEVELOPMENT ON KHONORULLO VARIETY
70
60
50
40
30
20
10
60
75
105
135
180
DAYS OF INOCULATION
1
2
3
4
5
6
7
8
9

Fig. 4·6

Fig. 4.7: Effect of different treatments of Trichoderma viride viz., 1) after inoculation with pathogen sprayed spore suspension of T. viride twice, 2) after inoculation with pathogen sprayed spore suspension of T. viride four times, 3) Before inoculation with the pathogen sprayed T. viride spore suspension twice, 4) Before inoculation with pathogen sprayed T. viride spore suspension four times, 5) Mycelial mat containing spores of T. viride inoculated together with the pathogen, 6) After inoculation with the pathogen 30 days old cell-free culture filtrate of T. viride sprayed, 7) Before inoculation with the pathogen sprayed 30 days old cell-free culture filtrate of T. viride, 8) After inoculation with the pathogen sprayed benomyl fungicide, 9) After inoculation with the pathogen sprayed both benomyl fungicide and the spore suspension of T. viride, on the percentage inhibition of lesion development on leaves of Mirikrak variety of paddy at different days of inoculation.

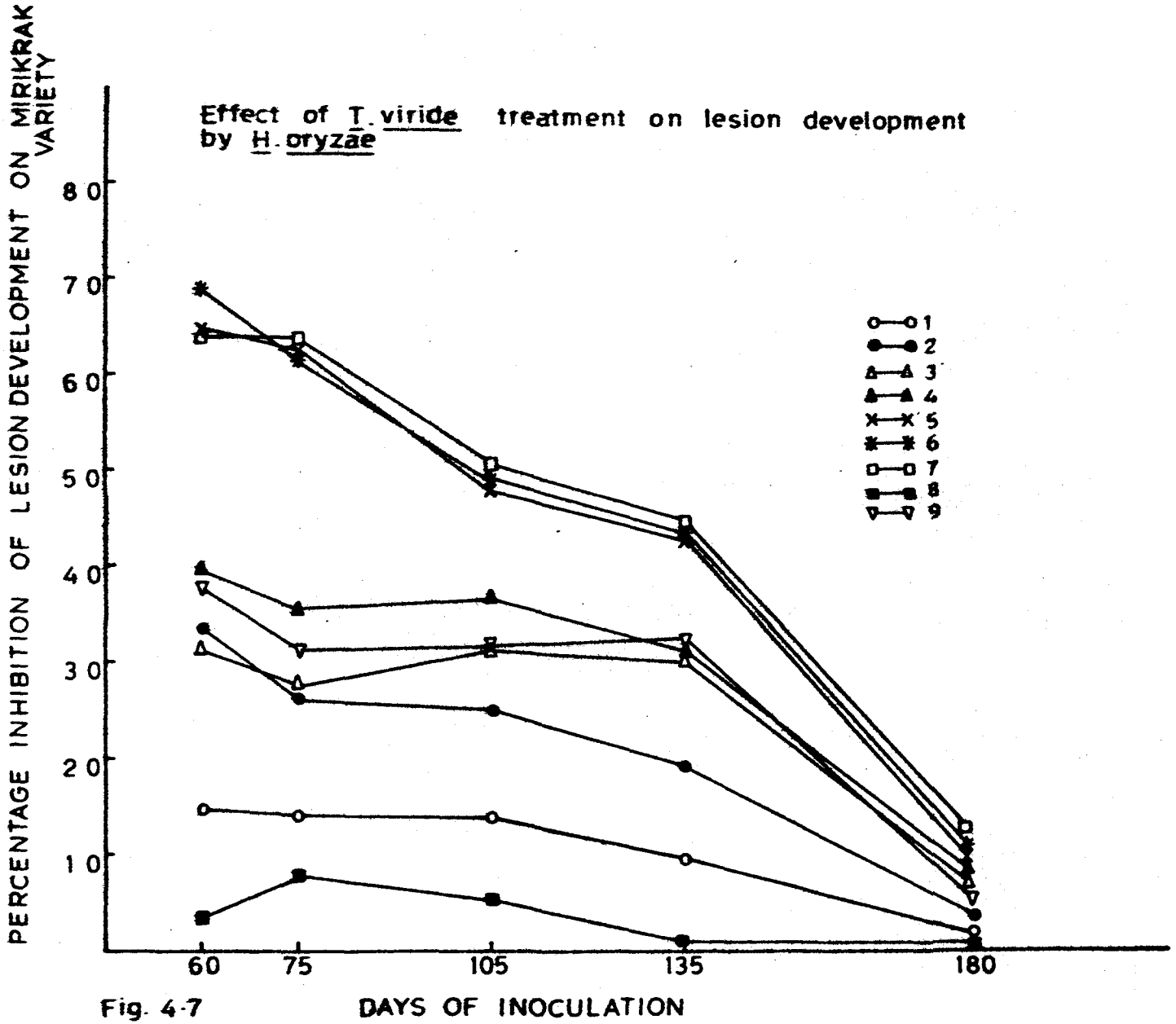
Effect of T. viride treatment on lesion development by H. oryzae
PERCENTAGE INHIBITION OF LESION DEVELOPMENT ON MIRIKRAK VARIETY
80
70
60
50
40
30
20
10
60
75
105
135
180
DAYS OF INOCULATION
1
2
3
4
5
6
7
8
9

Fig. 4-7

Further, the lesion-length was more on Mirikrak variety than on Khonorullo and the percentage inhibition of the lesion development of the pathogen by T. viride treatments was more pronounced on Mirikrak variety than Khonorullo.

II) When inocula of T. viride and H. oryzae were mixed with soil in different porportions, lesser number of plants as well as leaves was infected by the pathogen in the presence of T. viride (Table 4.9a and b). However, there was no significant difference between the high and low dose of T. viride. Further, in case of Khonorullo, T. viride was more effective at 2:1 ratio (T. viride:H. oryzae) than in the other two varieties. T. viride alone did not have any effect on the plants.

DISCUSSION

Every method to determine the inhibitory capacities of microorganisms may have its limitations (Heuvel, 1970).

The inverted agar discs punched from 7 day old colonies of epiphytes were suitable to study effects on germination of H. oryzae spores. The spores were exposed to a substrate which might have obtained an unfavourable pH or which might contain antifungal substances such as antibiotics. Labile or volatile compounds might still be in production and also exert their influence.

The inhibition percentage was calculated from countings after an incubation period of 5 hours, it remains unknown whether inhibition was due to sporostatic or fungicidal action, allowing only a certain percentage of spores to germinate or resulting into a delay of the germination. The results from these studies indicate that the most effective antagonist was T. viride which showed the highest inhibition.

When spores of both the test fungi and the pathogen were were mixed in different proportions the most effective inhibition was with spores of T. viride when its population was higher than the pathogen. Other fungi such as Alternaria alternata, Cladosporium herbarum and Penicillium funiculosum also showed significant inhibition of the pathogen.

When spores of both the saprophyte and the pathogen were mixed in equal numbers, then also T. viride was the most effective in inhibiting the pathogen spores followed by fungi like Penicillium funiculosum and Cladosporium herbarum. Upadhyay (1980) had observed that when germinating spores of phylloplane microfungi and Pestalotiopsis funerea, a leaf spot pathogen of Eucalyptus were studied in vitro by mixing them in equal porportions, Aspergillus flavus, Fusarium oxysporum and Penicillium oxalicum were found to be most effective in causing more than 60% inhibition of spore germination of the pathogen.

To see the effect on mycelial growth, dual culture method was followed as used by previous workers (Porter, 1924; Heuvel, 1970; Skidmore, 1976; Rai and Singh, 1980). In this case also Trichoderma viride was the most potent antagonist since it showed a highly significant inhibition of the radial growth of the pathogen on agar. Other fungi such as Penicillium sp., Cladosporium herbarum, Verticillium sp., and Gliocladium sp. also inhibited the radial growth of the pathogen to some extent. Trichoderma viride may be a mycoparasite because it was observed to be overgrowing the pathogen colonies (Plate 4.1, 1,2) but no distinct hyperparasitism could be observed under the microscope. The use of Trichoderma spp. as a biocontrol agent of soil borne and foliar pathogenic fungi has been known for quite a long time (Baker and Cook, 1974). The result of the present studies is in agreement with the studies of previous workers (Mitchell, 1973; Roy, 1977; Tsuneda and Skoropad, 1980; Upadhyay and Rai, 1980; Dutta, 1981) in that Trichoderma viride could suppress the growth of different pathogen by dual culture method.

When tested for antibiotic activity by different experiments it was found that T. viride produced some antibiotic substance(s) which could inhibit the growth of the pathogen which was clear from experiments with cell free culture filtrate of the fungus. When spores of pathogen were grown in culture filtrate of T. viride they showed some deformations (Plate 4.4, 3,4). T. viride is known to secrete some antibiotic substances

(Allen and Haenster, 1935; Docea et al., 1974; Volovik et al., 1975). The autoclaved culture filtrate of T. viride slightly reduced germination of the pathogen spores and also the percentage inhibition in radial growth. It appears that the active principle is partially thermolabile. In the present study, however, no effort was made to isolate the active principle because it is a known fact that T. viride produce antibiotics such as trichodermin and viridin which inhibit the growth of the microorganisms (Dennis and Webster, 1971).

Interaction studies in vitro suggest that some of the fungi like Trichoderma viride, Gliocladium sp., Penicillium spp. and Cladosporium sp. may be important antagonists of the brown spot pathogen.

T. viride was not so effective in inhibiting lesion development in vivo although it showed significant inhibition during the early stages. It is also clear from the present studies that T. viride may be producing some antibiotic substances which inhibited the brown spot pathogen in pot tests which was clear when culture filtrate of this fungus was sprayed instead of the spore suspension. However, when the inocula of both T. viride and H. oryzae were put together, then also a significant inhibition, in the early stages, was observed. This may be because T. viride may check the growth of the pathogen by hyperparasitism. The mechanism of action of T. viride was not clear, i.e. whether it inhibited the

pathogen by hyperparasitism or by production of antibiotics. Although the antagonistic effect may not be due to competition for nutrition alone, since the culture filtrate can also reduce disease incidence on the leaf surface to a significant extent. Similar results have been obtained by previous workers with other pathogens (Roy, 1977; Elad *et al.*, 1980). The decrease in the effectiveness of *T. viride* in the later stages in inhibiting the pathogen may be because of the reduced population of *T. viride* which have got washed out after some time or there may not be continuous physical contact with the pathogen or the less favourable environmental conditions. In the Khonorullo variety the lesion length was lesser than Mirikrak variety because the host itself may be providing some resistance to the entry of the pathogen in the resistant variety.

Further, *T. viride* could check *H. oryzae* to some extent when incorporated into soil although the effect was not very significant. Physical and chemical properties of soil may play a dominant role in parasitism by *T. viride* (Darpoux, 1960) and these may account for its partial effectiveness in the present test. These observations were similar to that obtained by Roy (1977) who also observed some inhibition of sheath blight fungus of rice by *T. viride* when incorporated into soil.

The antagonistic role of Trichoderma viride, a common leaf surface fungus has been recognized by several workers (Ale-Agha et al., 1974; Roy, 1977; Chet et al., 1979; Hadar et al., 1979). Differences in antagonistic behaviour of leaf surface microfungi in vivo and in vitro has been reported by many workers (Bhatt and Vaughan, 1963; Akai and Kuramoto, 1968; Heuvel, 1969; Pace and Campbell, 1974; Rai and Singh, 1980). Akai and Kuramoto (1968) has reported that a Candida sp. although was effective in controlling brown spot disease of rice in vivo but no inhibition could be observed in vitro studies.

The failure of Trichoderma sp. to exhibit germination on the leaf surface was probably due to the absorption of the toxic substances by the host, to evaporation of volatile substances or to neutralization of toxins by leaf surface chemicals (Mishra and Tewari, 1976c). This may also be due to absence of physical contact between the two organisms viz., the antagonist and the pathogen. The present results fail to explain the mechanism of operation and this aspect needs further study.

Moreover, in the present study, when a fungicide benomyl was used it was not effective for controlling the brown spot disease and when the fungicide and T. viride were combined then also there was no synergistic effect such as occurred when T. harzianum was combined with PCNB (Chet et al.

1979; Hadar *et al.*, 1979). Probably, Benlate may not be effective against the pathogen, *H. oryzae* although it is a broad spectrum fungicide and it is also known to control blast and sheath blight diseases of paddy.

From the present studies it can be concluded that under ideal conditions (as *in vitro*) selected fungal antagonists such as *Trichoderma viride* can be used as a biological control agent for brown spot disease of paddy although it is only partially successful in pot experiments. This may be because of gap in knowledge which relates the impact of natural environment, antagonistic selection and application technology (Spurr, 1981). Further studies are needed to test the effectiveness of *T. viride* against the brown spot pathogen of paddy in field and thus the biological control of leaf pathogens on a field scale offers a great challenge for future investigation.

CHAPTER V

COMPETITIVE ABILITY OF THE PHYLLOPLANE FUNGI

INTRODUCTION

An important aspect of phylloplane is the production of self inhibitory and self stimulatory compounds as leaf leachates or by the microorganisms present on the leaf surface (Tyagi and Chauhan, 1982).

There is now a wealth of evidence which shows that leaf surfaces exude substances in the form of leaf leachates which may have a direct effect on growth and colonization of microorganisms. When the effect is stimulatory to spore germination it is usually regarded as nutritional and is attributed largely to the carbohydrates and amino acids present in the environment (Godfrey, 1976). The host tissues are also known to exude the phytoncides which are inhibitory/stimulatory to the invading fungi.

The influence of leachates on plant surface microbial populations is very complex. Composition of leachates varies from plant to plant and also with the age of the plants. Blakeman (1972) found higher quantities of aminoacids in leachates of old beetroot leaves than the young ones. Microorganisms themselves respond in different ways to leachates from different plants (Chet et al., 1973). Furthermore, Purnell (1971) provided evidence to suggest that leachates may contain some components which stimulate growth and at the same time others which are inhibitory. Pero and Howard (1970) found

that leaf washings from Juniperus virginiana susceptible to attack by Phomopsis juniperovora, stimulated the germination of P. juniperovora conidia, whereas, leachates from J. chinensis, a non-susceptible species did not stimulate germination of P. juniperovora.

Evidence exists to show that exudates contain specific substances active against certain microorganisms. Brillova (1971) showed that germination of Cercospora beticola conidia was inhibited by 3-hydroxytyramine present in sugar beet leaf washings and suggested that resistant strains produced more of the inhibitor than susceptible strains. Schneider and Sinclair (1975) also showed that leachates from leaves of resistant varieties inhibited germination of pathogen spores. Irvine and Dix (1978) and Godfrey and Clements (1978) detected several water soluble inhibitory substances in leaf washings. Among the inhibitory substances produced by plants are the phyto-alexins whose production may be stimulated by the active growth of phylloplane fungi on senescent leaves.

The effect of a leachate on one microorganism may be modified by the interactions between it and other members of the microbial population (Blakeman, 1973). Leaf leachates sometimes may also modify the efficacy of fungicides (Dunn et al., 1971).

There is no doubt that aqueous leachates from aerial plant surfaces are important in relation to microorganisms resident there. Leachates may encourage growth by virtue of the nutrients they contain but they may, at the same time, include substances which inhibit microbial growth. Although some information about the composition of leaf leachates from some plants are available, the overall effect of the leaf and leaf surface nutrients on their phylloplane fungi is not studied in detail and there are still gaps in our knowledge. Only few workers (Mishra and Tewari, 1978) have worked on this aspect. Furthermore, most of the work reported has involved only leaf leachates and it would be of interest to know about the effect of leaf extracts on the leaf surface microorganisms. This stimulated to undertake the present investigation to see the effects of leaf leachate and extract of the three varieties of paddy (Khonorullo-disease resistant, Ngoba-moderately susceptible, Mirikrak-disease susceptible) on the spore germination and growth performance of certain fungi which were frequently isolated from the leaf surface of paddy.

MATERIALS AND METHODS

I) Germination behaviour of fungal spores in the leaf leachates and extracts of paddy plants.

Collection of leaf leachate:

Potted plants of paddy varieties were heavily sprayed with sterile distilled water and kept covered with moist

polythene bags for 24 hours. The leaf surface of these plants was then sprayed with sterilized distilled water and the washings were collected in sterilized containers. The washings were seitz filtered and concentrated to 1/4 of their volume.

Collection of leaf extract:

The leaf extract was obtained by crushing the leaves of the plants in mortar and filtered through whatman filter paper No. 1. The extract was again filtered before use.

The collection of leachates and extracts was done at different developmental stages i.e., seedling (15 days old), mature (90 days) and senescent (180 days).

The spore germination of different fungal species viz., Mucor hiemalis, Rhizopus nigricans, Aspergillus nidulans, A. flavus, A. niger, Alternaria alternata, Chrysosporium pruinosum, Cladosporium herbarum, Curvularia lunata, Epicoccum nigrum, Fusarium moniliforme, Gliocladium penicilloides, Memnoniella echinata, Helminthosporium oryzae, Nigrospora oryzae, Penicillium funiculosum, P. chrysogenum, Phoma glomerata, Stachybotrys atra, Starkeomyces koorchalomoides, Torula herbarum, Trichoderma viride, Trichothecium roseum and Verticillium albo-atrum was studied in the leaf leachates and extract of all the three varieties of paddy. The

germination of fresh spores obtained from 10 days old culture (on Czapek's Dox agar) medium was studied by hanging drop method using cavity slides. The slides with spores were incubated at room temperature (approx. 20°C) for 10 hours and the germination of spores was counted. Three replicates were maintained for each treatment. In control set the spore germination of the fungi was tested in sterilized distilled water.

2) The growth performance of fungal species in Czapek's Dox liquid medium containing leaf leachates and extracts:

The leachates and extracts were collected as described before and were kept in freezer and were mixed separately for each variety in equal quantity to get separate composite sample of leachates and extracts. 5 ml of leachate and extract was added to each flask containing 20 ml of liquid Czapek's Dox medium. To each flask were added 3 discs (4 mm diameter) of fungal inoculum obtained from 8 days old culture on Czapek's Dox agar medium. The fungi which were used in the previous experiment were used for this also. The flasks were incubated at 25±1°C for 10 days and the mycelial mat harvested. The mycelial felt was filtered through Whatman filter paper No.1, dried at 60°C for 3 days, cooled at room temperature and weighed.

3) Germination of fungal spores on detached leaf surface

The spore germination of 16 fungal species viz., Mucor hiemalis, Rhizopus nigricans, Aspergillus nidulans, A. flavus,

A. niger, Alternaria alternata, Cladosporium herbarum, Curvularia lunata, Fusarium moniliforme, Gliocladium penicilloides, Helminthosporium oryzae, Nigrospora oryzae, Penicillium funiculosum, Trichoderma viride, Trichothecium roseum and Verticillium albo-atrum was tested on the surface of detached leaves of the three paddy varieties. Fresh leaves were collected at random at different developmental stages of the plants. The leaves were cut into pieces of 2 cm length and kept in sterilized moist chambers. The spore suspension (5 x 10^4 spores/ml) of the fungi was pipetted on the leaf pieces and the plates were incubated for 10 hours at 25 ± 1°C. Thereafter, the spore germination was studied by collecting the drops of the spore suspension from the leaf surface with the help of micropipettes and mounting on the slide. Separate sterilized micropipettes were used for each fungi. In control set, the spore germination was studied in sterilized distilled water in cavity slides.

RESULTS

1) Germination of fungal spores in leaf leachates and extracts

Germination percentage of fungal spores in leaf leachate and leaf extract of paddy varieties collected at different ages varied significantly (P = 0.01) (Table 5.4).

From Table 5.1 it is clear that in case of Mucor hiemalis, Rhizopus nigricans, Asperigillus nidulans, A. flavus,

A. niger, Alternaria alternata, Stachybotys atra, Starkeomyces koorchalomoides, Torula herbarum, Trichothecium roseum and Verticillium albo-atrum, the germination percentage was low in the beginning when the leaf leachate was used from young plants. The germination, however, increased gradually and maximum was generally in the end, in the leachates of senescent stage. In Cladosporium herbarum, Curvularia lunata, Fusarium moniliforme, Memnoniella echinata, Penicillium funiculosum, P. chrysogenum and Trichoderma viride also a gradual increase was recorded but due to considerably higher germination percentage even in the earlier stages the increasing tendency was not so well marked as in the above cases. In case of Chrysosporium pruinosum, Epicoccum nigrum, Gliocladium pencilloides, Nigrospora oryzae and Phoma glomerata there was a decreasing tendency in leachates of later stages. In all those fungal species excepting Phoma glomerata the spore germination decreased in leaf leachates collected from mature stage and increased at the senescent stage or sometimes vice versa. In the case of the pathogen, Helminthosporium oryzae, however, the percentage germination increased gradually and the maximum was obtained in the exudate from the senescent stage which was more apparent in the susceptible variety than in the resistant one.

In general, the percentage spore germination of most of the antagonistic fungi like Trichoderma viride, Cladosporium herbarum, Nigrospora oryzae and Penicillium funiculosum against

the pathogen, H. oryzae, was higher in leaf leachates of the resistent variety (Khonorullo) than in the susceptible ones (Ngoba and Mirikrak). In addition, most of the fungi had high germination rate in the resistant variety. The percentage spore germination of the pathogen (H. oryzae) was maximum in leaf leachate fof the susceptible variety (Mirikrak) and minimum in case of the resistant variety (Khonorullo) while the moderately susceptible variety (Ngoba) occupied an intermediate position.

Germination behaviour of the spores of the above fungi differed significantly in the leaf extract of different developmental stages (Table 5.4). In most of the cases, as in the leaf leachates, the percentage germination increased gradually and the maximum was generally obtained in the extract of the later stages. In some cases, however, there was a gradual decrease in the spore germination in the later stages.

It is clear from Table 5.1 that the percentage germination of various fungi was always higher in the leaf extract than in the leaf leachates.

The pathogen showed maximum germination in the extract of the disease susceptible variety and on the other hand, most of the other fungi had higher germination rate in the leaf extract of disease resistant variety than in the susceptible one.

Germination of the spores in control was considerably high in most cases and sometimes it was higher in the corresponding exudate of extract treated sets.

2) Growth performance of certain fungi in Czapek's liquid medium containing leaf leachates and extract.

Growth behaviour of certain leaf surface fungi when studied in liquid Czapek's Dox medium containing a composite sample of leaf leachates and extracts of different ages separately revealed a characteristic pattern (Table 5.2). The dry weight of mycelial mat was always higher in the set containing leaf extract. In Mucor hiemalis, Rhizopus nigricans, Gliocladium pencilloides, Nigrospora oryzae, Phoma glomerata and Starkeomyces koorchalomoides, the degree of variation in the amount of dry mycelial felt in the treated and control sets was not much appreciable. The differences between the two corresponding treated sets was also not so high as compared to respective control. Higher mycelial felt in treated sets was obtained in most of the other fungi, viz., Aspergillus nidulans, A. flavus, A. niger, Alternaria alternata, Chrysosporium pruinosum, Cladosporium herbarum, Curvularia lunata, Epicoccum nigrum, Fusarium moniliforme, Gliocladium pencilloides, Nigrospora oryzae, Memnoniella echinata, Penicillium funiculosum, Cladosporium herbarum, Trichothecium roseum, Verticillium albo-atrum showed highest mycelial mat in the treated sets of the resistant variety.

Variation in the amount of mycelial felt harvested from exudate set and extract sets for different fungi was statistically significant at 5% probability for all the three varieties but it was significant at 1% probability also only in Khonorullo variety for both in exudate and extract sets.

3) Germination of fungal spores on detached leaf surface

Germination behaviour of 16 fungi viz., Mucor hiemalis, Rhizopus nigricans, Aspergillus nidulans, A. flavus, A. niger, Alternaria alternata, Cladosporium herbarum, Curvularia lunata, Fusarium moniliforme, Gliocladium pencilloides, Helmithosporium oryzae, Nigrospora oryzae, Penicillium funiculosum, Trichoderma viride, Trichothecium roseum and Verticillium albo-atrum when tested separately on the leaf surface of the three paddy varieties exhibitted varying pattern (Table 5.3). The spore germination in majority of the fungi, viz., Mucor hiemalis, Rhizopus nigricans, Aspergillus nidulans, A. flavus, A. niger, Alternaria alternata, Curvularia lunata, Fusarium moniliforme, Gliocladium pencilloides, Nigrospora oryzae, Penicillium funiculosum, Trichothecium roseum and Verticillium albo-atrum on leaf surface of paddy varieties was low in the beginning, increased continuously to the stage of maturity of the plants and decreased later on. In case of Cladosporium herbarum the germination percentage was not affected to a remarkable degree on the leaf surface of the three varieties varying in age although it increased slightly at the stage of maturity but it decreased at the stage of

Table 5.1: Percentage spore germination of certain fungi in leaf leachates and leaf extracts at different stages of plant growth collected from the three varieties of paddy (Mean of 3 replicates)

Fungi	Control	Young stage (15 days)						Mature stage (90 days)						Senescent stage (180 days)					
		Leaf leachate			Leaf extract			Leaf leachate			Leaf extract			Leaf leachate			Leaf extract		
		K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
Mucor hiemalis	89.1+	32.1	30.8	31.9	46.3	42.5	40.1	71.2	70.5	70.0	78.6	77.1	78.4	60.3	61.1	58.6	86.3	86.8	81.2
Rhizopus nigricans	90.5	34.5	32.1	36.3	48.0	49.2	41.0	68.0	69.1	65.0	76.1	75.9	76.0	89.3	90.1	89.4	90.2	90.0	88.4
Aspergillus nidulans	88.5	53.2	50.9	52.6	52.5	52.1	55.8	66.4	65.8	63.2	72.4	73.6	71.0	78.9	75.0	73.5	86.5	82.1	84.5
A. flavus	89.6	64.2	65.1	60.0	65.2	66.1	64.9	75.5	81.3	79.0	81.2	82.0	83.6	79.6	76.1	75.4	86.9	84.5	85.2
A. niger	92.3	35.4	36.0	32.4	38.6	37.5	38.1	62.3	65.1	63.4	71.2	74.9	72.6	75.3	76.9	72.8	91.2	86.9	90.8
Alternaria alternata	96.3	40.9	34.8	36.5	71.5	73.1	70.9	72.9	73.3	71.3	86.5	89.2	83.4	89.8	85.1	80.3	94.3	90.7	89.5
Chrysosporium pruinosum	82.5	54.5	50.2	56.1	62.1	64.2	61.9	48.5	41.6	40.2	76.3	75.1	76.2	52.3	54.6	51.2	79.1	76.9	78.6
Cladosporium herbarum	92.3	42.1	40.6	38.5	46.9	43.5	40.9	71.3	62.5	60.0	82.1	71.2	67.5	90.5	82.3	71.5	91.3	83.2	73.8
Curvularia lunata	89.6	52.4	53.2	56.8	58.1	54.1	57.8	78.6	80.1	71.2	81.8	81.6	80.5	71.2	73.4	70.5	80.6	81.7	81.2
Epicoccum nigrum	91.1	31.9	32.3	30.6	42.1	43.6	42.8	58.6	56.5	58.1	62.4	63.8	61.2	42.3	41.8	42.4	59.4	61.2	60.5
Fusarium moniliforme	91.5	71.4	68.5	82.1	78.6	71.2	85.4	83.2	90.5	91.0	86.8	91.0	91.2	90.2	89.5	90.9	91.2	90.8	91.3
Gliocladium penicilloides	96.2	32.5	35.0	38.1	43.1	40.9	39.7	42.1	43.5	44.6	44.6	43.8	43.9	39.0	35.2	30.0	41.1	37.5	32.4
Memnoniella echinata	95.0	35.8	36.1	35.4	42.8	41.9	40.6	41.5	42.1	40.0	43.1	43.0	42.6	48.6	42.0	43.9	53.5	51.1	50.9
Helminthosporium oryzae	98.1	17.6	21.3	37.5	38.6	38.8	41.7	41.1	63.2	65.0	45.5	65.6	68.1	50.9	89.4	93.4	51.2	90.1	97.8
Nigrospora oryzae	97.0	65.1	56.3	50.9	71.2	57.5	52.0	58.6	50.2	47.5	61.2	55.4	48.6	61.9	60.0	48.3	76.5	63.9	59.6
Penicillium funiculosum	93.8	43.1	39.5	36.1	56.5	53.0	50.9	79.5	63.1	62.0	84.6	79.3	62.9	93.6	82.0	71.5	93.7	83.5	78.9
P. chrysogenum	96.4	45.6	42.1	51.2	56.9	51.3	48.6	85.2	78.2	75.0	87.1	81.2	79.3	95.2	90.0	78.6	96.1	92.0	84.5
Phoma glomerata	62.1	52.1	53.4	59.0	61.7	58.6	60.4	43.4	42.1	49.3	46.6	43.8	52.1	36.5	34.3	31.1	38.5	37.9	37.6
Stachybotrys atra	96.3	38.5	41.0	40.9	40.6	42.1	41.3	69.1	75.2	78.4	76.2	76.8	81.2	85.3	85.0	83.2	93.2	94.0	91.8
Starkeomyces koorchalomoides	94.1	27.1	18.5	28.5	31.9	26.1	32.4	45.8	47.6	48.5	52.3	51.9	53.4	51.2	48.3	48.2	81.2	80.9	80.0
Torula herbarum	90.8	18.5	21.2	28.6	25.3	22.9	32.4	48.6	50.5	52.1	54.6	56.1	55.9	63.6	61.8	63.5	82.4	83.2	81.6
Trichoderma viride	96.8	53.4	50.1	48.1	56.6	53.2	49.6	68.6	52.4	43.0	79.8	56.5	49.2	95.1	58.0	52.4	96.3	72.1	53.9
Trichothecium roseum	93.6	36.2	37.5	35.4	38.2	41.3	39.2	48.5	62.1	54.5	52.3	65.0	56.1	86.5	87.2	86.1	90.2	90.5	90.0
Verticillium albo-atrum	94.7	19.1	32.1	12.6	25.9	33.1	24.6	56.2	53.1	48.2	71.6	67.2	65.4	91.5	83.2	78.8	93.8	89.1	79.0

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety

+ = Percentage germination based on 100 spores.

Table 5.2: Growth performance of certain fungi on Czapek's liquid medium incorporated with leaf leachates and leaf extracts of paddy varieties.

Fungi	Control	Cz + LL			Cz + LE		
		K	N	M	K	N	M
Mucor hiemalis	0.54	0.51+	0.49	0.50	0.52	0.51	0.51
Rhizopus nigricans	0.61	0.58	0.59	0.56	0.60	0.61	0.59
Aspergillus nidulans	0.34	0.36	0.38	0.37	0.41	0.43	0.42
A. flavus	0.28	0.32	0.30	0.31	0.36	0.37	0.35
A. niger	0.31	0.39	0.38	0.40	0.42	0.40	0.42
Alternaria alternata	0.29	0.32	0.31	0.36	0.46	0.42	0.38
Chrysosporium pruinosum	0.30	0.38	0.39	0.35	0.48	0.49	0.52
Cladosporium herbarum	0.32	0.45	0.41	0.37	0.52	0.48	0.45
Curvularia lunata	0.38	0.41	0.44	0.46	0.48	0.49	0.52
Epicoccum nigrum	0.31	0.33	0.32	0.31	0.41	0.43	0.42
Fusarium moniliforme	0.36	0.46	0.42	0.43	0.52	0.54	0.53
Gliocladium pencilloides	0.39	0.41	0.38	0.42	0.48	0.45	0.43
Helminthosporium oryzae	0.58	0.59	0.62	0.65	0.60	0.64	0.69
Memnoniella echinata	0.42	0.43	0.46	0.41	0.52	0.48	0.45
Nigrospora oryzae	0.51	0.48	0.50	0.51	0.53	0.52	0.52
Penicillium funiculosum	0,28	0,42	0.41	0.38	0.51	0.49	0.43
P. chrysogenum	0.24	0.43	0.40	0.42	0.48	0.43	0.45
Phoma glomerata	0.18	0.19	0.18	0.19	0.21	0.19	0.20
Stachybotrys atra	0.34	0.36	0.37	0.32	0.41	0.39	0.40
Starkeomyces koorchalo-moides	0.19	0.21	0.18	0.20	0.23	0.20	0.24
Torula herbarum	0.29	0.31	0.30	0.30	0.37	0.35	0.38
Trichoderma viride	0.48	0.59	0.57	0.49	0.62	0.59	0.51
Trichothecium roseum	0.51	0.54	0.53	0.51	0.61	0.59	0.59
Verticillium albo-atrum	0.42	0,45	0.42	0.41	0.58	0.46	0.45

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety
+ expressed in oven dry weight of mycelial felt in gms.

Table 5.3: Percentage spore germination of certain fungi on detached leaf surface of the three varieties of paddy at three **developmental stages.**

Fungi	Control	Young stage (15 days)			Mature stage (90 days)			Senescent stage (180 days)		
		K	N	M	K	N	M	K	N	M
Mucor hiemalis	90.2	72.1+	72.6	52.4	90.3	85.1	82.6	68.2	65.1	62.5
Rhizopus nigricans	91.6	81.2	83.6	72.4	90.8	86.5	88.5	48.3	32.5	51.9
Aspergillus nidulans	90.5	42.3	35.5	52.1	78.6	81.2	72.9	30.5	18.6	19.0
A. flavus	86.2	71.5	75.4	83.5	82.4	83.6	85.6	62.1	52.6	60.9
A. niger	68.2	68.4	43.6	71.9	61.2	38.7	62.1	29.4	21.5	18.1
Alternaria alternata	95.3	35.4	38.4	32.6	79.5	72.4	61.5	39.9	37.0	37.2
Cladosporium herbarum	92.5	86.9	80.2	76.4	90.5	81.2	76.1	71.2	62.4	57.5
Curvularia lunata	90.8	42.7	40.8	41.8	60.4	58.1	58.3	40.6	42.7	38.2
Fusarium moniliforme	91.1	61.3	50.9	60.3	82.1	72.9	61.8	31.2	35.0	30.5
Gliocladium penicilloides	95.5	38.6	40.2	35.0	85.2	67.5	52.5	35.0	30.2	18.6
Helminthosporium oryzae	98.1	16.0	18.0	21.2	16.7	49.3	66.1	11.2	52,4	86.0
Nigrospora oryzae	95.0	52.1	53.6	50.9	63.1	56.3	61.2	28.5	20.0	19.5
Penicillium funiculosum	95.5	42.9	36.5	25.3	96.4	75.2	61.5	32.1	28.6	17.2
Trichoderma viride	96.8	52.3	51.9	45.0	89.5	56.5	54.2	75.0	53.1	28.5
Trichothecium roseum	93.0	38.5	36.1	37.5	83.2	81.5	89.9	28.5	16.2	23.4
Verticillium albo-atrum	95.0	29.6	35.2	12.5	48.5	53.2	41.9	31.2	29.5	30.8

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety; + Average germination based on 100 spores and mean of 3 replicates.

Table 5.4: Analysis of variance for the spore germination of certain fungi in leaf leachate and leaf extract of paddy varieties at different growth stages of plant using 'F' test.

Factors	Growth stages								
	Young stage			Mature stage			Senescent stage		
	K	N	M	K	N	M	K	N	M
Leaf leachate	2.76**	3.33**	3.23**	2.94**	3.26**	2.79**	4.25**	4.21**	3.2**
Leaf extract	4.07**	5.78**	4.23**	3.25**	3.61**	3.42**	5.16**	5.10**	3.6**

** Significant both at 1% and 5% probability levels; K = Khonorullo variety; N = Ngoba variety M = Mirikrak variety.

Table 5.5: Analysis of variance for growth performance of certain fungal species in culture medium containing leaf leachate and extract of the three varieties of paddy.

Factors	F values		
	K	N	M
Leaf leachate	3.17**	2.09*	2.03*
Leaf extract	4.12**	3.62*	3.19*

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety; * Significant at 5% probability; ** Significant at both 1% and 5% probability.

Table 5.6: Analysis of variance for spore germination of certain fungal species on leaf surface of paddy varieties at three growth stages (observed 'F' values).

Variety	Young stage (15 days)	Mature stage (90 days)	Senescent stage (180 days)
K	8.9**	32.9**	2.59*
N	13.1**	13.5**	12.8**
M	18.3**	3.61**	10.1**

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety;

* Significant at 5% probability; ** Significant at both 1% and 5% probability levels.

senescence.

The pathogen, H. oryzae, however, showed an increasing trend only on the leaves of disease susceptible variety (Mirikrak) and the moderately susceptible variety (Ngoba). On the leaves of the resistant variety (Khonorullo) although it increased slightly at maturity but decreased at the senescence stage. On the other hand, Trichoderma viride an important antagonist of the pathogen, showed maximum percentage germination on the leaves of resistant variety. It increased considerably at maturity but decreased slightly at senescent stage. In the other two cases (Ngoba and Mirikrak) although it increased considerably on leaves from maturity stage but decreased considerably on the leaves of senescent stage.

The variation in percentage of spore germination generally was statistically significant (at $P = 0.01$ and $P = 0.05$) at different stages of the plant growth in all the three varieties of paddy (Table 5.6).

DISCUSSION

Significant variation was marked in the germination percentage of the fungal species in the leaf leachate and extract of paddy varieties. A perusal of Table 5.1 indicates that germination behaviour of the fungi differed in the leaf leachate and extract of the plants of same developmental stages.

Similar results were also obtained by Mishra dand Tewari (1978) working with wheat and barley.

Increase in spore germination of certain fungi (Table 5.1) in the leaf leachate and leaf extract of senescent plants may be accounted to the presence of higher amount of nutrients such as sugars, amino-acids and organic acids in the exudates and extracts of older leaves (Godfrey, 1976; Mishra and Tewari, 1978). Release of leachates in higher amount from older leaves has been reported by various workers (Colodny, 1932; Arens, 1934; Tukey et al., 1958). Increase in permeability of the cell membrane of older cells (Kerling, 1964) coupled with loose arrangement of the cell during this stage possibly accounted for the higher amount of leaching from the leaf surface.

The results also show that the percentage germination of the pathogen, H. oryzae was higher in leaf leachates and leaf extracts of the susceptible (Mirikrak) variety than in the leachates and extracts of the resistant variety (Khonorullo). These might be attributed to the presence of more phenolic substances or other fungitoxic compounds in leaf leachates of resistant cultivars, which have inhibitory effect on pathogenic fungi. Similar results were also obtained by Tyagi and Chauhan (1982) working with the pathogen, Alternaria solani in the leaf exudate of chilli varieties and also by Topps and Wain (1957), Dix (1974), Schneider and Sinclair (1975), Carrasco et al. (1978) and Godfrey and Clements (1978) working with different

pathogens.

The percentage germination of most of the antagonistic fungi, viz., Trichoderma viride, Penicillium funiculosum and Cladosporium herbarum was highest in the leaf leachates and extracts of the resistant variety and less in the susceptible one. This is in accordance with the results of previous workers (Tyagi and Chauhan, 1982). The increase in percentage germination of some fungi in resistant variety may be because of availability of optimum nutrients in the exudates and extracts.

Variation in the spore germination in the leachate and extract may be explained in the light of difference in the chemicals present in them (Table 5.2).

In most of the cases, addition of samples of leaf leachates and extracts of all the varieties separately to the Czapek's Dox liquid medium containing different fungal species favoured the growth of the fungi (Table 5.2). The leaf extract, however, resulted in higher mycelial felt for most of the fungi treated. This may be because there may be higher amount of nutrients in the extract than in the leachate which stimulated the growth of the fungi in a greater degree in the former case. Similar observations were also obtained by Mishra and Tewari (1978).

The pathogen, H. oryzae showed maximum growth in the

treated set of extract and leachate of the susceptible variety. This may be because of the presence of some toxins in the leachates and extracts of resistant variety which probably inhibited the growth of the pathogen.

Germination pattern of spores on the leaf surface of the three varieties varied significantly (Table 6.6). In most of the cases germination percentage increased upto the mature stage and decreased subsequently. Increment in the leaf leachates of the ageing plants accounted for higher germination on the leaves of advanced ages. At the senescent stage, however, the germination percentage decreased possibly due to the release of toxic substances on the leaf surface which overrides the nutrient level of the leaf surface. Deverall (1967) and Mishra and Tewari (1978) were also of the same opinion and suggested that inhibition level overtops nutrient level in its controlling effect on fungal development.

The pathogen, H. oryzae, however, showed highest germination on the leaves of susceptible variety and less on the resistant variety. This may be because the leaves of resistant variety may be providing some resitance to the growth of the pathogen. On the other hand, an important antagonist of the pathogen, H. oryzae, viz., T. viride showed highest germination rate on the leaves of the resistant variety.

From the results of the present investigation it is very clear that the germination and growth performance of certain selected fungi from the phylloplane of paddy varieties were enhanced in the leaf leachates, leaf extracts and on the surface of leaves. Further, the pathogen showed highest germination and growth performance in the leaf leachates, leaf extracts and on the surface of leaves of the susceptible variety (Mirikrak) and on the other hand, antagonistic fungi like T. viride showed maximum growth in the leaf leachates, extracts and on leaf surface of resistant variety (Khonorullo).

CHAPTER VI

BIOCHEMICAL ANALYSIS OF THE LEAF LEACHATES AND EXTRACTS

INTRODUCTION

There is now a wealth of evidence which shows that a plant's susceptibility or resistance to a disease may, in part, be determined by conditions prevailing on the surface of the leaf prior to attempted infection (Blakeman, 1971).

Substances, both organic and inorganic accumulate in water which is in contact with plant surfaces. Some of these materials orginate outside the plant as deposits from the atmosphere, greater proportions, however, have their origin within the plant and pass through the outer tissues into water in contact with the surface layers. This process is usually referred to as leaching. Since leachates contain components which can be utilized as nutrients by microorganisms and may also contain substances which inhibit their germination and growth, they are of considerable importance to the microbial populations of plant surfaces (Ruinen, 1961; Last and Deighton, 1965; Blakeman, 1971; Godfrey, 1976).

Substances leached from plants include a great diversity of materials (Morgan and Tukey, 1964). Inorganic nutrients leached include all the essential minerals and some other elements commonly found in plants, including both the macro-and micro-elements. In addition, large amounts of organic substances have been noted including free sugars, pectic substances and sugar alcohols. All of the amino acids found in plants and many of the organic acids have been detected in

leachates. Many growth regulating chemicals, vitamins, alkaloids and phenolic substances are also reported to be leached from plants (Tukey, 1971). Despite the large quantities of inorganic nutrients which can be leached, organic substances, principally carbohydrates, account for the major quantity of leached materials.

In addition, substrates utilized by pathogenic fungi may have undergone conversion from the forms in which they were originally present by microbial epiphytes. Sucrose is believed to be the major carbohydrate exuded from leaves, however, in analysis of foliar exudates, glucose, fructose and many other sugars have been shown to be present in almost equal quantities. This may be due to conversion by bacteria (Blakeman, 1971).

Leaching may induce diseases in some plants. Mann and Wallace (1925) observed a spotting disease in apples during rain when there was more leaching of potassium. Similarly Dalbro (1958) also observed a positive correlation between high leaching during rains with the spotting disease of apples. Thus nutrient compostion of the host may also affect susceptibility to disease. On the other hand, increased leaching of substances on leaves may also enable the epiphytic flora which includes saprophytes to increase and in turn may play a significant role in imparting resitance to foliar infection by forming a microbial barrier or buffer (Kumar and Balasubramanian, 1981).

The capacity of plant organs to release leachates increases with age (Stenlid, 1958). As the leaf begins to age, the amounts of nutrients on the leaf surface also increase (Tukey, 1971). Mishra and Tewari (1976b) showed that the amount of sugars and aminoacids leached from the leaf surface increased with age of the plants. Higher amounts of sugars and aminoacids were usually present in the leaf exudate and extract of the older plants. Thus the phylloplane microbial population is affected directly by nutrient level of the leaf surface which changes with the release of the substances from the leaf.

Many workers (Chet et al., 1973; Mishra and Tewari, 1978; Bakshi, 1981) have done quantitative analysis of leaf leachates at different growth stages of the plants. Tewari (1973) has reported that total soluble sugars in wheat and barley ranged from 148-196 mg/gm of dry weight of leaves and the sugar content increased continuously with age of the plants. But the quantity of total nitrogen and proteins decreased in post-flowering stages. The amount of amino acids, however, increased with maturity of the leaves (Mishra and Tewari, 1976b).

Although leaching is important and it has direct effect on the pathogens and other microbes present on the leaf surface, the influence of leachates on plant surface micro-organisms is complex in the sense that it may be modified by

interactions between them. The microbes also respond to leachates of different plants in different ways (Tukey, 1971).

Godfrey (1976) has thus stated that leaf leachates have an important role in shaping the environment available on the host leaf surface. Though there is a lot of information available on biochemical differences in leaf exudates of healthy and diseased host; but very little is known about the biochemical differences in resistant and susceptible varieties at different stages of plant growth during disease development. Thus there has been no effort to correlate the population dynamics with the physico-chemical characteristics of the leaf surface environment. This lacunae warranted a detailed investigation of the problem and with this aim in mind the present study was undertaken and biochemical changes in leaf leachates and leaf extracts of the three varieties of paddy differing in resistance towards the pathogen, H. oryzae at different growth stages of plant were studied and an attempt was made to correlate the chemical characteristics of the leaf surface environment with the population dynamics of the phylloplane microflora.

MATERIALS AND METHODS

Experimental plant: The three varieties of paddy viz., Khonorullo (disease resistant), Ngoba (moderately susceptible) and Mirikrak (disease susceptible) were used as

the experimental plant.

Collection of leaf leachates: Potted plants of the different varieties of paddy were heavily sprayed with sterile distilled water and covered with a moist polythene bag for 24 hours. Then leaf surface of these plants was sprayed again with sterile distilled water and the washings which appear as droplets at the leaf tips and margins were collected in sterilized containers by means of sterilized micropipette. The leachates of different plant varieties collected at different stages of plant growth, were stored in freezer and further used for chemical analysis of sugars, aminoacids, organic acids and phenols.

Collection of leaf extract: Leaves were collected from paddy varieties at different stages of plant growth. Fresh leaves were steam killed for 15 minutes and were dried at 60°C in hot air oven for 24 hours. These dried leaves were used to determine their total sugars, total aminoacids and total nitrogen.

For analysis of total phenols fresh leaves were used directly.

1) Qualitative analysis of leaf leachates for aminoacids, sugars, organic acids and phenols by paper chromatography

(I) Aminoacid analysis: Aminoacids of leaf leachates of different growth stages of the plant were determined by

undirectional chromatographic method (Smith, 1958). Solvent used was a mixture of n-butanol, glacial acetic acid and distilled water in the ratio of 4:1:1 v/v respectively. Ninhydrin (0.3% in acetone) was used as a spraying reagent for the appearance of the spots. The chromatograms were air dried at room temperature and finally at 100 - 110°C for 5 - 8 minutes. Identification of amino acids was done by running the known aminoacids as markers.

(II) Sugar analysis: The evaluation of quality of sugar was carried out by unidirectional paper chromatography using n-butanol, ethanol, water as solvents in the ratio of 5:1:4 v/v and ammoniacal silver nitrate reagent as the spray reagent (Mahadevan and Sridhar, 1982). This reagent was prepared by adding 5 N ammonium hydroxide to 0.1 N silver nitrate till the solution was clear. After spraying, the paper was kept at 100°C for 5 mins. Reducing sugars were detected by this method which exhibited brown to black colour. Standard chromatograms were run side by side and spots developing on known and unknown samples were compared by calculating their Rf values (Smith, 1958).

(III) Detection of organic acids: The evaluation of quality of organic acids was carried out by unidirectional paper chromatography using Isopropanol-conc. ammonium hydroxide-water as solvents in the ratio of 20:1:2 v/v and bromothymol blue as the spraying reagent (Mahadevan and Sridhar, 1982).

This reagent was prepared by dissolving 100 mg of bromothymol blue dye in 1 ml of 0.02 M NaOH and then raising the volume to 250 ml with distilled water. The organic acids gave a yellow colour. The standard chromatograms were run side by side and the spots developing on unknown samples were identified by comparing the Rf values of known and unknown samples (Smith, 1958).

(IV) Detection of phenols: The qualitative analysis of phenols was carried out by unidirectional paper chromatography using n-Butanol-acetic acid-water as solvent in the ratio of 4:1:1 v/v and diazotised sulphanilic acid as the spray reagent. The reagent was prepared by dissolving 50 gm of sulphanilic acid in 250 ml of 10% KOH, cooled and added 200 ml of 10% $NaNO_2$. Then this mixture was added in drops to 120 ml of conc. HCl (a mixture of 80 ml of conc. HCl and 40 ml of water). Precipitated diazonium salt was filtered with ice water, washed the precipitate with ethanol followed by ether and and then dried in air. The dried salt was stored at 2 - 4°C and 50 mg of the salt was mixed in 20 ml of 20 percent Na_2CO_3 for spraying. After spraying, the paper was dried at room temperature, phenols appeared as deep yellow, light red and other patterns.

2) Quantitative analysis of leaf extract for total nitrogen, sugars, aminoacids and phenols

(i) Estimation of total nitrogen and protein: Total

nitrogen was estimated by microkjeldahl method as given by Jackson (1967). 0.2 gm of plant material was taken in Kjeldahl digestion flask to this 0.4 gm of mixed catalyst ($CuSO_4$:K_2SO_4: Selenium dioxide in the ratio of 1:8:1) and 3 ml of concentrated H_2SO_4 was added. The mixture was then heated until dark brown colour of the solution changed to green. It was then allowed to cool and the digested sample was taken in the funnel of distillation apparatus. In a flask, 15 ml of boric acid and two drops of mixed indicator (0.5 gm Bromocresol and 0.1 gm methyl red dissolved in 100 ml 95% alcohol) were taken and placed under the delivery tube of the condenser, so that the tip was below the liquid surface. Then 80% NaOH was added to the funnel of distillation apparatus by slowly rotating the stopper. NaOH was allowed to fall drop wise so that it got mixed with the digested sample in the distillation apparatus. When the colour of the indicator changes and all the distillate was collected it was titrated against $\frac{N}{14}$ HCl until greenish colour of the solution turned red after neutralization.

Percentage total nitrogen was calculated from the following formula,

$$\% \text{ total } N_2 = \text{volume of HCl used} \times \text{Normality of acid} \times \frac{1.4}{\text{Weight of sample.}}$$

Percentage total protein was calculated from the formula,

Protein % = % Nitrogen x 6.25 which is based on the assumption

that plant proteins contain 16 percentage of nitrogen.

Estimations were done in triplicate and average readings taken.

(II) Estimation of total sugars and amino acids: The total sugars and amino acids were determined by the method given by Peach and Tracey (1955). 100 mg of powdered dried leaves were crushed in a mortar and pestle in 80% ethanol and filtered through Whatman filter paper No. 1. A slightly yellow coloured filtrate was obtained. The filtrate was treated with activated charcoal and centrifuged at 6000 rpm to get a clear supernatant. Ethanol was boiled off from the clear filtrate in a hot water bath and the volume was made upto 5 ml with the addition of double distilled water. 3 ml of this solution was taken in a test tube and 6 ml of anthrone reagent (0.4% in H_2SO_4 w/v) was added gently to the test tube from the side while the test tube was kept in a cold water bath. Then the test tube was gently shaken and warmed on a cold water bath for 3 mins. A green colour was obtained. The transmittance of this coloured solution was observed in spectrophotmeter at 610 mμ. Standard curve was obtained from transmittance of varying concentrations of glucose solution treated with anthrone reagent. From the standard curve the values of the total sugar of the samples were expressed in μg/100 mg dry weight of the samples.

For total amino acid estimation, to the rest of the 2 ml extract (which was left after estimating total sugars) 2.5 ml

of acetate buffer (40 g NaOH dissolved in distilled water, 100 ml of glacial acetic acid was added to it and the volume was made upto 1000 ml with distilled water) and 2.5 ml of ninhydrin solution (1% in isopropyl alcohol) were added simultaneously. The test tube containing the solution was placed in hot water bath for half an hour, a light purple colour was obtained. The transmittance of this coloured solution was observed in spectrophotometer at 540 nm. Standard curve was obtained from the transmittance of the varying concentration of a mixture of amino acids (1 mg of each alanine, aspartic acid, Tryptophan, Proline/lysine and to make 5 mg of composite mixture of aminoacids which were dissolved in 0.1 HCl) treated with acetate buffer and ninhydrin solution. From the standard curve the values of the total amino acids of the samples were expressed in µg/100 mg dry weight of the samples. The estimations in case of sugars and amino acids were done in triplicate and average readings taken.

(III) Estimation of total phenols: For estimation of total phenols (Bray and Thorpe, 1954) 100 mg of fresh leaves were crushed in a mortar and pestle in 60% ethanol and filtered through a whatman filter paper No. 1. 1 ml of the extract was taken in a test tube and 1 ml of Folin reagent and 2 ml of Na_2CO_3 solution were added. The tube was shaken and kept in boiling water bath for 1 minute. The tube was cooled in

running tap water and the volume was made up to 25 ml with distilled water. The transmittance of this solution was observed at 650 nm in a spectrophotometer. Standard curve was prepared from the transmittance of varying concentration of catechol.

RESULTS

1) Qualitative analysis of leaf leachates for aminoacids, sugars, organic acids and phenols

(I) Aminoacid analysis: A perusal of qualitative analysis of aminoacids indicate that a total of eighteen aminoacids was detected at different stages of plant growth from all the paddy varieties (Table 6.1). Threonine, tryptophan and leucine were detected almost throughout the growth stages of the plant. Methionine was detected only in the susceptible variety (Mirikrak). Glutamic acid, valine, aspartic acid, proline and hydroxyproline were detected only in the resistant (Khonorullo) and moderately susceptible (Ngoba) varieties. Nor-leucine was detected only in the moderately susceptible (Ngoba) variety. The remaining amino acids viz., Tyrosine, threonine, phenylalanine, leucine, serine, amino-n-butyric acid, tryptophan, arginine, cystine, iso-leucine and glyoine were detected in all the three varieties of paddy at different stages of plant growth.

From Fig. 6.1 it is clear that the number of amino-acids varied with age of the plants. Further, it is also clear that higher number of aminoacids was detected in the leaf leachates of resistant variety (Khonorullo) than in the other two varieties (Ngoba and Mirikrak).

(II) Sugar analysis: A perusal of the qualitative analysis of sugars of leaf leachates collected at various growth stages of the plant indicates that a total of 16 sugars was detected (Table 6.2). Lactose and xylose were detected throughout the study period in all the three varieties. Most of the sugars viz., lactose, xylose, glucose, rhamnose, sucrose, mannose, melibiose, cellobiose, galactose, trehalose and arabinose were detected in all the paddy varieties studied. Fructose, ribose, raffinose and sorbose were detected only in resistant (Khonorullo) and moderately susceptible (Ngoba) varieties. Maltose was detected only in Khonorullo, the disease resistant variety. No sugar was observed to be exclusive to the susceptible variety.

From Fig. 6.1 it is clear that the number of sugars increased with plant-age and the maximum was detected at 180 days in Khonorullo variety. Further, it is also clear that the leachates of disease resistant had more sugars than in the susceptible varieties (Ngoba and Mirikrak) and the least number of sugars was present in the leaf leachates of the disease susceptible variety.

(III) Organic acid analysis: Table 6.3 shows that 12 organic acids were detected in leaf leachates of the three varieties of paddy at different stages of plant growth. Most of the organic acids viz., palmitic acid, caprylic acid, malic acid, oxalic acid, capric acid, myristic acid, tartaric acid and succinic acid were detected in all the three varieties of paddy. Stearic acid and lactic acid were detected only in the disease resistant (Khonorullo) and the moderately susceptible (Ngoba) varieties. Citric acid and maleic acid were detected only in the susceptible variety (Mirikrak). No organic acid was exclusively isolated from the resistant variety.

From Fig, 6.1 it is clear that number of organic acids varied with plant-age and more number of organic acids was detected in the resistant (Khonorullo) variety than in the other two varieties (Ngoba and Mirikrak).

(IV) Analysis of phenols: Table 6.4 shows that 9 phenols were detected in leaf leachates of the three paddy varieties. Among the phenols detected, chlorogenic acid, phloroglocinol and catechol were commonly found in all the three varieties, whereas, caffeic acid, isochlorogenic acid and resorcinol were detected only in the resistant (Khonorullo) variety and in the moderately susceptible (Ngoba) varieties. Protocatechuic acid was detected only in the disease resistant (Khonorullo) variety and dinitrophenol was detected only in the susceptible varieties (Ngoba and Mirikrak).

From Fig. 6.1 it is clear that number of phenols increased with age of the plants in all the varieties of paddy. Moreover, there were more phenols in the disease resistant (Khonorullo) variety than in the other two varieties (Ngoba and Mirikrak). The least number of phenols was detected in the disease susceptible variety (Mirikrak).

2) Quantitative analysis of leaf extracts for total nitrogen, sugars, amino acids and phenols

(I) Total nitrogen and protein: The quantity of total nitrogen and protein was initially almost the same in all the three varieties of paddy but with increase of age of the plants it declined and became quite low at senescence. The nitrogen and protein content although initially was lower in the disease resistant (Khonorullo) variety than in the other two varieties (Ngoba and Mirikrak) but at later stages it was higher than in the later case (Fig. 6.2).

(II) Total sugars and aminoacids: The amount of total sugars and amino acids increased with age of the plants and maximum was in Khonorullo, the disease resistant variety and less in the other two varieties (Ngoba and Mirikrak) (Fig. 6.2).

(III) Total phenols: The phenolic content was higher in the disease resistant variety (Khonorullo) than in

Fig. 6.1: Histograms depicting a comparison of number of amino acids, number of sugars, number of organic acids and number of phenols in the leaf leachates of different varieties of paddy viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

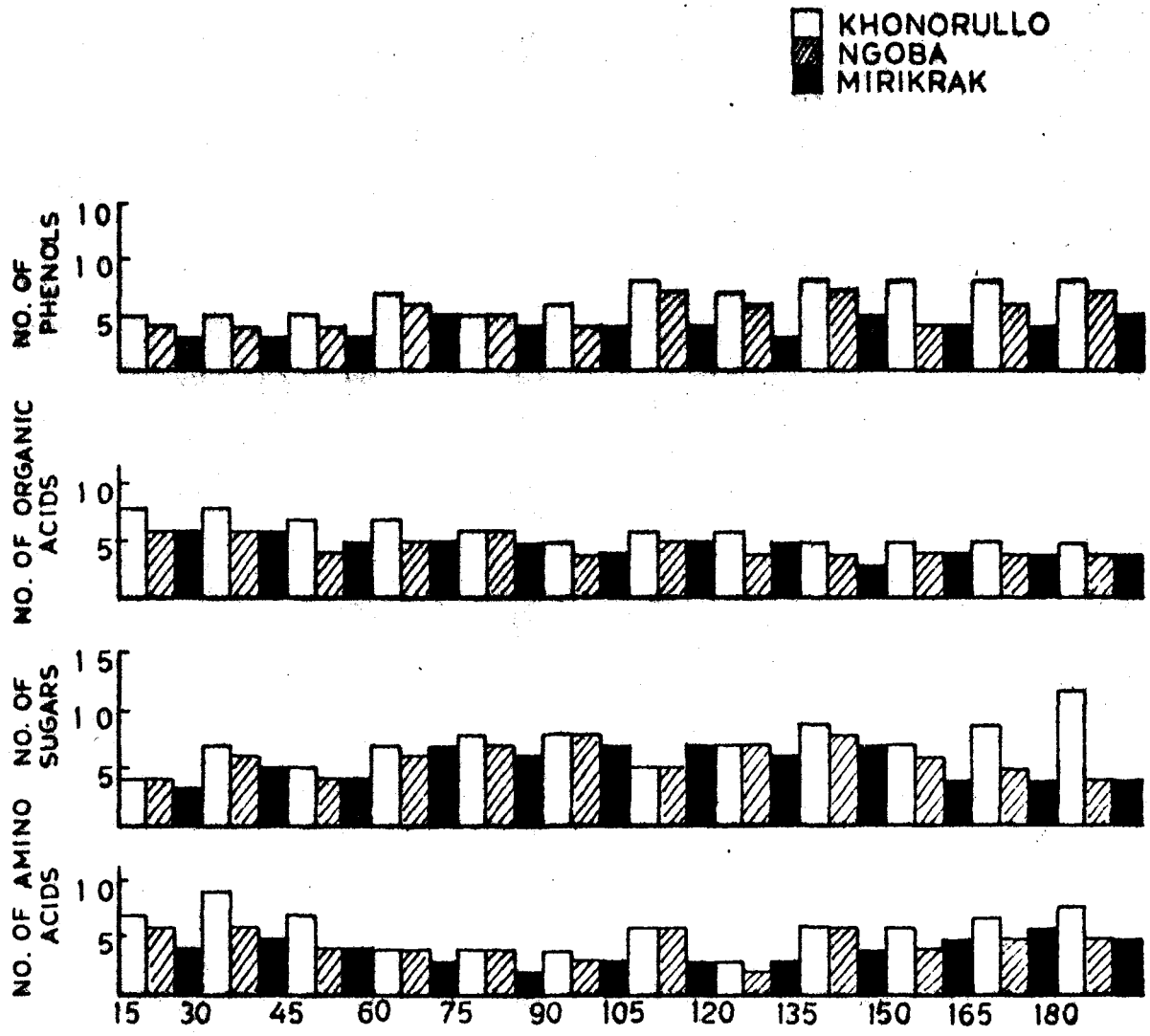
KHONORULLO
NGOBA
MIRIKRAK
NO. OF PHENOLS
10
10
5
NO. OF ORGANIC ACIDS
10
5
NO. OF SUGARS
15
10
5
NO. OF AMINO ACIDS
10
5
15 30 45 60 75 90 105 120 135 150 165 180
AGE OF THE PLANT IN DAYS

Fig. 6·1

Fig. 6.2: Histograms depicting a comparison of percentage total nitrogen, percentage total protein, total sugars (in µg/100 mg), total amino acids (in µg/ml) and total phenols (mg/gm) in the leaf extracts of paddy varieties viz., Khonorullo, Ngoba and Mirikrak at different ages of the plant.

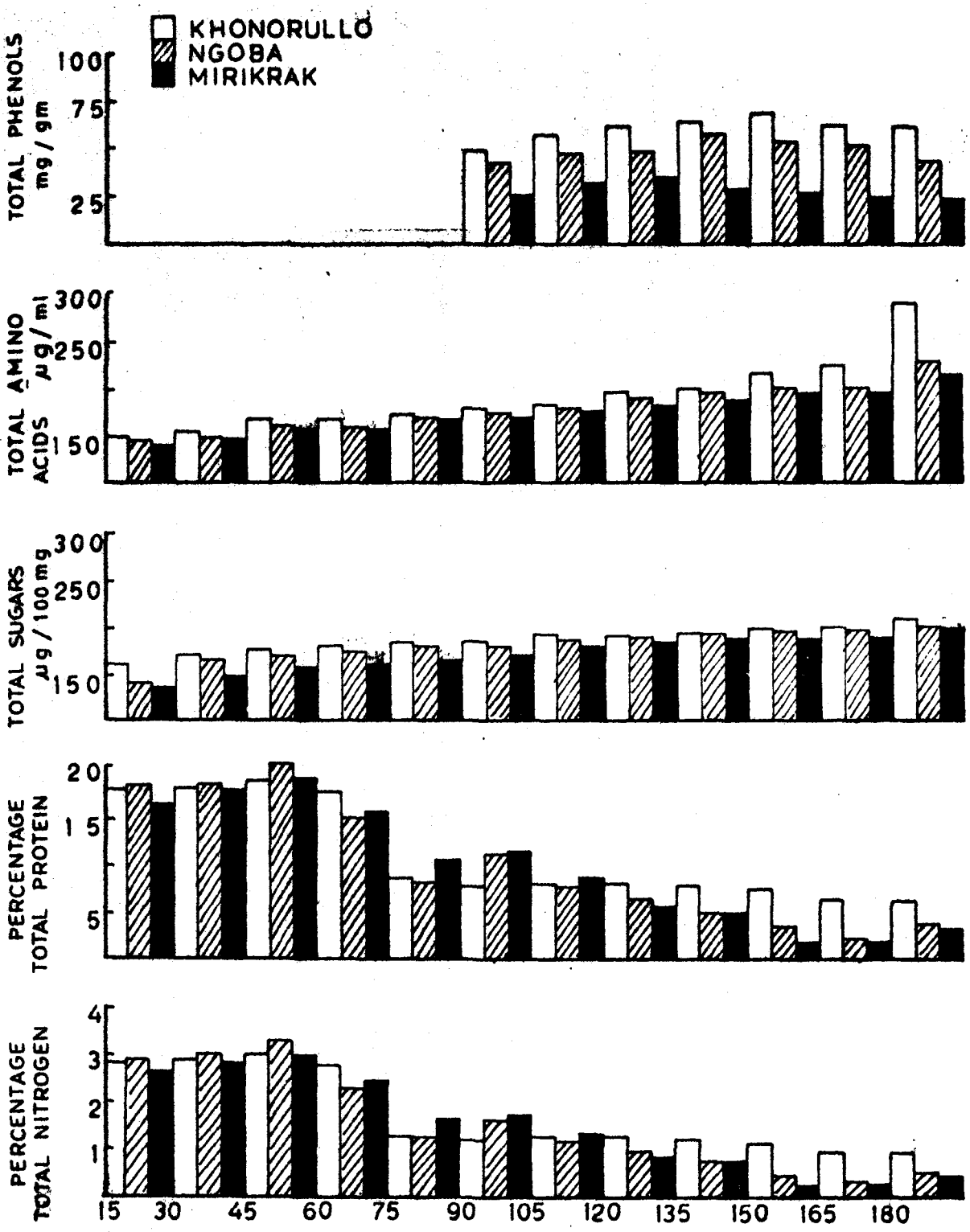
KHONORULLO
NGOBA
MIRIKRAK
TOTAL PHENOLS mg / gm
100
75
25
TOTAL AMINO ACIDS µg/ml
300
250
150
TOTAL SUGARS µg / 100 mg
300
250
150
PERCENTAGE TOTAL PROTEIN
20
15
5
PERCENTAGE TOTAL NITROGEN
4
3
2
1
15 30 45 60 75 90 105 120 135 150 165 180
AGE OF THE PLANT IN DAYS

Fig. 6.2

Table 6.1: Showing the amino acid composition of leaf leachates of three paddy varieties at different ages of the plant.

Amino-acids	AGE OF THE PLANT IN DAYS																																			
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
Tyrosine	+		+			+							+	+	+							+	+	+	+		+	+	+	+		+	+	+	+	+
Methionine			+			+			+												+			+			+			+			+			+
Threonine	+	+	+	+	+		+		+	+	+	+	+			+		+	+	+				+		+	+	+		+	+			+	+	+
Phenylalanine	+								+												+			+			+			+					+	+
Leucine	+	+	+	+	+	+	+							+		+	+	+	+	+	+				+					+	+					
Serine		+								+			+																		+	+					
Proline	+	+		+	+		+						+	+						+	+				+	+										
Amino-n-buytric acid	+	+		+						+								+	+					+								+	+			
Tryptophan	+	+		+			+	+		+	+	+					+			+	+					+					+	+			+	+
Arginine				+			+	+	+		+	+															+	+	+	+			+			
Glutamic acid				+			+															+			+			+			+			+	+	
Cystine				+		+										+								+						+			+			
Iso-leucine				+	+	+													+	+																
Hydroxy proline					+					+	+																									
Valine					+			+		+							+																			
Aspartic acid							+															+			+	+					+			+		
Nor-leucine		+														+				+																
Glycine														+	+								+			+			+			+			+	

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety.

Table 6.2: Showing the sugar composition of leaf leachates of three varieties of paddy at different plant age.

Sugars	AGE OF THE PLANT IN DAYS																																							
	15			30			45			60			75			90			105			120			135			150			165			180								
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+			+		+	+	+	+	+	+	+	+	+	+	+	+	-	+					
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	-						
Glucose	+	+	+									+						+			+	+		+	+	+	+			+		+				+						
Rhamnose	+	+		+	+	+		+		+	+					+	+			+	+							+	+		+			+							
Sucrose				+	+	+	+	+		+	+	+	+	+	+	+	+	+			+	+	+	+			+	+			+			+							
Mannose				+	+					+									+						+	+	+			+			+								
Melibiose				+	+		+		+			+	+			+	+	+				+	+						+				+			+					
Fructose				+													+							+			+			+			+	+							
Cellobiose							+		+			+	+	+	+	+	+	+	+	+	+	+	+	+			+			+			+								
Galactose								+		+			+	+											+	+	+		+			+			+							
Maltose													+									+			+			+			+			+							
Trehalose										+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					+	+		+			+	+					
Ribose				+							+					+			+																						
Arabinose														+	+										+						+			+							
Raffinose																+									+	+								+							
Sorbose																						+			+	+								+							

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety.

Table 6.3: Showing the organic acid composition of leaf leachates of paddy varieties at different ages of the plant.

Organic acid	AGE OF THE PLANT IN DAYS																																			
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
Palmitic acid	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+		+	+	+	+	+													
Caprylic acid	+	+	+	+		+	+		+	+	+	+	+	+	+									+												
Stearic acid	+			+			+			+			+			+			+			+	+		+			+			+			+		
Malic acid	+		+	+	+	+	+		+	+	+	+	+	+		+			+	+	+	+		+	+		+	+		+		+	+		+		+
Oxalic acid	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+		+			+			+			+	
Capric acid	+	+		+		+	+			+																										
Myristic acid	+	+	+	+	+	+				+	+		+	+		+																				
Lactic acid	+	+		+			+	+		+			+												+	+		+	+		+	+		+	+	
Citric acid			+									+			+						+			+												
Tartaric acid					+			+		+			+	+		+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+
Succinic acid					+													+	+	+	+				+	+	+	+	+	+	+	+	+	+	+	+	+
Maleic acid																		+				+								+			+			+

K = Khonorullo variety; N = Ngoba variety;
M = Mirikrak variety

Table 6.4: Showing the phenol composition of leaf leachate of three paddy varieties at different ages of the plant.

Phenols	AGE OF THE PLANT IN DAYS																																			
	15			30			45			60			75			90			105			120			135			150			165			180		
	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M	K	N	M
Chlorogenic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catechol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+		+	+	+	+
Caffeic acid	+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+		+	+	
Protocatechuic acid	+			+			+			+									+						+			+			+			+		
Isochlorogenic acid	+			+			+			+	+		+			+			+	•		+	+		+	+		+	+		+	+		+	+	
Dinitrophenol		+	+		+	+		+	+		+	+		+	+		+	+		+	+		+			+	+			+			+	+		+	+
Phloroglocinol										+	+	+	+	+	+	+		+	+	+	+	+	+		+	+	+	+	+	+	+	+		+	+	+
Resorcinol										+						+			+	+		+	+		+	+			+		+	+		+	+	
Metacresol												+							+		+	+			+		+	+		+	+		+		+		+

K = Khonorullo variety; N = Ngoba variety; M = Mirikrak variety.

the other two varieties (Ngoba and Mirikrak) and the least was recorded in the disease susceptible variety (Mirikrak). The phenolic content increased with age till 150 days and later decreased.

DISCUSSION

Results of biochemical analysis of leaf leachates indicate that eighteen aminoacids, sixteen sugars, twelve organic acids and nine phenols were recorded in leaf leachates from the three paddy varieties. The chemical spectra of these varieties were different. These results are in accordance with those of Mace and Beech (1973) and Tyagi and Chauhan (1982) who showed that the chemical spectra of leaf exudates depend upon the host cultivars.

There is a change in the aminoacid spectrum of the leaf leachates of paddy varieties at different stages of plant growth. The maximum number of aminoacids was obtained at the later stages of plant growth (Fig. 6.1). This could be due to degradation of some of the aminoacids by microorganisms and synthesis of new aminoacids by the host as the plants mature (Bakshi, 1981). The sugars also followed the same trend as the aminoacids and the maximum number of sugars was detected at the senescence stage in Khonorullo variety (Fig. 6.1). The occurrence of more aminoacids and sugars in the resistant

variety may be because these chemicals play a part in imparting resistance to the plant providing nutrition to the growth of saprophytes which forms a microbial barrier or buffer to foliar infection by pathogens. Similar results have also been observed by Kumar and Balasubramanian (1981). Changes in aminoacids, sugars, organic acids and phenols have already been reported in diseased plants of paddy because of infection by H. oryzae (Dasgupta and Chattopadhyay, 1975). The decrease in the amount of aminoacids and sugars in the susceptible variety may be because the pathogen may be utilizing some of the aminoacids, causing a reduction in its number.

On the other hand, qualitative study of organic acids reveals that the organic acids were not exuded in large numbers and their number decreased with age of the plant. This may be due to their low concentration, too dilute to be detected by chromatographic technique or they are bound up in some form which is not easily exuded, however, differences were observed at different stages of plant growth indicating changes in host physiology as organic acids are important sources in respiratory cycle (Bakshi, 1981).

Moreover, the presence of more phenols in resistant or moderately susceptible variety is attributed to their role in conferring resistance to the plant from brown spot disease which supports the work of Tyagi and Chauhan (1982). Edreva (1976) showed two main aspects of the functions of phenols in

resistance, the first is due to the toxic effect on the pathogenic fungi and the second with the reaction of hypersensitivity. Isochlorogenic acid and caffeic acid were uniformly recorded in the resistant variety. Kuc et al. (1956) and Tyagi and Chauhan (1982) also demonstrated the presence of isochlorogenic acid in potato and chilli cultivars and showed that it played a definite role in disease resistance.

The quantitative study of sugars and aminoacids also reveal that their amount increased with age of the plants. Higher amount was mostly obtained in the extract of older plants (Fig. 6.2). The quantitative differences in sugar content at different stages of plant growth could be correlated with microbial activity on leaf surface as sugars are most easily utilized as a source of energy (Tukey, 1971).

On the other hand, the quantity of total nitrogen and protein decreased with age and became very low at senescence stage. Similar results were also obtained by Mishra and Tewari (1976b) working with leaf extracts of wheat and barley.

The quantitative analysis of phenols of paddy varieties indicate that their amount increases with age of the plant till 150 days but later on decreases. The maximum phenol content was obtained from resistant variety.

From the studies of fungal population of phylloplane of the three varieties of paddy (Chapter I) it was clear that the fungal population in the beginning when the plants were young was low, increased gradually with the age of the plants and maximum was at the senescence stage. Moreover, the fungal population both in total number of species isolated and total fungal colonies was higher in the resistant (Khonorullo) variety than in the other two varieties and the least fungal population was on the leaf surface of susceptible variety (Mirikrak). From biochemical studies of leaf leachates and leaf extracts of paddy varieties for aminoacids and sugars, reveal that they also followed the same trend as the fungal population with maximum amount at later stages. The phylloplane population thus seems to be directly governed by the leaf exudation and nutrient content of the leaves. Similar results were also obtained by previous workers (Mishra and Tewari, 1976b). Increase in permeability of the cell membrane of older cells (Kerling, 1964) coupled with loose arrangement of the cells during this stage accounted for the higher amount of leaching from the leaf surface.

In the resistant variety (Khonorullo) high nutrient content facilitates the development of higher fungal population which provides as a suitable microbial barrier against invasion by pathogens. On the other hand, in the susceptible variety because of domination by pathogen on the leaf surface, it was not conducive for the growth of other fungi due to decline in

nutrient content.

Moreover, the quantitative study of phenols reveals that the maximum phenolic content was obtained from the resistant variety (Khonorullo) which supports the work of Sathiyanathan and Vidyasekaran (1981) who demonstrated the presence of more phenolics in the leaves of resistant rice cultivars and their considerable increase in the resistant variety suggests a positive role of phenolics in the resistance of rice to brown spot disease caused by H. oryzae. Further, Akai and Ueyama (1955) also observed increase in catalase in rice leaves inoculated with H. oryzae.

The results of the present investigation clearly indicate that the colonization of fungi on the leaf surface is directly controlled by nutrient level of the leaf surface which changes with the release of substances from the leaf.

Moreover, some phenolic compounds may also provide resistance to the plant to brown spot disease of paddy.

CHAPTER VII

EFFECT OF POLLEN ON THE SAPROPHYTIC AND PATHOGENIC MYCOFLORA OF THE PHYLLOPLANE

INTRODUCTION

It has been known from earlier studies (Fokkema, 1971; Sainger et al., 1978; Garg and Sharma, 1982) that pollens might bring about qualitative as well as quantitative alterations in the phylloplane microflora. Artificial inoculation of pollen grains on to leaf surfaces is shown to enhance the growth of folicolous pathogens, thus enhancing the infection rate to considerable extent (Fokkema, 1968; 1971). Moreover, the presence of pollen on surfaces of flag leaves in triticale had marked influence on populations of phylloplane microfungi (Garg and Sharma, 1982).

Further, Kerling (1964) also observed a sudden increase in the development of sparophytic fungi shortly after flowering which may be due to the presence of pollen that has fallen on leaves after flowering. Fokkema (1968) also reported increased development of Cladosporium herbarum in the presence of pollen on rye leaves. He could also show later (Fokkema, 1971) that C. herbarum and many other fungal spp. occurred in higher number on rye leaves with pollen. The stimulatory influence of pollen on phylloplane microflora has been stressed by several workers (Warren, 1972, 1976, Fokkema et al., 1979).

It is known already from earlier studies (Fokkema, 1976, 1978; Skidmore, 1976; Blakeman and Brodie, 1976) that the nonparasitic microorganisms in the phylloplane control the

incidence and development of leaf diseases through antagonism. Since pollen is an important factor which favours the development of saprophytes especially on older leaves during crop growth (Kumar and Balasubramian, 1981) it might be of significance in disease development.

Considering the significant role played by pollen on pathogenic and saprophytic flora of phylloplane, the present investigation was undertaken to see the effect of paddy pollen on its mycoflora.

MATERIALS AND METHODS

Research plant: The three varieties of paddy viz., Khonorullo (disease resistant), Ngoba (moderately susceptible) and Mirikrak (disease susceptible) were used as the experimental plant.

At 105 days of plant age when the flowering just begins, ears of some plants of the three varieties of paddy were tightly wrapped with clean polythene bags to check the presence of their own pollen on leaves. The rest of the plants of the three paddy varieties were not wrapped with polythene bags to allow pollen deposit on their leaves under natural conditions. Occassionally mature ears of plants in untreated lot were also gently agitated over surfaces of leaves to ensure a heavy pollen deposit.

After 15 days when the plants were at 120 days of plant age, the samples of flag leaves were taken from plants of both from treated and untreated sets for studying the phylloplane mycoflora. Phylloplane fungi was isolated by dilution plate technique as described in Chapter 1. Petri-dishes were incubated at 25 ± 1°C for 7 days and colonies were examined chiefly for those fungi recorded as dominant as seen from the studies of phylloplane mycoflora (Chapter 1). The samplings were done again at 150 days and 180 days of plant age. Results for each fungus were expressed as fungal population x 10^3 per gm fresh weight of leaves.

RESULTS

From Table 7.1 it is clear that presence of pollen strongly increased the population of each member in the phylloplane in the three paddy varieties and leaves with pollen possessed more fungal population than leaves without pollen. The pollen effect was more pronounced on mature leaves at 180 days of plant age.

The presence of pollen also increased the total fungal population in all the three varieties (Fig. 7.1). It is more pronounced at 180 days of plant age.

Moreover, the fungal population was always more on Khonorullo variety (disease resistant) than in the other two

Fig. 7.1: Comparison of total fungal population (x 10^3 per gm fresh wt. of leaves) of phylloplane fungi on leaves with and without pollen deposit of different varieties of paddy viz., Khonorullo, Ngoba and Mirikrak at three growth stages of the plant.

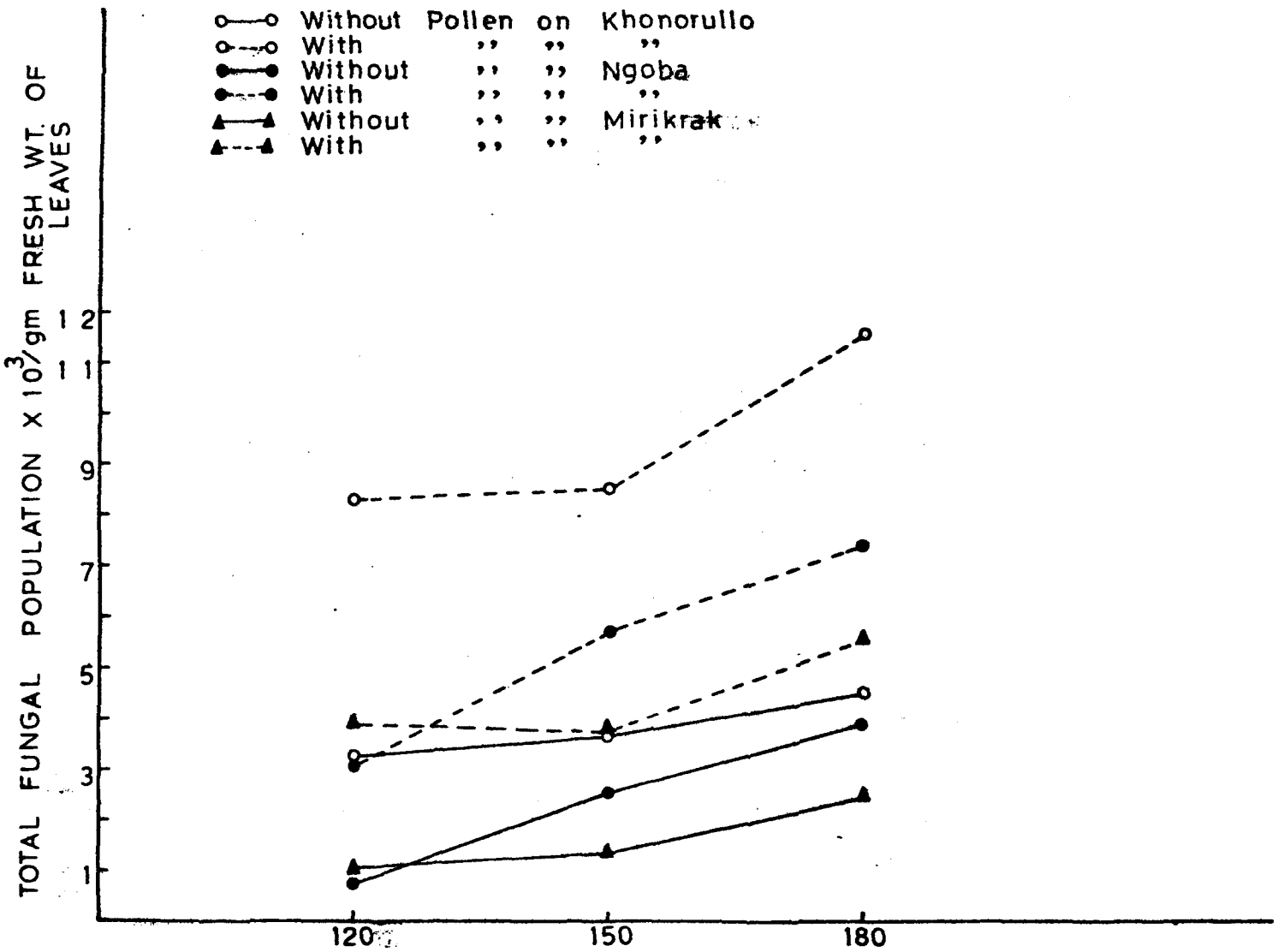
Without Pollen on Khonorullo
With " " "
Without " " Ngoba
With " " "
Without " " Mirikrak
With " " "
TOTAL FUNGAL POPULATION X 10^3/gm FRESH WT. OF LEAVES
12
11
9
7
5
3
1
120
150
180
AGE OF THE PLANT IN DAYS

Fig. 7.1

Table 7.1: Showing the population of common phylloplane fungi on leaf surface with (+) and without (-) pollen deposit after flowering. Population is expressed as average number of colonies x 10^3 per gm fresh weight of leaves.

Fungi	120 days						150 days						180 days					
	K^-	K^+	N^-	N^+	M^-	M^+	K^-	K^+	N^-	N^+	M^-	M^+	K^-	K^+	N^-	N^+	M^-	M^+
Alternaria alternata	.06	.25	-	-	-	-	.13	.38	-	-	-	-	0.31	1.6	.25	.31	.08	.19
A. solani	.08	.21	.05	.13	.04	.10	-	-	-	-	-	-	-	-	.18	.26	.12	.21
Arthrinium sp.	0.61	1.8	.34	1.2	.29	1.1	.82	2.1	.74	1.8	.51	1.7	.56	1.5	.42	.98	.09	.26
Aspergillus flavus	.08	.32	.01	.04			.26	.42			.02	.13						
A. nidulans	.62	.89	.08	.15	.32	.55												
A. niger	.26	.42					.016	.09	.01	.12	.08	.16			.06	.18		
Aureobasidium pullulans													0.9	1.8	1.1	2.0	1.0	1.9
Cladosporium herbarum	.08	.41	.06	.32	.02	.06	.28	.86	.16	.43	.09	.18	.46	1.2				
C. cladosporoides	.06	.26	.05	.3	.04	.11			.08	.15								
Curvularia lunata					.19	1.25							.11	.21	.08	.15	.08	.12
Epicoccum nigrum	.07	.19	.08	.24									.06	.16				
Fusarium moniliforme	.08	.23					.35	.52	.14	.26			.05	.11				
Helminthosporium oryzae					.08	.26											0.21	1.2
Nigrospora oryzae							.08	.20					.04	.12	.03	.07		
Penicillium funiculosum	0.5	1.8	0.08	.65	.05	.32	0.72	1.86	0.6	1.5	0.58	1.45	0.85	2.2	0.8	1.5	0.76	1.52
Trichoderma viride	0.7	1.4					0.82	1.8	0.7	1.2			1.1	2.4	0.9	1.8		
Verticillium albo-atrum	0.06	.12			.08	.16	.19	.28	.12	.25	.09	.17	.09	.32	.09	.15	.095	.19

K^- without pollen on Khonorullo variety; K^+ with pollen on Khonorullo variety; N^- without pollen on Ngoba variety; N^+ with pollen on Ngoba varity; M^- without pollen on Mirikrak variety; M^+ with pollen on Mirikrak variety.

varieties (Ngoba and Mirikrak). The least fungal population was obtained from leaves of the susceptible variety (Mirikrak).

Further, some of the most dominant fungi in the phylloplane viz., Cladosporium spp., Alternaria spp., Arthrinium sp., Aspergillus spp. and Penicillium sp. also showed an increase in their population in the presence of pollen (Table 7.1). Besides the dominant fungi some other fungi such as Aureobasidium pullulans, Curvularia lunata, Epicoccum nigrum, Fusarium moniliforme, Nigrospora oryzae and Vertioillium albo-atrum were also stimulated.

In addition, the pollen also affected the pathogen, H. oryzae. It was seen that in the susceptible variety, Mirikrak presence of pollen stimulated the pathogen population which was more pronounced at the later stages. An important antagonist of the pathogen, T. viride also showed high population in the presence of pollen especially on the resistant variety, Khonorullo.

DISCUSSION

From the results obtained from the present investigation (Table 7.1) it is clear that presence of pollen strongly increased the population of each member of the fungal community formed on the phylloplane habitat. Moreover, the total fungal population (Fig. 7.1) also increased in the presence of pollen.

Similar results were also obtained by previous workers (Garg and Sharma, 1982). This pollen effect became more pronounced on mature leaves (Fig. 7.1).

Further, the increase in the dominant fungi such as Cladosporium spp., Alternaria spp., Arthrinium sp., Aspergillus spp., Penicillium sp. in the presence of pollen, supports the work of Fokkema (1968, 1971); Warren (1972, 1976); Garg and Sharma (1982). Some of the less dominant fungi such as Aureobasidium pullulans, Curvularia lunata, Epicoccum nigrum, Fusarium moniliforme, Nigrospora oryzae and Verticillium albo-atrum also showed increase in their population in the presence of the pollen. Fokkema (1971) and Warren (1972, 1976) also observed an increase in number of Aureobasidium pullulans and some other yeasts in presence of pollen on leaves of rye, lime and birch.

In Khonorullo variety, the population was maximum in the presence of pollen. This habitat formed by the leaf surface of the resistant variety was more conducive for the growth of fungal flora than in the other two varieties (Ngoba and Mirikrak). On the other hand, the pathogen H. oryzae showed higher population on the susceptible variety (Mirikrak) than in the other two varieties (Ngoba and Khonorullo). The pathogen, also showed an increase in their number in the presence of pollen. Fokkema (1971) also observed an increase in infection by Helminthosporium sativum with the influence

of pollen on rye leaves. Many other workers (Ogawa and English, 1960; Bachelder and Orton, 1963; Chu-chou and Preece, 1968; Chu-chou, 1970; Warren, 1972) also demonstrated that presence of pollen affected infection of leaves by parasites.

It has been demonstrated by a number of workers (Fokkema, 1976; 1978; Skidmore, 1976; Fokkema et al., 1979) that non-parasitic micro-organisms in the phylloplane control the incidence and development of leaf diseases through antagonism. Thus any factor such as pollen which might alter the balance between saprophytes and pathogens on leaf surface may be of significance in disease development.

Thus, pollen may be one of the important factors which favours the development of saprophytes on older leaves (Kumar and Balasubramanian, 1981). Pollen which is shed and is carried away by wind and is deposited on the leaf surface may add to the nutrient level available on the leaf surface and in turn may have a stimulatory influence on the phylloplane microflora.

Results obtained from the present study thus clearly demonstrate that pollen may have a stimulatory effect on both saprophytic and pathogenic flora of phylloplane of paddy varieties. Such study may help in understanding the pathogenesis and development of effective means for control of leaf diseases of paddy such as brown spot.

GENERAL DISCUSSION

The results obtained from the studies on phylloplane fungi of paddy clearly indicate many similarities with the phylloplanes of other angiosperms both in taxonomic composition and in pattern of development of microbial populations. The phylloplane mycoflora of paddy examined in the present study appears to be closely similar to those previously described on other plants by Dickinson (1967); Hislop and Cox (1969); McBride and Hayes (1979); Hayes (1982) and include a number of typical dominant fungi occurring on this habitat viz., Cladosporium spp., Penicillium spp., Aspergillus spp., Alternaria spp., Aureobasidium sp. and some Ascomycetes belonging to Chaetomium sp., and some other common fungi such as Fusarium moniliforme, Nigrospora oryzae, Trichoderma viride, Trichothecium roseum, Verticillium albo-atrum, etc. A total of 88 species belonging to 53 genera of fungi was isolated from the phylloplane of paddy by using different techniques of isolation. The results obtained from the different techniques clearly suggest that the study of the leaf surface population needs a combination of various methods for assessing the complete picture of the phylloplane fungal flora. This is in accordance with the view of previous workers (Dickinson, 1971; Sharma et al., 1974; Mishra and Dickinson, 1981). Earlier workers have obtained more Mucorales and Hyphomycetes and very few Ascomycetes in their studies on phylloplane mycoflora (Mehrotra and Aneja, 1979; Singh and Rai, 1980). Whereas, in the present studies although the majority of the forms

isolated from the leaf surface of paddy belonged to Deuteromycetes, Ascomycetes were also isolated in large number in addition to phycomycetes and mycelia sterilia. This may be because of use of cellulose agar medium in addition to Czapek's Dox agar medium and also the use of moist chamber technique which enabled the isolation of decomposers (Tables 1.4 - 1.7). Tsao (1970) was of the opinion that the nutrient medium used plays an important role in isolation of fungal species.

The results of the experiments on the distribution of the microorganisms in relation to host species especially varietal characters, leaf maturity, weather changes and also by the pathogen, H. oryzae have brought out certain facts of importance. As with other plants, leaf age probably has an important role in influencing the development of the phylloplane microflora (Fig. 1.4 - 1.6). This may be due to increased leaching of nutrients from ageing plants. Various workers have reported the release of leachates in higher amount from older leaves (Colodny, 1932; Arens, 1934; Tukey et al., 1958; Last and Deighton, 1965; Mishra and Tewari, 1978). Similar results were also obtained in the present studies (Fig. 6.2) where the amount of sugars and amino acids increased with the age of plant and the maximum was obtained at the senescent stage.

Ever since Last (1955) and Ruinen (1961) introduced the concept of phylloplane, many investigators have observed changes

in the microflora of phylloplane of crop plants which is influenced by varietal characters and also by plant pathogens (Kumar and Balasubramanian, 1981). In the present studies, when the leaf surface of three varieties of paddy differing in resistance to the pathogen, H. oryzae were compared, the leaf spot disease resistant variety (Khonorullo) supported a significantly higher population of fungi than the moderately susceptible (Ngoba) and susceptible (Mirikrak) varieties (Tables 1.3 - 1.7). The higher population could play a significant role in imparting reistance to foliar infection by forming a microbial barrier or buffer. The microflora present in the phylloplane is recognized as the fourth dimension (Last, 1971) and dictates the development of the diseases in addition to the virulent and aggressive pathogen, susceptible host and favourable environmental conditions. Certain fungi may extensively colonize a substrate to the exclusion of others which arrive later (Brueh and Lai, 1966). Similar results have also been observed by various workers (Balagopal and Oblisami, 1973; Kumar and Balasubramanian, 1981). However, some workers (Kumar and Gupta, 1974; Tyagi, 1980) have observed higher fungal population in susceptible varieties than resistant ones. The decrease in the fungal population observed on the leaf spot susceptible variety (Mirikrak) in the present studies could be due to the domination of the pathogen, H. oryzae resulting in the

reduction of available nutrients on diseased leaf surface or by eliciting antifungal compounds. In addition, a naturally occurring biological control may be present in the different varieties of paddy studied. It was seen that in resistant varieties (Ngoba and Khonorullo) when T. viride population increased it inhibited the growth of H. oryzae to some extent. Similar natural biological control was also observed by Fokkema et al. (1975) while studying the buffering capacity of the microflora of rye leaves to artificial infection by Cochliobolus sativus. In addition, the distribution of fungi may greatly depend on weather changes. Meterological factors such as atmospheric temperature, humidity and rain are important factors (Fig. 1.1) influencing the fungi on the leaf surfaces and this is in agreement with the data of Sinha (1971) and Hayes (1982) who also found that such factors determine the periodicity and affect the trapping of spores on leaf surface. The total number of microorganisms and also the specific composition of the mycoflora was affected by the weather conditions, some being present throughout the growing period whereas others were exclusively associated with a particular set of climatic factors (Tables 1.4 - 1.7).

From a study on the airspora of the the experimental plots where the three paddy varieties were grown it is quite clear that the fungal spores present in the air showed a great impact on the leaf surface of paddy since a significant direct

correlation existed between them (Table 2.4). Most of the spores of air were also trapped on the leaf surface (Table 2.1). However, some of the forms maintained their specificity in the specialized environment. The specificity of forms to the two environments, viz., air and phylloplane was due to change in nutritional status, micro-ecology and the other unknown conditions of the different environments. This accounted for the restricted occurrence of certain forms in the phylloplane and air of paddy plants. Similar results have also been obtained by Sinha (1971) and Mishra and Tewari (1976a). Further, the pathogen, H. oryzae was present in the air of all the three varieties of paddy at an early stage of the crop growth (Fig. 2.8 and Table 2.3) but the visible symptom of the brown spot disease was seen only from July-August when the optimum conditions for its survival were prevalent. Moreover, the pathogen was conspicuously absent from the leaf surface of Khonorullo variety probably because of its disease resistant nature although it was isolated in the air. On the other hand, T. viride an important antagonist of H. oryzae was although present in almost the same proportions in the air of all the three paddy varieties, was dominant on leaf surface only in disease resistant variety (Khonorullo) and subdominant on the moderately susceptible (Ngoba) variety. Such aerobiological and phylloplane studies thus may be of suggestive value in the control measures of the pathogen since the occurrence of the pathogen at an early stage of the crop in the air may be helpful

in making a forecast regarding the occurrence of the brown spot disease of paddy.

By using Koch's Postulates for doing pathogenicity test it was confirmed that the causal organism for disease occurring on Ngoba and Mirikrak varieties of paddy was Helminthosporium oryzae which caused brown spot disease on paddy. Further, from the studies it was also clear that the optimum temperature for its growth was around 25-35°C (Table 3.4). Padmanabhan (1973) in his analysis of the great Bengal famine caused by the brown spot disease of paddy had hypothesized that the disease assumed such epiphytotic proportions in 1942 primarily because of the existence of optimum weather conditions such as high temperature (35°C) and high moisture along with a cloudy weather during the period. Similar results were also obtained in the present investigation. Further, for studies involving host-parasite interactions when H. oryzae, the pathogen population has to be taken then spores from 15 day old cultures with a concentration of 2×10^5 spores/ml suspended in 0.01 M phosphate buffer at pH 6 if incubated at 35°C may give good results. Similar results were also obtained by Purkayastha and Mukhopadhyay (1974). Moreover, the susceptibility to the pathogen, increased with age which is clear from the pathogenicity studies conducted in vitro and in vivo.

From interaction studies done *in vitro* and *in vivo* between certain selected epiphytic fungi and the pathogen, it is clear that under ideal conditions (as *in vitro*) selected fungal antagonists such as *Trichoderma viride* may be used as a biological control agent for brown spot disease of paddy although it was only partially successful in pot experiments. The antagonistic role of *T. viride*, a common leaf surface fungus has been recognized by several workers (Ale-agha *et al.*, 1974; Roy, 1977; Chet *et al.*, 1979; Hadar *et al.*, 1979). Differences in antagonistic behaviour of leaf surface fungi *in vivo* and *in vitro* have been reported by many workers (Bhatt and Vaughan, 1963; Akai and Kuramoto, 1968; Heuvel, 1969; Pace and Campbell, 1974; Rai and Singh, 1980). Akai and Kuramoto (1968) tried to control this important disease of paddy biologically. They have reported that a *Candida* sp. although was effective in controlling brown spot disease of rice *in vivo* failed to inhibit *in vitro*. Moreover, the exact mechanism of operation of *T. viride*, one of the important antagonists of the pathogen is not very clear from the present studies. It is not clear whether *T. viride* inhibited the pathogen by hyperparasitism or by producing antibiotics although it is a known fact that members of the genus *Trichoderma* are active both as hyperparasites and as antibiotic producers (Hadar *et al.*, 1979) Although significant progress has already been made in the development of biological control of plant diseases, still there are some gaps in our knowledge which relates the impact of

natural environment, antagonistic selection and application technology (Spurr, 1981). It is difficult to speak of biocontrol in the same terms as chemical control since biocontrol is diverse and heterogenous and a choice must be made of the most appropriate means available and the disease situation in question (Dubos and Bulit, 1981).

To see the competitive ability of the phylloplane fungi on the leaf surface of paddy varieties, the spores of various fungi were germinated in leaf leachates, leaf extracts and on the detached leaf surface of the three varieties. It was clear from the results that the germination and growth performance of these fungi were enhanced in the leaf leachates, leaf extracts and on the surface of leaves. Further, most of the antagonistic fungi like T. viride showed maximum growth in the leaf leachates, extract and on leaf surface of resistant variety (Khonorullo) and on the other hand, the pathogen, H. oryzae showed highest germination and growth performance in the case of susceptible variety (Mirikrak). Moreover, most of the other fungi were more stimulated in the leaf leachates and leaf extracts of the resistant variety than in the susceptible ones. Similar results were also obtained by Tyagi and Chauhan (1982) working with the pathogen, Alternaria solani in the leaf exudates of chilli varieties. There was also an increase in spore germination of certain fungi (Table 5.1) in the leaf leachates and leaf extracts of senescent plants. This may be

accounted to the presence of higher amount of nutrients such as sugars, amino acids and organic acids in the exudates and extracts of older leaves (Godfrey, 1976; Mishra and Tewari, 1978) which was also confirmed from the biochemical studies of the leaf leachates and leaf extracts.

In addition, the results of the biochemical analysis of the leaf leachates and leaf extracts clearly confirms the fact that the colonization of fungi on the leaf surface is directly controlled by nutrient level of the leaf surface which changes with the release of substances from the leaf. Moreover, some phenolic compounds may also provide resistance to the plant against brown spot disease of paddy as is seen from the present studies (Table 6.4 and Fig. 6.2). This is in agreement with the work of Sathiyanathan and Vidyasekharan (1981) who demonstrated the presence of more phenolics in the leaves of resistant rice cultivars and their considerable increase in the resistant cultivars suggest a positive role of phenolics in the resistance of brown spot disease caused by H. oryzae. In addition, enriched nutrient level on the leaf surface of resistant variety (Khonorullo) may also attract the colonization of more saprophytic fungi on this habitat (Table 1.4 - 1.7) which may provide resistance by forming a microbial barrier or buffer to foliar infection by the pathogen. However, in the susceptible variety (Mirikrak) on the other hand, because of domination of the pathogen, H. oryzae may decrease the amount of nutrients to

a considerable degree which makes it non-conducive for the colonization and growth of the fungal saprophytes to a maximum level. Thus a low fungal population was correlated with the presence of low nutrient level in the susceptible variety.

Another factor which favours the development of saprophytes on older leaves during crop growth is pollen. The stimulatory influence of pollen on phylloplane microflora of paddy was clearly observed during the present studies (Fig. 7.1 and Table 7.1). Presence of pollen, heavily increased the population of each member of the fungal community formed on the phylloplane habitat. Many other workers (Garg and Sharma, 1982) have also obtained similar results. In addition to change in saprophytic flora, the pathogen also showed an increase in its population in the presence of pollen which was distinct on the susceptible variety, Mirikrak. Fokkema (1971) also observed an increase of infection on rye leaves in the presence of pollen. Many other workers (Ogawa and English, 1960; Bachelder and Orton, 1963; Chu-chou and Preece, 1968; Chu-chou, 1970; Warren, 1972) have also demonstrated that presence of pollen affected infection of leaves by parasites. The resitant variety, Khonorullo on the other hand, showed maximum stimulation of the fungal population in the presence of pollen. This habitat formed by the leaf surface of the resistant variety may be the most suitable for the growth of the fungal communities.

CONCLUSION AND SCOPE FOR FURTHER RESEARCH

From the present investigation on ecological studies of phylloplane fungi of paddy it is clear that the distribution of fungi on leaf surface was mainly dependent on host species, leaf maturity and weather conditions. Pollen was another factor which might alter the balance between the saprophytes and pathogen on leaf surface since it stimulates the pathogenic and saprophytic mycoflora and thus may be of significance in disease development. Moreover, in the present studies since the source of inoculum and climatic conditions prevailing were similar for all the three varieties of paddy (since they were grown together in the same locality) the differences observed in quantitative and qualitative composition of these communities are mainly as a result of habitat characteristics, i.e., leaf morphology and physiology and also by the presence of plant pathogens. The leaf surface of resistant variety provided the most suitable habitat for the growth of maximum saprophytic fungi because of the presence of more nutrients such as sugars, aminoacids, organic acids but on the other hand, was not at all conducive for the growth of the pathogen, H. oryzae because of the presence of more phenols and also may be because of the presence of microbial barrier provided by the presence of saprophytes. Further, from the present studies it is clear that H. oryzae, the most destructive pathogen of paddy which causes brown spot disease was prevalent in this locality where the present investigation was conducted because of the

presence of optimum conditions for its growth such as temperature and moisture. Moreover, although the spores of the pathogen are present in the air very early during the crop season but the visible symptoms of the disease occur only later and that also only on the leaf surface of the susceptible varieties. On the other hand, T. viride was found to be a potential antagonist of the pathogen, which could efficiently control the pathogen to some extent in vitro studies but was not that successful in vivo. Thus from the present studies it could be concluded that some antagonists such as T. viride could be used as a biological control agent for brown spot disease of paddy.

Further studies are needed to test the effectiveness of T. viride against the brown spot pathogen of paddy in field and thus the biological control of leaf pathogens on a field scale offers a great challenge for future investigation. Although it is unlikely, however, that microbial antagonism could be a total substitute for chemotherapy, biological control is a satisfactory control system whose exploitation is turning out to be full of promise. Current knowledge is too limited to accurately assess the different aspects of the antagonism phenomena which have an opposing impact on crop production. Further research, therefore, is urgently needed to quantify the role of phylloplane fungi in field situations.

SUMMARY

Microbial communities on the leaf surface of three varieties of paddy differing in resistance to the leaf spot pathogen, Helminthosporium oryzae, viz., Khonorullo (disease resistant), Ngoba (moderately susceptible) and Mirikrak (disease susceptible) were studied in detail using five different techniques of isolation (direct observation, moist chamber, leaf impression, washed leaves plating and dilution plate methods). The microflora of the phylloplane of the paddy plants is mainly influenced by varietal characters and also by the pathogen, H. oryzae. In addition, distribution of fungi on leaf surface was also dependent on leaf maturity and weather changes. Analysis of phylloplane mycoflora showed that in the resistant variety there was a significantly higher population of fungi as compared to the susceptible one. Further, the total number of fungal colonies was observed to increase significantly with the age of the plant and was independent of plant variety. Among the fungi isolated Cladosporium spp., Penicillium spp., Alternaria spp. and Aspergillus spp. dominated the leaf surface of both resistant and susceptible cultivars. Species diversity among fungal members as calculated by Sorenson's index was found to vary with plant age. A natural antagonism was found on the resistant varieties between Trichoderma viride and the leaf spot pathogen, H. oryzae.

From a study on the airspora of the three experimental plots where the paddy varieties were grown it was found that the fungal spores present in the air exerted a great

impact on the leaf surface of paddy. Most of the spores of air were also trapped on the leaf surface. Some of the forms however, maintained their specificity in the two environments viz., air and phylloplane. The spores of the pathogen were found in the air of all the three paddy varieties very early in the crop season but the visible symptom of the disease was observed later only on the leaf spot susceptible variety. T. viride, a possible antagonist of the pathogen was found only on the leaf surface of the resistant variety although it occurred in the air of all the paddy varieties.

Pathogenicity test was conducted by using Koch's postulates both in vitro and in vivo and it was confirmed that the causal organism for the disease occurring on the susceptible varieties was definitely Helminthosporium oryzae Breda de Haan which caused brown spot disease of paddy. This pathogen was also isolated in pure culture. The susceptibility to the pathogen increased with age which was clear from the pathogenicity studies done in vivo and in vitro. The pathogen spores from 15 day old cultures with a concentration of 2×10^5 spores/ml suspended in 0.01 M phosphate buffer at pH 6 if incubated at 35°C showed maximum spore germination and germ tube length.

From interaction studies done in vitro and in vivo between certain epiphytic fungi and the pathogen, it was observed that in vitro some selected fungal antagonists such

twelve organic acids and nine phenols were recorded in leaf leachates of the three paddy varieties. The number of amino-acids, sugars, organic acids, and phenols increased as the plants grew older and more amino acids, sugars, organic acids and phenols were detected in the exudate of the resistant variety. The quantitative study of leaf extracts also indicate that more quantity of sugars, amino acids and phenols were detected in the resistant variety than in the susceptible one and the amount increased with plant age. The results of the biochemical analysis of the leaf leachates and extracts clearly confirm the fact that the colonization of fungi on the leaf surface is directly controlled by nutrient level of the leaf surface which changes with the release of substances from the leaf. In addition to high nutrient level in the resistant variety which enable this habitat to attract more saprophytic fungal colonization thereby forming a microbial barrier to foliar infection, some phenolic compounds may also provide resistance to the plant to brown spot disease of paddy.

Another factor which favours the development of saprophytes on older leaves during crop growth was found to be pollen. The presence of pollen on surfaces of leaves had marked influence on phylloplane fungal population. The total fungal population was highly stimulated on leaves with pollen especially on mature leaves and the effect was much pronounced on the leaves of resistant variety. In addition to change in

saprophytic flora the pathogen population also increased in the presence of pollen on the leaves of susceptible variety. This study suggests a possible role of pollen in pathogenesis and disease development.

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