ECOLOGICAL STUDIES ON THE MICROBIAL AND BIOCHEMICAL ASPECTS OF FOREST LITTER DECOMPOSITION AT HIGHER AND LOWER ALTITUDES OF MEGHALAYA

ABSTRACT

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THESIS SUBMITTED IN FULFILMENT OF
THE REQUIREMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY

To

DEPARTMENT OF BOTANY
SCHOOL OF LIFE SCIENCES
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The study was conducted in order to find out the effect of altitudinal variation and vegetational cover on microbial succession, decomposition and enzymes under forest ecosystem. The study sites were situated at two altitudes i.e. at higher altitude (1500m MSL) at Shillong and at lower altitude (100m MSL) at Byrnihat. At each altitude two forest stands of different regenerational stages were selected. Two plant species, dominant and codominant, were selected in each forest stand. At lower altitude, Ageratum conizoides and Mallotus philippinensis in young stand and Holarrhena antidysenterica and Vitex glabrata in old stand were selected. At higher altitude, Alnus nepalensis and Pinus kesiya were selected in old forest stand whereas, Myrica esculenta and Pinus kesiya were taken from young stand.

Quantitatively the bacterial population was more than the fungal population at both the altitudes. Bacterial and fungal populations were less in the beginning of litter decomposition but increased with the progress of decomposition. Microbial population was minimum at the end of litter decomposition. At lower altitude, A.conizoides litter harboured maximum fungal and bacterial populations followed by M.philippinensis, V.glabrata and H.antidysenterica. In young and old stands the microbial (fungal and bacterial) population attained its peak in May-June. The litters at lower altitude harboured more microbial population than at higher altitude.

At higher altitude, litter of A.nepalensis supported more fungal and bacterial population compared to M.esculenta and P.kesiya. A marked seasonality in microbial population was observed on all the litters. Bacterial population exhibited peaks in July and September, while the fungal population showed its

peaks in June and September. During winter months, the litter harboured minimum microbial population.

Eighteen fungal species were isolated from litter at higher altitude and twenty five species at lower altitude. Penicillium chrysogenum was dominant at lower altitude, while Trichoderma viride at higher altitude. The weedy leaf litter harboured more of phycomycetous fungi compared to its woody counterparts where the primary colonizers were mainly deuteromycetes followed by ascomycetes and few phycomycetes.

Fungal and bacterial populations showed significant positive correlation with the moisture content of litter at both the altitudes.

The herbaceous litter decomposed faster (K=2.930) at lower altitude compared to woody litters of M.philippinensis (K=1.587), H.antidysenterica (K=1.279) and V.glabrata (K=1.139). The decomposition of plant litter at higher altitude was slow than at lower altitude. Litter of A.nepalensis (K=0.810) decomposed faster than M.esculenta (K=0.526) and P.kesiya (K(old)= 0.398 and K (young)= 0.372). The relative weight loss of all the litters except M.philippinensis was correlated significantly with fungal population and bacterial population.

The litter at lower altitude were less acidic than at higher altitude which became more acidic towards the end of decomposition. The moisture content of litters ranged from 17.8 to 66% at lower altitude and 13.9 to 72% at higher altitude.

Quantitatively organic constituents were maximum in the early stage of decomposition than its final stage. The amount of cellulose, hemicellulose, total soluble sugars and amino acids was more in A.conizoides than M.philippinensis, H.antidysenterica and V.glabrata litters. The lignin content was, however, more in V.glabrata. Lignin degradation started towards the later part of litter decomposition. At higher altitude the lignin content was maximum

in pine litter and minimum in A.nepalensis. The latter had more cellulose, hemicellulose, total soluble sugars and amino acids. At both the altitudes the absolute weight loss of different litters showed a significant negative correlation with the weight remaining of different organic constituents. While lignin content was correlated positively with absolute weight loss.

The initial nitrogen content was more in A.conizoides followed by M.philippinensis, H.antidysenterica and V.glabrata. The phosphorus content, however, was maximum in H.antidysenterica and minimum in A.conizoides. At higher altitude A.nepalensis had maximum initial nitrogen and phosphorus contents followed by M.esculenta and P.kesiya. Unlike nitrogen, which was retained for some time in the litter, phosphorus was released along with the decomposition of litter at lower altitude. At higher altitude, however, the retention of nitrogen and phosphorus was for longer period. The absolute weight loss of different litters showed negative correlation with their nitrogen and phosphorus contents.

Both amylase and cellulase activities were less in the beginning of decomposition and increased with time, while a reverse pattern was observed for invertase. At lower altitude, the cellulase activity was more in M.philippinensis followed by H.antidysenterica, V.glabrata and A.conizoides. Amylase activity was more in M.philippinensis and H.antidysenterica followed by V.glabrata and A.conizoides. Unlike amylase and cellulase, the invertase activity was more in A.conizoides and less in V.glabrata. All the enzymes showed a marked seasonal variation. Cellulase and amylase showed a significant positive correlation with fungal and bacterial populations of litter. A positive correlation was established between cellulase, amylase and absolute weight loss of different litters. The invertase activity, however, showed a negative correlation with fungal and bacterial populations and absolute weight loss. Maximum cellulase, amylase and invertase enzymes were extracted from the litter of A.nepa-

lensis followed by M.esculenta and P.kesiya at higher altitude. The seasonal variation in microbial enzymes was similar to lower altitude. Cellulase and amylase activities showed a significant correlation with fungal and bacterial populations. However, absolute weight loss showed a positive correlation with cellulase and negative correlation with amylase activity. A negative correlation was also observed between weight remaining of cellulose and cellulase. The invertase activity showed a positive correlation with fungal and bacterial populations. The weight remaining of total soluble sugars was correlated significantly with invertase activity of all the litters.

Litter of H.antidysenterica decomposed faster than P.kesiya under laboratory conditions. The rate of decomposition showed a negative correlation with temperature. The optimum temperature for decomposition was observed at 25°C. The mixture of the two test fungi i.e. T.viride and P.chrysogenum ameliorated the decomposition compared to their individual rate. The decomposing ability of different fungal species varied significantly ($P < 0.05$) at different temperatures. Maximum weight loss in litter of H.antidysenterica occurred after 120 days at 5°C and 15°C, while after 90 days at 25° and 35°C. On the contrary, it could be achieved after 150 days at 5° and 15°C and after 120 days at 25° and 35°C respectively in P.kesiya litter.

Litter quality had a marked effect on the rate of decomposition. The duff decomposed much faster than either fresh fragmented or intact litter. Maximum loss of duff (partially decomposed litter) occurred after 60 days in H.antidysenterica and 90 days in P.kesiya. Intact litter of H.antidysenterica and P.kesiya took more than 90 and 120 days respectively. The decomposition constant (K) of duff, fresh fragmented and intact litters varied significantly. The test fungi differed in their decomposing ability. Sugars and amino acids along with cellulose and hemicellulose were microbially degraded rapidly in the beginning of decomposition. The decomposition of different organic con-

stituents was faster at 25°C compared to 5°C, 15°C and 35°C. Lignin decomposition took place only towards the end of the biodegradation process.

Total sugars and amino acids, hemicellulose and cellulose were estimated less in duff compared to fresh litter. However, lignin was more in partially decomposed litter than fresh ones. The decomposition of lignin in partially decomposed H.antidysenterica litter started after 90 days while in case of P.kesiya litter it began after 120 days.

Maximum endo-β-glucanase and β-glucosidase were produced on 15th day of microbial inoculation after which it decreased gradually. Production of exo- β-glucanase was, however, maximum on 10th day of fungal inoculation. The p^H of the medium inoculated with T.viride, F.oxysporum and mixture became more acidic while in P.chrysogenum inoculated medium slight increase in p^H was observed. The dry weight of mycelium of all the test fungi increased with increase in the length of incubation. It was maximum for T.viride. The maximum xylanase activity was recorded on 10th day of incubation in all the test fungi. Mycelial dry weight was minimum on 5th day which increased till 10th day and declined again. The maximum mycelial dry weight was obtained in culture filtratesof T.viride and F.oxysporum.

Different test fungi showed different p^H optima for the production of cellulases. Endo- β-glucanase activity was maximum at p^H - 5.5 for all the test fungi, while the production of exo- β-glucanase varied with p^H. T.viride and P.chrysogenum produced maximum exo-β-glucanase at p^H 6.0, while F.oxysporum and the mixture respectively at p^H 6.5 and 5.5. T.viride, F.oxysporum, P.chrysogenum and their mixture produced optimum β-glucosidase respectively at p^H 5.0, 5.5, 6.5 and 5.0. Maximum soluble protein was produced at p^H - 5.5 by T.viride and at p^H - 6 by F.oxysporum and P.chrysogenum. The variation in cellulase production at different test p^H was statistically significant. The mixture of these fungi preferred slightly more acidic condition (p^H -5.5) for

the optimum production of protein. At all the different test p^H, the p^H of the medium drifted towards acidity. Mycelial dry weight varied from p^H to p^H and the specific test fungi.

Fungi showed differential xylanase activity at different p^H. T.viride produced maximum xylanase at p^H -5.5 whereas, P.chrysogenum and F.oxysporum at p^H -6.5 and their mixture at p^H -6. Like cellulases, even in xylanase the p^H of the test medium became acidic towards the end of the sampling period. The mycelial dry weight, produced by different fungal species growing on a medium supplemented with xylan as a carbon source, varied with p^H and fungal species.

All the test fungi produced minimum cellulases and xylanase at 5°C. Cellulases and xylanase activities increased with increase in temperature till 25°C. At the highest temperature i.e. 35°C, the activities again declined. T.viride produced maximum cellulases, followed by mixed inoculum, F.oxysporum and P.chrysogenum. Besides, T.viride also produced maximum xylanase as compared to other test fungi. Optimum amount of soluble protein and mycelial dry weight was also obtained at 25°C. A significant variation was observed in the xylanase and cellulase activities, both due to different temperatures and different test fungi.

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C E R T I F I C A T E

We certify that the thesis entitled, ECOLOGICAL STUDIES ON THE MICROBIAL AND BIOCHEMICAL ASPECTS OF FOREST LITTER DECOMPOSITION AT HIGHER AND LOWER ALTITUDES OF MEGHALAYA, submitted by Miss Seema Kshattriya 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 our 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.

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C O N T E N T S

GENERAL INTRODUCTION

Decomposition is a process in which dead plant material is biologically disintegrated from a stage where it is still attached to the living plant upto the humus stage. Forest litter is an unique component of the forest biogeocoenosis and one of its major indicators of energy and mass transfer. The functioning of an ecosystem can be recognised from three distinct subsystems, i.e., primary producers, consumers and decomposers. To sustain the integrity of an ecosystem harmonious transfer of matter and energy among these subsystems is necessary. The litter on the soil surface acts as an input-output system and is important in the nutrition of woodlands, particularly, those on soils of low nutrient status, where the plants rely to a great extent upon the recycling of nutrients.

A large amount of plant litter is added annually to the soil through leaffall and death of plants. In deciduous forest ecosystem leaves constitute about 70% of the litter on the soil surface (Jensen, 1974). In forest ecosystem, litter decomposition is considered as an indispensable factor influencing the growth and development of forest stand. This is relevant especially in nutrient deficient coniferous forests where decomposition is slow and availability of nutrients to plants is low.

The bulk of the above ground annual net primary productivity of most forest ecosystem is tranferred directly to the decomposer subsystem via litterfall (Swift et al, 1979 and Seastedt and Crossley, 1987). Role of decomposition in terrestrial ecosystem has been comprehensively reviewed by Swift et al (1979), but the pattern of litterfall and subsequent nutrient release are important determinants to understand the function of forest ecosystem.

The process of decomposition is mainly accomplished by two important phases - physical breakdown of litter and breakdown of structural organic com-

pounds by soil microbes. The plant materials are not decomposed at once. Various chemical constituents are attacked by microbes at different rates. Some of them are able to degrade only a few, while others can attack a considerable number of constituents. Biodegradation is influenced by different environmental conditions like climate (Shukla and Singh, 1984), soil microbial population (Witkamp, 1963) and litter moisture, which varies with rainfall pattern besides the effect of diurnal drying. These factors affect the colonization of microbes, their activity, mechanism of degradation, besides the nature of the products formed. Obviously, any influence on these factors during decomposition shall affect the cycling of nutrients. The rate of decomposition also depends on the quality and quantity of organic constituents of litter (Christensen, 1986), age of the litter and the nutritional requirements of the heterotrophs. It is also obvious that different elements are retained with different strength in litter structure. These factors cause a differential pattern of release of elements over time. Besides, it also depends upon the immobilization of nutrients by microbes (Gosz et al, 1973; Swift et al, 1979 and Singh et al, 1989).

The litter quality is considered to be related with the nitrogen requirement and successional status of plant species (Aber and Melillo, 1982). Nitrogen and lignin are the major substrates controlling the rate of decomposition (Berg and Wessen, 1984). The early successional plant species have higher nitrogen content compared to the late successional species which are rich in lignin content. The nitrogen rich litter decomposes at a faster rate than lignin rich litter. Consequently, nitrogen is retained in the litter to a certain minimum concentration and thereafter is released at the same rate as organic matter loss, while the elements in non-limiting concentrations are released through out the decomposition phase (Berg and Staaf, 1981).

The chemical constituents of fresh litter can broadly be divided into three groups; the readily degradable or soluble compounds, insoluble polymer carbohydrates and insoluble phenolic compounds mostly lignins. The leaching of soluble plant nutrients (Nitrogen and Potassium etc.) controls the initial weight loss, While lignin controls the humification. The readily soluble compounds, mainly the monomers which serve as an unit of polymers, that are the basis of cell structure i.e., various sugars, simple aromatic structures, amino acids and nucleotides are the building blocks of polysaccharides, lignins, proteins and nucleic acids. Polysaccharides are most abundant component of the cell wall. They vary in complexity from those containing simple and single linkage sugars than those sugars with several types of linkages.

The polysaccharides are the major products of photosynthesis and considerable amount of them, after microbial degradation, returns to the soil. Plant biomass is primarily cellulose (30-40%), hemicellulose (20-30%) and lignin (5-10%) (Ladisch et al, 1983). Therefore, there is a need to contemplate over this vast food energy potentially locked up in the hemicellulosic residue. Cellulose, a widely distributed polysaccharide is the vast replenishable resource and is recycled by the microbes. Cellulosic portion of the biomass can be converted to glucose by enzymatic hydrolysis. Chemical conversion of cellulose to glucose is accomplished by acid hydrolysis which is not practicable on a large scale due to its limited efficiency (Cuskey et al, 1982). In addition to cellulose, lignin decomposition is difficult due to their heterogeneity. Lignins remain closely associated with cellulose and hemicellulose (Lundquist et al, 1980). Sometimes the lignin degradation products particularly, oxidised phenolics react with nitrogenous compounds and limit the availability of nitrogen to decomposers.

The microbes play an indispensible role in the transformation of organic matter owing to the liberation of nutrients and their effect upon soil ferti-

lity. Through biodegradation a huge amount of nutrients locked up in the organic matter are made available for new plant growth. The transformation of carbon, nitrogen, phosphorus etc. involves directly or indirectly the activities of various saprophytic groups of microbes.

Of all the microbes, fungi are the most active decomposers of plant litters. Infact, the decomposition starts before the different plant parts fall as litter on the soil. Later, after falling on the ground, the plant remains are subjected to successive and overlapping layers of different saprophytic forms. Each of them alters the microhabitat and makes way for the next. The quality and quantity of the microbes depends upon the nature of soil, plant vegetation and various environmental factors notably temperature, moisture (Rai, 1973 and Jensen, 1974) and aeration (Mikola, 1954 and Witkamp, 1966). A definite sequence of microbial colonization on litters occur in a given time during decomposition, which resembles an autogenic succession of higher plants except that there is a progressive depletion of nutrients. The soil faunas break down the litter physically into small pieces exposing fresh surfaces to microbial attack. The role of fungi in decomposition process has been reviewed by various workers like Pugh(1980) and Wicklow and Carroll (1982) and the role of bacteria by Skinner (1975) and Shelley et al (1983).

Measurement of the volume of colonizers and their activity is important in evaluating their contribution in biological cycling of nutrients in fields. The difference in the diversity of colonizers reflects the potential difference in the rate of decomposition (Hutchinson and King, 1989). The dominant factor controlling the microbial density is the different composition of plant species.

The degradation of polysaccharides is accomplished by the microbial enzyme complexes. The primary colonizers produce enzymes necessary for the hydrolysis of common polysaccharides. It is an established fact that most of the

hydrolysis processes are localized at the microbe-substrate interface. Probably little enzyme diffuses to act on substrates some distance away from the organisms. The enzymatic transformations are carried out in a chain like reactions, where one organism utilizes the product of other, one reaction makes the way for another reaction.

The polysaccharases are of two general types i.e., endopolysaccharase and exopolysaccharase. The endopolysaccharases are characteristic random acting enzymes responsible for the hydrolysis of higher molecular weight long-chained polymeric carbohydrates. Different enzymes are required for the hydrolysis of various homopolymers like glucans, galactans, etc. due to their diverse linkage types. Only a few organisms are capable of producing required enzymes. Then these organisms become dominant member of the microflora (Reese, 1968).

The exopolysaccharases act only after the endo-enzymes bring about a considerable increase in polymer chains. They act upon the non - reducing terminal units of the chain. One sugar unit is removed at a time. They act efficiently on longer chains and consequently the rate falls off from tetramer to trimer to dimer & to monomer. Like endoenzymes, exoenzymes are also substrate specific (Reese, 1968). Other enzymes which are widely distributed are invertase and amylase (Skujins, 1978), involved in the hydrolysis of carbohydrates as a part of organic matter mineralization (Skujins, 1967, Hankin et al, 1976 and Spalding, 1977).

The importance of microbes and their activity in decomposition is undoubtedly unparallel as far as release of various locked up nutrients and release of energy for plant growth is concerned. This has emphasised the need of exploitation of efficient microbes for biodegradation purposes both in natural as well as in man made environments.

In the North-Eastern part of India, people still use the primitive form of agriculture known as shifting cultivation. After one or two years of cropping the land is abondoned as Jhum fallow. The fertility of soil built up over the years owing to successive litter decomposition is exhausted soon. Besides, much of the nutrients are lost due to the steepness of hill slope along with the surface run-off due to heavy rainfall. Probably, a fraction of the nutrients are utilized by the growing seedlings. In addition, the recent population pressure has caused rapid destruction of forest resources and shortening of Jhum cycle. The high rainfall and extreme climatic fluctuations have also necessitated the studies pertaining to the role of microbes in litter decomposition and nutrient release.

A number of studies have been conducted on the microbial degradation of plant leaf litter (Frankland, 1966; Ivarson, 1973; MacLean and Wein, 1978, Edmonds, 1979; Das 1980; Rai and Srivastava, 1982 and Deka 1981. Very few of these studies pertain to the biochemical aspects of litter decomposition (Triska et al, 1975; Hankin et al,1976; Spalding,1977, 1978, 1980 and Sinsabaugh et al, 1981).

The present investigation was undertaken with a view to understand the microbial and biochemical aspects of litter decomposition in terms of ecosystem functions of different secondary successional forests at two altitudes of Meghalaya. To achieve the objectives set in, the following aspects were undertaken:

-Microbial population and succession of fungi on different plant leaf litters in young and old forest stands at two altitudes.

-Microbial decomposition of different leaf litters under field conditions.

-Microbial activities in terms of their different enzymes viz: cellulase, amylase and invertase.

-Effect of resource quality and temperature on microbial litter degradation.

-Effect of temperature and p^H on the synthesis of cellulases and xylanase by certain dominant fungi.

REVIEW OF LITERATURE

FUNGAL SUCCESSION AND MICROBIAL POPULATION

The succession on litter by microbes has been highlighted by many workers. Garrett (1951) reviewed the relationships of certain soil fungi to their substrate and pointed out that the first invaders of injured or dead plant tissues are the sugar fungi belonging to phycomycetes followed by cellulose decomposing ascomycetes and deuteromycetes. The lignin decomposers are the last to develop in such a habitat and belongs mainly to basidiomycetes. This was followed by most significant work of Webster (1956, 1957) who studied the microfloral succession on decaying cocksfoot culms (Dactylis glomerata).

Pugh (1958) studied the decomposition of Carex paniculata L. leaf litter and observed a definite change in the fungal flora which he attributed to temperature and moisture content of the litter.

Kendrick and Burges (1962) summarized the study of fungal succession on decomposing coniferous litter.

Caldwell (1963) observed a definite course of fungal succession on the decomposing beach leaf litter. Most of the fungi were present from the earliest to the latest stage of decomposition without much change in the frequency.

Hering (1965) while studying the fungal succession on plant litter observed a common pattern of distribution of fungal species. The primary fungal flora was replaced and dominated by Trichoderma and Penicillium species.

Hogg and Hudson (1966) investigated the microfungal succession on Fagus sylvatica leaves from the time of unfolding upto eighteen months after leaf fall.

Macauley and Thrower (1966) worked on the succession of five different groups of fungi during fifteen months of decomposing Eucalyptus regnans leaf

litter. They observed moniliales as the primary colonizers followed by the species of Penicillium and mucorales at later stage. The disappearance of certain fungal species during the course of investigation even when the nutrients were sufficient was attributed to the antagonistic effect of some fungal species.

Chang and Hudson (1967) studied the fungal succession on wheat straw compost and observed a regular pattern of fungal succession.

Hudson (1968) reviewed the ecology of fungi on above ground remains and presented a general scheme for the colonization of decaying leaves where the succession begins with parasites followed by primary saprophytes. Later on, the primary saprophytes are overriden by secondary saprophytes such as ascomycetes and deuteromycetes. Phycomycetes are the last to colonize the litter.

Brandsberg (1969) conducted a qualitative study of the microflora associated with the degradation of different coniferous litters. Altogether, he isolated 128 fungal species without any pronounced difference in the microfloral composition on the duff of different tree species. He also observed a definite successional pattern on the decomposing leaf litters.

Sharma and Dwivedi (1972) examined the fungal succession on decaying grass (Setaria glauca) and observed differences in the total number of fungi at different time intervals on different plant parts i.e. stem, sheath and blade. They attributed the fluctuation in fungal population to various factors like the moisture content of the substrate, temperature, humidity and competition between the colonizers.

The microflora of Eucalyptus maculata leaf litter was studied by Eicker (1973) using three different isolation techniques. He observed a total of 45 species representing 22 genera from various litter horizons.

Watson et al (1974) studied the fungal succession on loblolly pine litter and foliage in North Mississippi. They observed dominance of deuteromycetes,

phycomycetes, ascomycetes and basidiomycetes on F-layer while, only basidiomycetes in the H-layer.

The classification of the colonizing fungi into dominant, common, frequent, occassional and rare types depending upon their frequency was proposed by Vittal (1976).

The pattern of colonization of Scots pine litter by soil fungi was studied by Black and Dix (1977). They observed a difference in the colonizing ability from species to species. Trichoderma sp. Penicillium sp. and members of mucorales were the surface colonizers and their ability to colonize was dependent on the internal tissues of the litter.

Lehman and Hudson (1977) observed that urea treatment had an obvious effect on the fungal succession of pine needles. Application of urea resulted into a changed successional pattern and also stimulated the growth of some uncommon fungal species instead of typical pine needle fungi.

Mehrotra and Aneja (1979) studied the succession of fungi while investigating the microbial decomposition of Chenopodium album litter. They observed the dominance of deuteromycetes during the initial phase of decomposition while the final stage was dominated by ascomycetes and deuteromycetes.

Sandhu and Sidhu (1980) studied the fungal succession on decomposing bagasse for twenty weeks. They isolated nine fungal species in which Aspergillus fumigatus and A.terreus were common.

Das (1980) studied the fungal succession and microbial population of Pinus kesiya litter of different plantations. He observed Trichodermaviride and Penicillium chrysogenum as dominant microflora during decomposition.

Similar results were recorded by Deka (1981) while working on the decomposition of bamboo leaf litter.

Mishra and Dickinson (1981) studied the phylloplane and litter fungi of Ilex aquifolia. They obtained a marked seasonal variation in the mycoflora.

The bacterial dynamics was studied by Kjoller et al (1985) during the decomposition of alder litter. They observed high aerobic, amylolytic and proteolytic bacteria in the beginning which decreased with decomposition coinciding with depletion of readily available compounds in the litter.

Kuter (1986) isolated different fungal species from both green and senescent decomposing sugar maple leaves. A quantitative variation was observed in the fungal population which followed a consistent pattern.

Tiwari (1988) studied the fungal and bacterial population of Pine apple litter of different plantations. He recorded low bacterial and fungal population in the beginning which increased as decomposition progressed and declined towards the final stage. Altogether, 19 fungal species from decomposing leaf and root litters were recorded.

MICROBIAL LITTER DECOMPOSITION

Studies on litter decomposition on forest floor are important for understanding the soil fertility and nutrient cycling. Various workers have tried to understand the process of litter decomposition both in the field and laboratory conditions.

Shanks(1954) studied the role of microbial population in litter degradation at different altitudes. He observed an increase in microbial population and rate of decomposition with decrease in altitude which he attributed to temperature and humid climate.

Witkamp (1963) evaluated the role of microbial density on litter decomposition under the influence of various environmental conditions. He associated the rate of litter breakdown with dense microbial population. He further noted that the rate of degradation and microbial population were influenced by stage of decomposition, moisture of litter and p^H of the stand.

Ivarson (1973) observed that the rate of decomposition increased with increase in temperature.

The microbial decomposition of plant material in soil as influenced by some environmental factors was studied by Martyniuk and Myskow (1976).

Wani and Shinde (1977) studied the decomposition of wheat straw under laboratory conditions and recorded Aspergillus sp. to be the most effecient decomposer.

Brinson (1977) and Edmonds (1979) have suggested that temperature and moisture were the most important abiotic factors controlling the rate of decomposition.

MacLean and Wein (1978) were also of the opinion that the decomposition rate is controlled by factors like temperature, moisture regimes, substrate quality and microfloral and microfaunal populations.

Howard and Howard (1980) studied the effect of species, site, soiltype and climatic variation on leaf litter decomposition. They concluded that the site on which the tree grew and the soil on which the litter decomposed markedly influenced the weight loss, moisture content, respiration and P^H. They further added that the moisture content of litter affected the composition of microbial population.

Aneja (1981) determined the individual role of thirteen fungal species in decomposing Desmotachya and Chenopodium litter. He observed a considerable difference in the activity of the test fungi. Chaetomium erectum was most efficient in decomposing Desmotachya litter while, Aspergillusflavus was efficient in decomposing Chenopodium litter.

Panwar and Sharma (1981) have attributed the slow rate of decomposition of Scirpus tuberosus litter by fungi to high lignin, tannin and relatively poor nitrogen content beside, the abundance of sclerenchymatous tissues and unfavourable climatic conditions.

Berg et al (1982) studied the changes in the organic chemical constituents of pine needles during decomposition. They observed a marked variation in different organic constituent concentration with decomposition.

Deka and Mishra (1982) studied the decomposition of bamboo leaf litter in relation to age of jhum fallows. They observed an increasing trend in the rate of decomposition with increase in fallow age. They observed faster decomposition of sugar and aminoacid followed by hemicellulose, cellulose and lignin.

Rai and Srivastava (1982) while studying the decomposition of leaf litter in a tropical dry mixed deciduous forest observed that the rate of decomposition was high during rainy months and low during winter.

Schinner (1982) investigated the altitudinal effect on litter decomposition. The rate of decomposition decreased with increase in altitude.

Rai and Srivastava (1983) studied decomposition and competitive colonization of leaf litter by fungi in terms of carbondioxide evolution. They observed a high degree of decomposition by fast growing species like Mortierella subtilissima, Pestalotia macrotricha, Aspergillus niger and Trichoderma harzianum than slow growing Penicillium rubrum, Robillarda phragnitis and Myrothecium advena.

Woods and Raison (1983) assessed the rate of decomposition of summer leaf fall (abscised leaves), winter leaf fall containing some green leaves and mature green (picked) leaves in sub-alpine forests. They observed a positive linear relationship with initial concentration of nitrogen and phosphorus and rate of weight loss.

Decomposition and nitrogen dynamics of Myrica gale litter was studied by Schwintzer (1984). He observed that only 40% of the initial biomass and 10% of initial nitrogen were lost during first five years of decomposition, which was correlated to the high initial lignin content of litter.

Shukla and Singh (1984) studied the biodegradation of Shorea robusta leaf litter. They assigned the breakdown of litter to various environmental factors coupled with microfloral and microfaunal population.

The study on the decomposition of Scots pine litter was conducted by Jansson and Berg (1985) in central Sweden. They analysed the climatic influence on litter decomposition.

Stott et al (1986) studied the effect of low temperature and low water potentials on the microbial decomposition of wheat residue. They observed that low temperatures and low water potentials had a greater effect on microbial activity during the utilization of soluble components. Moore (1986) also conducted a similar study to see the effect of moisture and temperature on the decomposition rates of hardwood and coniferous litter.

Enright and Ogden (1987) while investigating the decomposition of litter from common woody species of kauri forest observed that the rate of phosphorus depletion varied according to the initial C:P ratio of the litter. Rate of P mineralization increased as C:P ratio decreased.

Decomposition of boreal forest litters under laboratory condition was studied by Fyles and McGill (1987). They observed that the cutting of Pine needles enhanced the decomposition rates indicating that physical constraints associated with litter structure exerts a strong influence on litter decomposition.

O'Connell (1987) investigated litter decomposition in three different sites in Karri forest of south-western Australia. He observed similar rate of weight loss and nutrient mobility at all the sites. Nevertheless, the changes in the amount of less mobile elements in litter differed significantly between the sites.

Singhal and Sharma (1987) investigated the biochemical and elemental composition of Shorea robusta litter from five different sites. The chemical com-

position varied marginally according to the age and forest stand.

The effect of initial nitrogen and initial lignin on the dynamics of leaf litters of five tree species was studied by Laishram and Yadava (1988). They observed an inverse linear relationship between the percentage of the original mass remaining and the nitrogen content. Significant positive correlation was also established between initial nitrogen and lignin content and remaining biomass.

Taylor and Parkinson (1988) explored the relative influence of substrate type, temperature and moisture on decomposition rates of Aspen and Pine litter in laboratory microcosm. They observed that temperature exerted more influence than watering rate for both the species.

In vitro the influence of environment, management practices and cultivar on the rate of cereal straw decomposition was also assessed by Summerell and Burgess (1989). They observed an increase in the proportion of lignin while, cellulose, hemicellulose and hot water soluble compounds decreased. They also observed an increase in the decomposition rate with increasing temperature within a range of 5-35^{o}C.

Upadhyay et al (1989) studied the decomposition of ten Central Himalayan forest litter in terms of temporal changes in nutrient concentration, weight loss and its spatial pattern. They observed a rapid loss of labile nutrients and concluded that the placement of the bags and the rainy season have maximum influence on litter breakdown.

Kalburtzi et al (1990) studied the decomposition of fababean and wheat straw and release of N,P,K, Mg and Ca under field conditions. They observed an increase in the concentration of N and P in the beginning of decomposing material which were decreased at the end of the experiment. The weight loss was neither affected by the straw species nor the container type.

MICROBIAL ENZYMES

The decomposition of litter is brought about by the extracellular enzymes secreted by the microbes.

Reese et al (1950) proposed C_1-Cx concept to account for the mechanism of cellulolysis by microorganisms.

Selby (1968) studied the mechanism of biodegradation of cellulose and showed that the insoluble cellulose substrate is first degraded to an intermediate soluble form and then to sugars.

Domsch and Gams (1969) investigated the ability of different fungal isolates for decomposing pectin, xylan and carboxymethylcellulose. They observed an insignificant variation in the decomposition of these three substrates. However, CMC was degraded rapidly by Truncatella truncata, xylan by Phoma eupyrena and pectin by Penicillium expansum.

Ghose (1969) studied the heterogeneity of cellulose and concluded that it was a multienzyme system which involves the concerted action of different enzymes.

Hancock et al (1970) studied the characteristics of pectate lysase formation by Hypomyces solani and concluded that production differed from organism to organism under the influence of temperature.

Stutzenberger et al (1970) observed a significant relationship between extractable cellulolytic activity and cellulose decomposition in municipal solid waste compost.

Effect of temperature, oxygen and carbondioxide on cellulolytic activity of some fungi was studied by Walsh and Stewart (1971). They screened cellulolytic fungal species like Chaetomium globosum, Memnoniella echinata and stachybotrys atra which acted at a temperature range from 15 to 35°C. They observed little effect of CO_2 on the cellulolytic activity of these organisms.

Pancholy and Rice (1973) studied various soil enzymes like amylase, cellulase, invertase, urease and dehydrogenase in two successional stages and a climax stand. The activities of amylase, cellulase and invertase were highest in the fast successional stage and lowest in climax stand. A reverse trend was observed for urease and dehydrogenase.

Berg (1975) examined the cellulase releasing systems in fungi and bacteria and observed a similar functional cellulase system in both.

Long et al (1975) studied the invertase activity and its relevance to the accumulation of storage polysaccharides in leaves infected by biotrophic fungi. They observed an increase in the acid invertase at the site of infection and very low activity in healthy leaves. The p^H optima from infected tissues ranged from 5.0-6.5.

Cellulase production by Trichoderma koningii and T.pseudokoningii was studied by Fanelli and Cervone (1977). They observed maximum cellulase activity at p^H-4 in presence of carboxymethylcellulose (1%). T.pseudokoningii was more efficient in producing cellulase than T.koningii.

Flannigan and Sellars (1977) studied the amylase, β- glucosidase and β-xylosidase activities of thermotolerant and thermophillic fungi isolated from barley. They obtained different p^H optima for different enzymes. Highest amylase, β-glucosidase and β- xylosidase activities was shown by Aspergillus fumigatus, Thermoascus crustaceus and Absidia corymbifera respectively.

Forbes and Dickinson (1977) determined the cellulolytic activity of three Fusarium isolates under a range of physical and nutrient conditions. They concluded that nitrogen source and concentration were major influencing factors for cellulolytic activities. They observed maximum cellulase activity at an initial or neutral p^H.

Spalding (1977) observed a significant correlation between CO_2 evolution and amylase, cellulase and xylanase (except invertase) activities in decompo-

sing coniferous leaf litter. He (1980) further observed a high degree of correlation among mannase, cellulase and xylanase activities.

Cellulase and hemicellulase activities of topsoils and tussock plant materials were investigated by Ross and Speir (1979). Neither cellulase nor hemicellulase activity was detected in the green leaves. They observed much higher enzyme activity in standing dead sheaths and blades than topsoil.

Araujo (1980) purified and determined the molecular weight and heterogeneity of cellulases from marine fungus Aspergillus terreus.

Cellulose decomposition by four isolates of Pyricularia oryzae was studied by Singh and Kunene (1980). They observed a suppression in the cellulolytic activity by glucose. Singh (1982) further studied cellulolytic ability of aquatic hyphomycetes and noted that the capacity to utilize cellulose was limited to few fungal species.

The invertase, cellulase, amylase, xylanase, acid phosphatase, peroxidase and polyphenol oxidase was measured in a Pahokee muck soil by Duxbury and Tate (1981). They observed a significant variation in these enzymes due to variation in soil depth and crop cover.

Mishra et al (1981) studied the cellulose degradation by Humicola lanuginosa.

Ross (1981) determined invertase and amylase activities in kauri plant material and in a moroid, strongly acid litter cone profile of Waipoua clay. He detected highest invertase activity in fresh leaves and twigs, and lowest in mineral soil horizons while, amylase activity was highest in litter horizons.

Cellulase activity associated with the decomposition of leaf litter in a woodland stream was studied by Sinsabaugh et al (1981). They observed a significant difference in endocellulase and exocellulase activies among oak, mapple and dogwood litters. They noticed a temperature profile for the enzymes

regardless of leaf species and length of stream incubation.

Speir and Ross (1981) studied the enzyme activities of tussock litter exposed around the base of tussock grass. They observed a significant correlation of urease, phosphatase, sulphatase, invertase, amylase, cellulase and hemicellulase activities with moisture contents, total nitrogen contents and C/N ratio of the litter. Except phosphatase all the enzymes were present in higher amount in exposed than unexposed litters.

Martinez et al (1982) studied the plant cell wall degrading cellulases produced by Botrytis cinerea. The endo-cellulase, exo-cellulase and xylanase activities were higher in culture liquid than mycelial extract.

The induction and inhibition of cellulase complex in Fusarium solani was studied by Bisen et al (1982).

D'Souza and Volfova (1982) observed that p^H played an important role in the biosynthesis of cellulase.

Mitchell (1982) investigated the invertase activity in wheat rust susceptible and resistant leaf tissues. He observed high concentration of sugars from infected tissues than healthy ones.

Moustafa and Sharkas (1982) isolated 15 cellulolytic fungal species from the tidal mud flats of Kuwait. They recorded new strongly cellulolytic fungal species like Arachniotus chankaliensis, Lasiobiolidium orbiculoides, Corynascus sepedonium and Pesotum sp.

Singh (1982) while investigating the effect of carbon and nitrogen sources on the production of cellulolytic enzymes in Acrocylindrium oxyzae observed that these nutrients had a considerable influence on growth, sporulation and production of cellulolytic enzymes.

Aneja (1983) studied the litter colonizing ascomycetes for their cellulolytic, pectolytic and lignolytic abilities and observed maximum cellulase activity in Chaetomium erectum while, Thielavia minor didn't show any activity.

The cellulase production by Rhizobium was investigated by Morales et al (1984). They found variation in cellulase activity from species to species depending on the culture medium used.

Biely et al (1985) used new chromogenic substrates for assaying endo -1,-4- β-xylanase and endo 1-4- β-glucanases. They recommended a method for assaying the activities in cell free exract and medium containing large amount of reducing compounds.

Awasthy (1987) investigated the nature and efficiency of some cellulolytic soil fungi to obtain an optimum culture conditions for overproduction of cellulase. She obtained Trichoderma sp. and T.koningii as the best cellulase producer among the four different Trichoderma sp. tested. An incubation period of 15 days alongwith p^H-5.5 and temperature 35 ± 1°C was found optimum for the production of cellulase.

Bhat and Maheshwari (1987) studied the cellulase activity of Sporotrichum thermophile. They noticed an appreciable difference in terms of extracellulase production. S.thermophile was more efficient in producing cellulase than T.reesei.

The cellulolytic and xylanolytic enzyme produced by an anaerobic rumen fungus grown on wheat straw were investigated by Lowe et al (1987). The CMC-ase and cellobiase activities were maximum at p^H-5.5 and xylanase at p^H-6 at a constant temperature (50°C).

Sandhu and kalra (1987) studied the production of cellulase, xylanase and pectinase by Trichoderma longibranchiatum grown on different substrates. Cellulase, xylanase and pectinase were produced on all C-sources except glucose, on which the production was minimum.

Sinsabaugh and Linkins (1987) studied the inhibition of T.viride cellulase complex by deciduous and coniferous litters of different degradational stages and observed that β -glucosidase activity was more susceptible to the

inhibitory effect of litter extracts than endo and exocellulases. Between pine and decidious leaf litter extracts, the pine litter extract was more effective.

Zare-Maivan and Shearer (1988) screened different fungal species for their ability to produce amylase, xylanase, cellulases, protease etc. in decomposing submersed wood. They observed that except Pythium sp. most of the fungal species were able to degrade a wide range of substrates.

Bachman and McCarthy (1989) purified and characterized a thermostable β-xylosidase from Thermomonospora fusca. They obtained maximum activity at a p^H range of 5-9 and temperature 40-60°C.

The regulation and production of hemicellulolytic and cellulolytic enzymes by a Streptomyces sp. growing on a lignocellulose substrate was studied by Bernard et al (1989).

Robson et al (1989) studied the effect of validamycin -A on the production of cellulase, xylanase and polygalacturonase activities by Rhizoctonia solani. Addition of antibiotics suppressed the production of CMC -ase and cellobiase while it could not affect xylanase and polygalacturonase activities.

Gokhale and Deobagkar (1989) studied the xylanase and endoglucanase activities in hybrid protoplast of cellulomonas sp. and Bacillus subtilis. Hybrid was more efficient than the individual species in producing these enzymes.

Sinsabaugh and Linkins (1989) studied the relative mobility of Trichoderma viride component in decomposing leaf litters. They observed that β -glucosidase enzyme was less mobile than endo and exoglucanase enzymes. Adsorptive capacity was changed while the relative mobility remained unchanged from senescent to partially decomposed litter.

STUDY AREA, CLIMATE AND VEGETATION

Soil degradation is defined as any change in physical and chemical properties of soil, which reduces the productivity of site. Soil of the North-East region is deficient in nutrients. Most of the nutrient capital of the site is concentrated in organic horizon, which takes years to accumulate and enrich the upper soil layers. Besides, soil organic matter also acts as a rooting substrata and supports natural regeneration. It also provides energy source for microbes which contribute to the release of nutrients. In N.E. regions of India, where slash and burn agriculture is the most prevalent form of agriculture destroys the organic layer and thus slows down the process of regeneration.

Keeping in view the above fact i.e. degree of degradation, the study areas were selected at two altitudes, one at lower altitude (100 m from MSL) at Byrnihat and another at higher altitude (1500 m from MSL) at Shillong. Both the sites were 90 km apart from each other. At each of the two altitudes, two forest stands showing different stages of regeneration were selected. While selecting the stands, care was also taken to ensure similarity in topography. In each of the four stands an area of 1 hectare was demarcated for detailed study.

The study areas were located between latitude $25^{o}34'$ N and longitude $92^{o}47'$E at Shillong and between latitude 26^{o}N and longitude $92^{o}.45'$E at Byrnihat.

Lower altitude:

Climate:

The climate of Byrnihat is of subtropical type. It remains warm and moist during May to September. Usually the lowest temperature is recorded in

the month of December and January. The climate of the whole year can broadly be divided into three main seasons: summer season (April to June), characterised by dry and warm weather and strong winds, rainy season (July to September), characterized by high temperature and heavy rainfall and Winter season (December to February) characterized by low temperature and occasional showers. Besides these three well marked seasons, March and October-November may be classified as pre-summer and pre-winter months respectively. The climate during these months is quite moderate.

The annual rainfall varied from 9mm to 473 mm while the maximum and minimum temperature ranged from 23°C to 35°C and 2.4°C to 26.7°C respectively (Fig.1).

Soil:

The soil of the study areas have originated from the hard rocks representing gneisses, schists and granite. The soil texture is sandy loam developed through laterization.

Vegetation:

The natural vegetation of the area is a forest which has been grouped into moist tropical forests. The natural forest is now confind to small areas. Whereas large area of this region is under secondary growth of forest on the abandoned 'jhum' land. Depending on the fallow age & degree of disturbance, the species composition of the two forest stands showed great variations.

The more degraded forest stand is dominated by weedy species namely Ageratum conizoides and Eupatorium odoratum besides few tree stumps like Mallotus philippinensis, Manihot esculenta, Accacia sp. etc. While the less degraded forest stand was dominated by tree species like Holarrhena antidysenterica and Vitex glabrata. The other species inhabitating the study area were Litsea

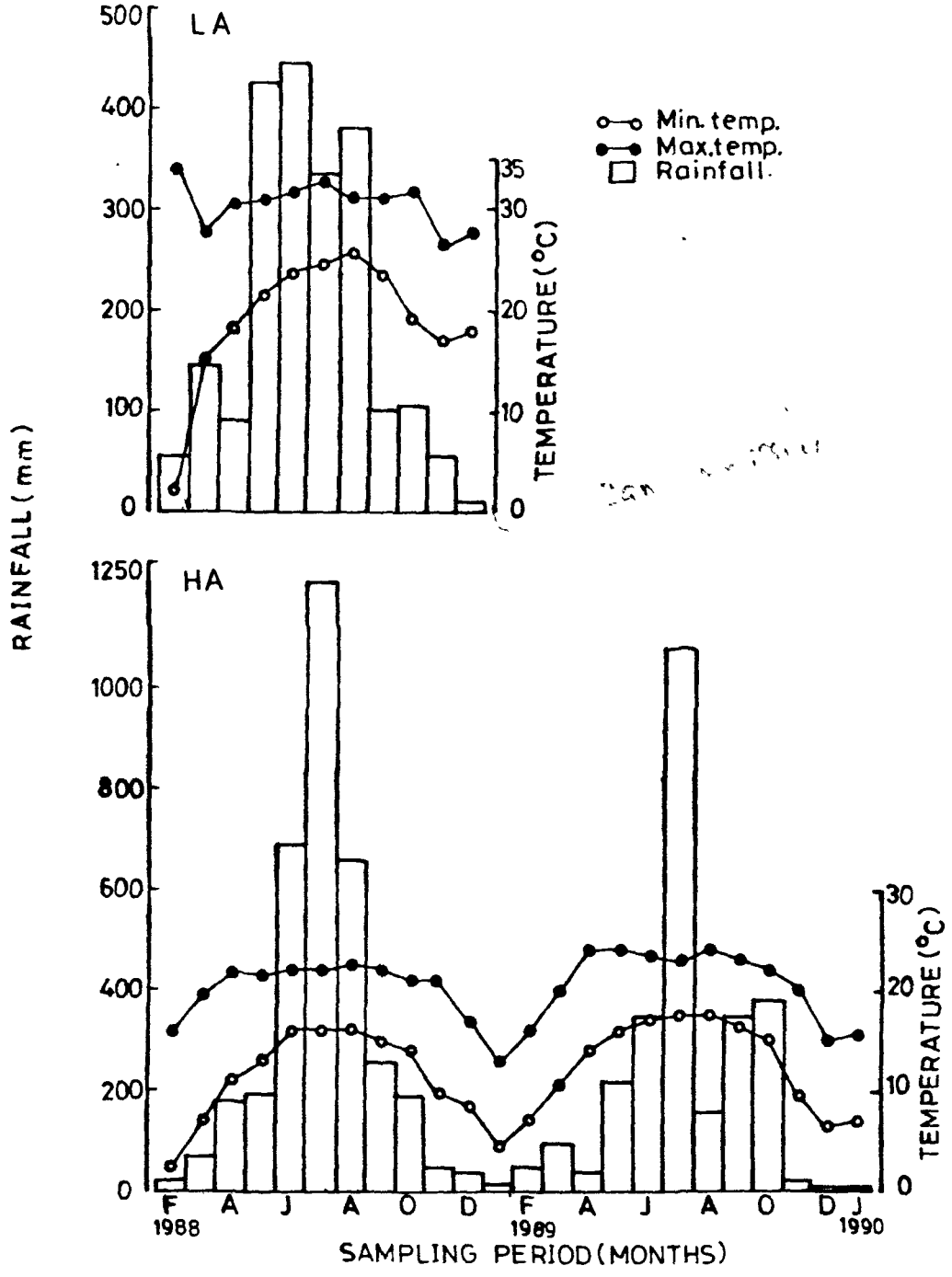


Fig. 1. Monthly variation in rainfall, maximum and minimum temperature of the study area at lower (LA) and higher (HA) altitudes.

monopetala, Bauhinia purpuria, Toona ciliata, Morus sp. besides the weedy species Ageratum conizoides, Eupatorium odoratum etc.

Higher altitude:

Climate:

The climate at Shillong is of tropical monsoonic type. The summer temperature recorded as high as 22°C and the mean winter temperature falling down to 6°C with periodic fluctuations below the freezing point, marked by appearance of ground frost at night and morning.

Based on the meterological conditions, the year may be broadly classified into rainy, winter and spring seasons. The rainy season (is comprised of April to October) months. The maximum rainfall (approximately 80%) occurs during June-July. While the winter season extends from November to middle of February. The period is characterized by low temperature and less rainfall. The spring season includes middle of February to March. The period is charac terized by high velosity of wind, less humidity and moderate temperature.

The annual rainfall ranged from 7mm to 1219mm during 1988 and 0.7mm to 1078 mm during 1989. The average maximum and minimum temperature varied from 14.9°C to 22.5°C and 0°C to 16.4°C respectively in 1988 and 13.1°C to 23.9°C and 4.5°C to 17.7°C respectively during 1989 (Fig. 1).

Soil:

The soil is red laterite under red loam or brown loam type. The texture ranged from very light (sand upto 90%) to very high clay contents. The soil is acidic.

Vegetation:

The vegetation at higher altitude is a forest of wet hill forest type. The forest stands are dominated by Pinus kesiya. The other species inhabitating the study area includes Alnus nepalensis, Myrica esculenta, Elaeagnus latifolia, Eurea japonica, Rhus javonica besides the herbaceous weedy Rubus ellipticus, Lantana camara, Osbekia crinata, Eupatorium adenophorum etc.

CHAPTER I

FUNGAL SUCCESSION AND POPULATION DYNAMICS OF MICROBES ON LEAF LITTER

INTRODUCTION

The term succession is defined as an orderly sequence of communities over a period of time at a definite place. In general, the microbial communities at any place due to their presence and biological activities modify the environment which is less conducive for the existing population but makes the way for future community. Thus, there is a continuous change in community structure, physiognomy of the associated flora and the environment of a place in course of time. Unlike autogenic succession in microbial succession there is a gradual depletion of nutrients. Nevertheless, the microbial succession plays an important role in ecosystems where the decomposers are an important biotic component. These bring about mineralization of dead organic matter thus releasing the mineral elements into the soil. The sequence of fungal succession upon a natural substratum reflects a complex interaction of nutritional relationship between each individual and the substratum together with competition between the individual fungi (Macauley and Thrower, 1966).

Micro-organisms are involved in successive phase of colonization, exploitation and exhaustion of organic substrates during decomposition. Greater the species richness of the saprobic microbial community colonizing the natural substrate the more rapid is the rate of decomposition (Waksman and Cordon, 1939). The diverse nature of microbial community results in a more enzymatic-biochemical diversity causing a rapid attack of the substrate.

The role of fungal succession in degradation process has been reviewed by many workers like Hudson (1968), Pugh (1980), Kjoller and Struwe (1982) and Wicklow and Carroll (1982). However, there are very few studies pertaining to the role of bacteria in decomposition (Skinner, 1975; Shelley *et al*, 1983 and Kjoller *et al*, 1985). Bacteria, despite their high number and diverse fun-

ction, play a secondary role in the decomposition of litter. The bacterial population plays an important role in the turn over of nitrogen (Struwe and Kjoller, 1985). The composition of bacterial flora also changes during decomposition but the number remains almost same relating their growth to the release of organic substances (Gunnarsson et al, 1988).

Shifting cultivation is a very primitive form of agriculture which involves clear cutting and burning of vegetation. The land abondoned as fallow is depleted of nutrients. During fallow period, accumulation of mineral nutrients and organic matter takes place under secondary growth of vegetation (Edmonds, 1979). Though studies have been conducted on decomposition and microbial colonization of leaf litter (Hering 1967; Kamal and Srivastava, 1975; MacLean and Wein, 1978 and Mehrotra and Aneja, 1979), but comparatively little information is available on microbial degradation process on forest litter of different successional stages (Deka, 1981). Therefore, a study to understand the microbial population dynamics and fungal succession on the leaf litters from the forests of different successional stages at different altitudes was carried out.

MATERIALS AND METHODS

Selection of Site:

Keeping in view the altitudinal variations and successional stages of forests, four forest stands of different ages were selected for the present study, two at higher altitude (Shillong, 1500m MSL) and the other two at lower altitude (Byrnihat, 100m MSL).

In each of the forest stand, two plant species (dominant and codominant, based on their diversity and dominance indices) were selected for the present investigation.

Pinus kesiya was dominant at higher altitude in both young and old for-

est stands. Alnus nepalensis was codominant in old forest stand while Myrica esculenta was codominant in young forest stand. At lower altitude, however, young forest stand was dominated by a herbaceous weedy species, Ageratum conizoides followed by a few tree stumps of Mallotus philippinensis which was codominant. The old forest stand was dominated by Holarrhena antidysenterica and codominated by Vitex glabrata.

Population dynamics of microflora and fungal succession on litter:
i) Collection of the Samples:

Nylon bag technique of Bocock et al (1960) was followed to study the saprophytic activity of microflora (fungi and bacteria) during decomposition of various leaf litters. Nylon litter bags of 20 x 20cms (mesh size 1 mm) containing 10g air dried leaf litter of the dominant and co-dominant plant species were spread randomly on the forest floor. At monthly intervals, 6 bags of each plant species were collected aseptically and brought to the laboratory. Three litter bags were used for the assessment of the microflora. The bags were kept at 4°C in fridge till they were processed (Ruscoe, 1971).

ii) Isolation of microflora from the litter:

For the isolation of fungi and bacteria from the decomposing litters dilution plate technique was followed (Waksman, 1922). 1g of litter was cut into small pieces with the help of sterilized scissor and transferred into a 250 ml sterilized conical flask containing 100ml of sterilized distilled water. Litter suspension of 1:100 was then obtained. 10 ml of this litter suspension was transferred aseptically into another sterilized 250ml conical flask containing 90ml of sterilized distilled water to get a dilution of 1:1000. The process was repeated once more to get a suspension of 1:10000 dilution. For the estimation of fungi and bacteria, 1:1000 and 1:10,000 dilutions respectively were found suitable for counting their population. Enumera-

tion of fungal and bacterial population was done on Rose bengal agar (Martin, 1950) and Nutrient agar medium (Difco-manual, 1953) respectively.

0.5 ml litter suspension was transferred aseptically from 1:1000 and 1:10,000 dilutions into each of the sterilized petriplates containing 15 ml of sterilized solidified Rose-bengal agar and Nutrient agar media for the estimation of fungi and bacteria respectively. The petri plates were shaken gently in order to disperse the suspension uniformly. Three replicates were maintained in each case. The entire isolation procedure was carried out inside a laminar flow chamber. For the isolation of fungi the petri plates were incubated at 25 ± 1°C in B.O.D. incubator for five days, while for bacterial isolation, the plates were incubated at 30 ± 1°C in bacteriological incubator for 24 hours.

The total number of fungi and bacteria were calculated per g dry litter, taking into consideration the dilution factor and moisture content of the litter. The pure cultures of fungi were maintained on slants of Malt - extract and Czapex-Dox agar media. The fungi were identified by consulting the manuals of Gilman (1957), Subramanian (1971) and Barnett and Hunter (1972).

iii) Determination of relative abundance:

The percentage relative abundance of fungi was calculated by using the following formula.

$$\text{Relative abundance (\%)} = \frac{\text{Total number of colonies of the individual species}}{\text{Total number of colonies of all the species}} \times 100$$

iv) Determination of moisture content and p^H:

The moisture content of the decomposing litters was determined by drying 1g of litter at 80°C until constant weight was obtained.

$$\text{Moisture content } (\%) \quad \frac{W_1 - W_2}{W_1} \times 100$$

Where, W_1 = Initial weight of the litter.

W_2 = Weight of the litter after oven drying.

The p^H of the litters was read on a digital p^H meter. 10g of litter was taken and mixed with water in a ratio 1:5. The litter was ground and kept for 1 hour and then the p^H was read by immersing the electrode of the p^H meter into the solution.

v) Climatic conditions:

The climatic conditions of the study area is given in details in chapter: Study area, Climate and Vegetation of the thesis.

vi) Statistical Analysis:

The coefficient of correlation (r) was calculated using Karl Pearson's coefficient of correlation (Zar, 1974).

RESULTS

Bacterial Population:

Quantitatively the bacterial population was more than the fungal population at both the altitudes. In the beginning of leaf litter decomposition, the bacterial population was less which increased as the decomposition progressed and again decreased towards the end of the decomposition.

At lower altitude the leaf litter of Ageratum conizoides harboured the maximum bacteria followed by woody leaf litters of Mallotus Philippinensis,

Vitex glabrata and Holarrhena antidysenterica.

In all the four leaf litters the bacterial population showed a marked seasonality. In young forest stand, the leaf litter of A.conizoides harboured the maximum bacterial population in the month of May, while in M. philippinensis the bacterial peak was observed in June. In old forest stand bacterial peak was observed in the month of June in both the litters. Thereafter, the population decreased in July and again increased slightly in August (Fig.2).

The number of bacteria was less at higher altitude as compared to lower altitude. Myrica esculenta litter harboured more bacterial population than Pinus kesiya litter in young forest stand, whereas in old forest stand the bacterial population on Alnus nepalensis showed a marked difference from P.kesiya litter. Like lower altitude, at higher altitude too, the bacterial population on the decomposing litters showed a marked seasonal variation. The bacterial population exhibited two peaks, the first peak was observed in the month of July followed by another in September. During winter months (December - February) the bacterial population was drastically reduced (Fig.3).

At higher altitude the bacterial population showed a significant positive correlation with moisture content and p^H of all the leaf litters (Table 9a, 9b,9c&9d). At lower altitude, the bacterial population showed a positive correlation with moisture content. The correlation, however, was significant only in H.antidysenterica and V. glabrata (Table 10a,10b,10c & 10d).

Fungal population:

Like bacterial population, the fungal population too was minimum on the first sampling which increased with the progress of decomposition and declined towards the end of the process. As compared to higher altitude, lower altitude exhibited more fungal population.

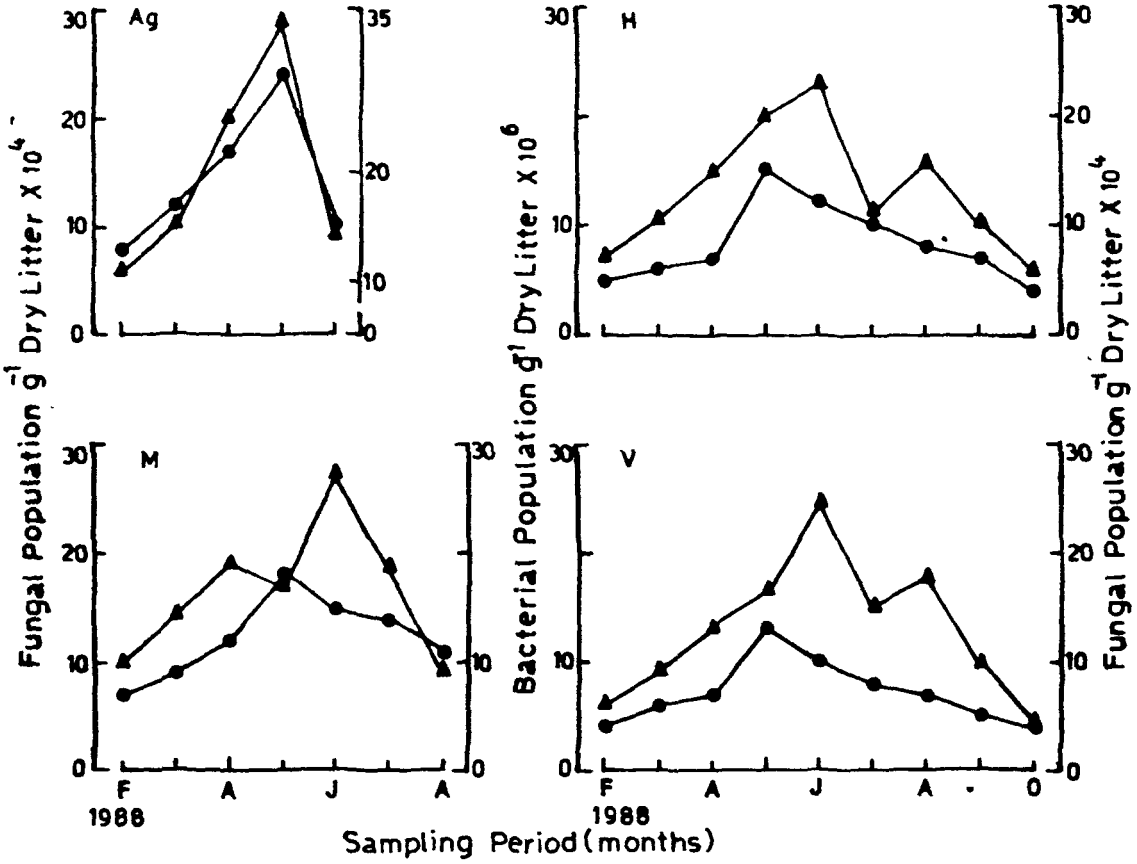


Fig. 2. Monthly variation in fungal (●–●) and bacterial (▲–▲) population of different leaf litters at lower altitude. Ag - Ageratum conizoides, M - Mallotus philippinensis, H - Holarrhena antidysenterica and V - Vitex glabrata.

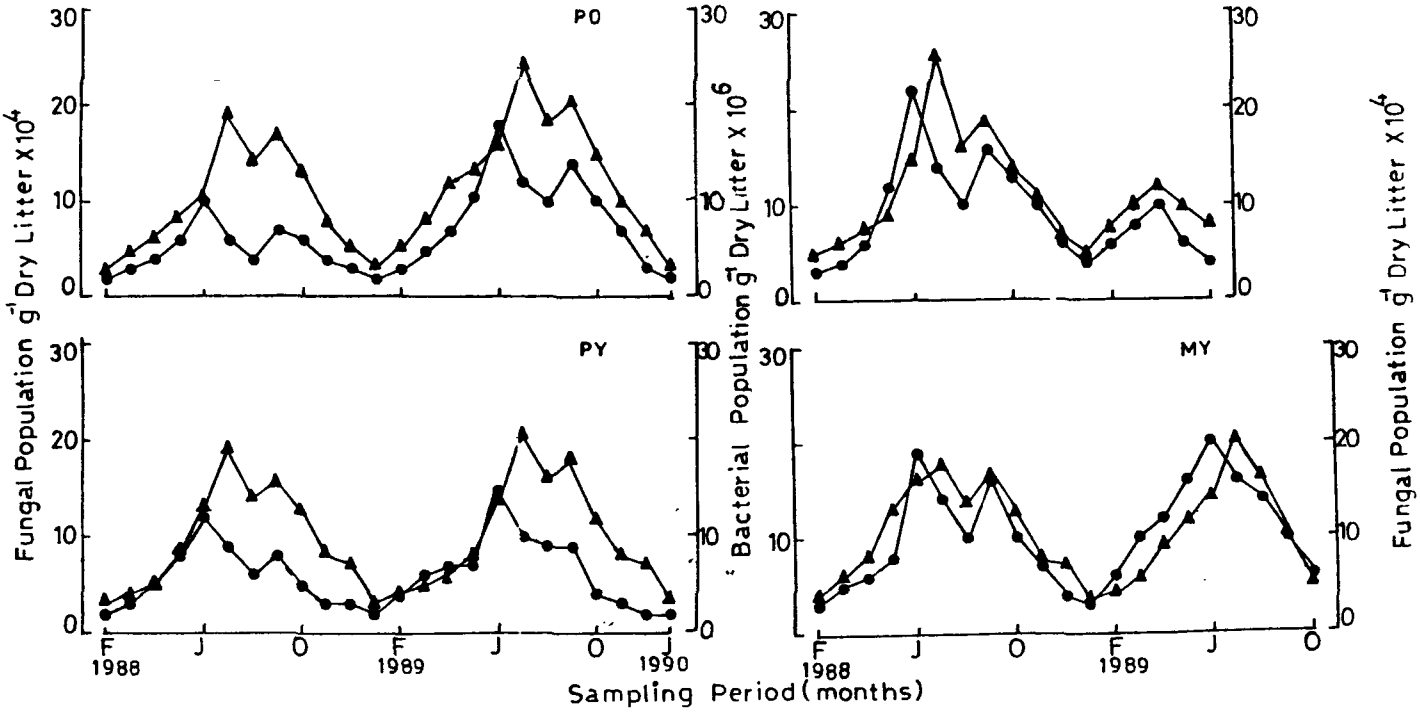


Fig. 3. Monthly variation in fungal (●-●) and bacterial (▲-▲) population of different leaf litters at higher altitude. PO - *Pinus kesiya (old)*, A - Alnus nepalensis, PY - Pinus kesiya (young) and MY - Myrica esculenta.

At lower altitude, in young forest stand the maximum fungal population was observed on A.conizoides litter followed by M.philippinensis. Whereas in old forest stand the litter of H.antidysenterica harboured more fungal population than V.glabrata. In A. conizoides and M. philippinensis population attained its peak in the month of May. Similarly in old forest stand the fungal peak was observed in May in both the litters. The population of fungi again decreased in the following months (Fig. 2).

At higher altitude, A.nepalensis harboured more fungal population followed by M.esculenta and P.kesiya litters. In all the litters a marked seasonal variation was observed. The maximum population was encountered in the month of June which subsequently decreased till August and the second peak of fungi was observed in the month of September which again decreased with the onset of winter. During second year the trend of variation in fungal population was almost similar to the previous year (Fig. 3).

Altogether 19 fungal species at higher altitude and 25 species at lower altitude were isolated. Penicillium chrysogenum was dominant species at lower altitude while Trichoderma viride at higher altitude. Some of the important fungal species isolated at lower altitude were Mucor hiemalis, Aspergillus niger, A.flavus, Acremonium sp., Alternaria alternata, Cladosporium cladosporoides, Curvularia lunata, Fusarium solani, P. chrysogenum, Penicillium sp., Trichoderma viride, T.koningii, T.harzianum, and white and black sterile mycelia (Table 1,2,3 and 4).

At higher altitude the important fungal species isolated were M.hiemalis, Rhizopus sp. Pythium sp., F.oxysporum P.chrysogenum, P.citrinum, P.rubrum, Penicillium sp., A.flavus, A.candidus, Cladosporium cladosporoides, T.viride, T.harzianum, Verticillium sp., Geotrichum candidum, Oidiodendron sp., Absidia cylindrospora, Paecilomyces sp., and white and black sterile mycelia (Table 5,6,7 and 8).

Table: 1. Distribution of percentage relative abundance of fungal species of *Ageratum conizoides* leaf litter at lower altitude.

Species	FEB	MAR	APR	MAY	JUNE
Mucor hiemalis	10.05	11.11	10.89	7.56	-
Actinomucor sp.	3.20	4.39	-	-	-
Zygorynchus sp.	8.64	2.65	-	-	-
Mucor racemosus	-	3.87	-	2.51	-
Rhizopus stolonifer	12.13	09.11	-	5.29	2.98
Absidia cylindrospora	-	6.64	8.25	3.68	-
Aspergillus flavus	7.08	7.36	12.57	11.47	10.9
A.niger	-	3.35	-	8.43	10.26
A.candidus	-	-	5.58	-	6.54
Pencillium chrysogenum	18.24	18.25	22.39	28.67	29.07
Cladosporium cladosporoides	-	-	4.83	4.09	-
Fusarium oxysporum	-	6.5	9.85	10.13	13.65
Trichoderma viride	16.29	14.23	15.39	13.5	11.96
T.harzianum	9.03	-	10.25	-	7.89
White sterile mycelium	10.37	12.24	-	3.97	6.75
Black sterile mycelium	4.97	-	-	0.7	-

Table 2. Distribution of percentage relative abundance of fungal species of Mallotus philippinensis at lower altitude.

Species	FEB	MAR	APR	MAY	JUN	JUL	AUG
Mucor racemosus	-	-	2.98	-	4.98	6.47	-
Mucor hiemalis	09.07	10.35	11.52	-	6.59	-	4.56
Rhizopus stolonifer	-	9.86	08.15	9.39	-	-	-
Aspergillus flavus	5.91	6.42	6.64	10.56	6.67	9.25	13.64
A.niger	5.13	-	-	7.12	6.84	7.98	10.56
A.fumigatus	3.08	-	4.15	-	-	-	-
A.candidus	-	6.68	-	6.98	-	6.43	-
Penicillium chrysogenum	14.45	16.27	19.45	23.57	26.34	30.09	27.98
Penicillium sp.	6.2	-	5.17	8.45	6.53	-	-
Acremonium sp.	6.69	3.25	5.31	-	-	6.23	-
Cladosporium cladosporoides	-	8.78	-	7.65	-	-	8.35
Fusarium solani	4.08	-	-	-	2.11	-	-
Fusarium oxysporum	-	4.92	-	-	8.42	11.64	9.87
Trichoderma viride	14.26	15.69	15.23	14.97	12.65	13.24	14.23
T.koningii	8.89	-	5.27	6.48	5.65	-	8.31
T.harzianum	10.68	8.97	6.35	-	4.64	-	-
Bromella sp.	-	1.23	-	-	0.89	-	-
White Sterile mycelium	11.56	-	4.65	-	6.86	8.67	-
Black Sterile mycelium	-	7.58	5.13	4.83	0.83	-	2.5

Table: 3. Distribution of percentage relative abundance of fungal species of Holarrhena antidysenterica leaf litter at lower altitude.

Species	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT
Mucor hiemalis	-	7.19	-	4.73	8.25	4.36	7.69	-	-
Rhizopus stolonifer	8.23	11.67	10.96	-	7.64	5.35	-	-	6.12
Cunninghamela sp.	-	3.65	-	5.68	-	-	-	4.36	-
Acremonium sp.	5.89	-	4.63	-	-	3.69	-	-	-
Aspergillus flavus	-	9.25	10.25	-	10.63	09.91	13.65	14.13	13.08
A.niger	-	5.18	5.78	7.21	8.12	9.45	12.23	-	9.87
Penicillium chrysogenum	17.38	19.56	23.46	26.37	33.89	30.69	25.98	23.56	19.56
Penicillium sp.	2.77	-	4.51	8.20	8.56	-	-	7.38	9.53
P.humicola	7.49	8.68	-	-	-	-	-	4.18	-
Cladosporium cladosporoides	3.36	-	4.14	-	1.25	2.08	-	3.49	-
Curvularia lunata	3.45	-	-	4.08	-	2.74	-	-	4.23
Paecilomyces sp.	9.83	-	2.56	-	-	1.08	-	-	-
Fusarium oxysporum	-	-	7.56	11.44	11.53	15.16	12.08	10.68	-
Trichoderma viride	19.19	19.19	15.23	13.52	5.36	5.58	10.36	15.37	17.56
T.koningii	-	6.09	-	6.34	2.28	5.02	-	10.26	11.19
T.harzianum	10.24	4.0	5.63	7.05	-	4.89	7.56	-	8.08
Monilia sp.	4.82	-	-	-	1.56	-	-	-	-
White sterile mycelium	-	4.26	1.83	4.24	-	-	6.26	6.56	0.78
Black sterile mycelium	4.35	1.28	3.45	1.14	0.93	-	4.19	-	-

Table: 4. Distribution of percentage relative abundance of fungal species of Vitex glabrata leaf litter at lower altitude.

Species	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT
Mucor hiemalis	-	11.19	10.65	-	6.42	-	7.21	5.98	-
Rhizopus stolonifer	10.83	9.98	-	5.24	-	7.17	5.36	3.65	4.18
Cunninghamela sp.	4.64	-	-	3.92	0.98	-	-	-	3.1
Acremonium sp.	5.26	-	-	5.68	-	5.06	-	-	-
Aspergillus flavus	-	6.54	10.13	-	14.25	09.92	12.55	11.97	16.59
A.niger	6.37	-	5.89	7.98	8.69	6.31	5.23	7.98	6.25
Penicillium chrysogenum	16.28	18.65	20.42	26.09	32.6	30.54	29.18	25.26	21.34
Penicillium sp.	-	-	6.24	7.54	7.24	-	6.54	8.65	-
P.humicola	6.49	5.24	-	-	-	-	2.37	4.28	-
Cladosporium cladosporoides	-	6.23	4.27	-	3.97	3.25	-	2.46	-
Curvularia lunata	2.89	-	8.23	-	-	3.48	2.32	-	2.98
Paecilomyces sp.	7.19	-	-	-	-	5.16	-	-	-
Fusarium oxysporum	-	6.37	5.82	9.72	12.45	14.6	10.2	-	-
Trichoderma viride	18.73	16.16	15.48	13.65	10.23	9.86	11.65	13.09	16.35
T.koningii	6.42	7.25	-	6.25	1.68	1.26	-	8.65	9.26
T.harzianum	3.10	5.14	5.31	8.29	-	3.37	1.73	-	8.24
Monilia sp.	-	3.16	-	-	-	-	-	-	3.37
White sterile mycelium	5.33	4.09	7.56	4.48	-	-	4.31	4.78	5.64
Black sterile mycelium	6.57	-	-	1.16	1.49	-	1.35	3.25	2.7

Table: 5. Distribution of percentage relative abundance of fungal species of *Myrica esculenta* leaf litter at higher altitude during 1988-1989.

Species	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
Mucor hiemalis	-	8.3	10.9	10.1	8.7	-	9.7	11.7	-	8.5	-	-	7.5	-	11.9	13.1	13.5	-	7.4	8.6	9.5
Rhizopus Stolonifer	6.6	8.9	-	7.7	5.5	9.3	-	-	-	6.3	-	4.8	4.3	9.5	4.7	-	8.2	6.6	-	-	10.6
Pythium sp.	-	-	8.7	5.9	7.3	7.2	5.2	-	-	-	-	-	-	6.8	9.5	11.1	9.5	8.6	6.3	-	-
Cladosporium cladosporoides	8.3	7.5	10.2	-	-	-	-	-	6.9	8.4	9.6	8.6	-	-	-	6.2	-	-	8.1	6.6	7.1
Fusarium Oxysporum	-	-	5.1	5.3	6.8	7.7	8.4	9.6	10.2	-	-	-	-	-	4.6	8.7	6.2	9.2	-	8.0	-
Aspergillus flavus	9.1	11.3	-	8.1	8.9	-	12.7	13.1	-	-	10.5	9.3	8.5	10.5	-	-	-	10.6	11.3	15.2	16.4
A.niger	4.6	5.5	-	6	-	8.2	10	6.7	8.6	9.8	8.2	8.1	-	8.7	9.5	-	9.2	10.4	7.9	-	-
Penicillium chrysogenum	14.9	16.3	18.9	19.6	23.2	22.5	20.1	20.1	18.4	17.7	15.5	15.4	13.3	15.7	17.4	19.7	21.9	22.0	19.8	19.0	17.4
P.citrinum	-	-	6.9	4.2	5.4	8.7	-	-	7.7	6.2	-	-	8.4	-	5.4	7.1	-	-	-	8.4	-
Trichoderma harzianum	7.1	-	-	-	3.2	4.1	-	-	8.3	7.2	13.7	10.2	12.3	10.1	-	-	-	-	-	-	9.2
T.viride	29.2	33.2	30.1	26.4	25.1	25	22.4	23.7	25.4	25.9	26.6	26.1	29.2	29.9	26.6	24.7	21.1	22.5	24.0	25.5	27.5
Verticillium sp.	-	-	3.8	6.7	-	3.1	4.4	4.9	6.9	7.3	6.4	4.9	5.1	-	-	2.4	5.1	-	-	-	-
Geotrichum candidum	-	-	-	-	1.1	-	2.4	2.7	1.0	-	2.1	-	0.9	-	-	-	-	-	5.5	-	
Oidiodendron sp.	4.4	4.3	-	-	-	-	-	-	-	2.5	-	2.5	1.1	-	-	-	0.9	-	-	-	
White sterile mycelium	7.1	4.7	5.3	-	-	2.3	5.1	-	6.6	-	5.3	6.4	9.5	8.9	6.7	-	4.4	10.1	9.7	8.7	2.4
Black sterile mycelium	8.6	-	-	-	4.6	1.9	-	7.5	-	-	2.2	3.8	-	-	3.9	6.9	-	-	-	-	-

Table: 6. Distribution of percentage relative abundance of fungal species of Pinus kesiya (young) litter at higher altitude during 1988-1989.

Species	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN
Mucor hiemalis	-	-	9.23	-	-	-	9.31	10.35	8.75	-	-	-	5.27	10.41	10.26	-	8.23	6.38	8.92	10.46	8.89	-	-	-
Rhizopus stolonifer	10.46	11.34	-	-	-	8.69	9.14	11.8	-	-	10.09	-	6.49	-	10.58	11.36	-	6.53	7.59	12.47	-	-	11.09	12.24
Pythium sp.	-	-	-	6.09	9.13	10.3	8.34	-	6.89	8.64	-	-	-	11.78	9.19	10.12	7.48	10.21	11.25	6.29	-	-	-	-
Cladosporium cladosporoides	8.09	-	6.65	-	8.96	-	1.68	-	-	9.64	8.08	6.43	-	-	-	4.88	5.01	-	-	1.86	4.58	10.48	6.58	-
Fusarium oxysporum	-	-	5.87	8.17	9.35	9.99	-	-	-	7.23	2.96	-	-	9.39	9.82	10.48	8.11	8.69	5.07	-	-	6.98	-	-
Aspergillus flavus	9.33	12.15	-	-	-	-	10.28	14.26	12.68	-	-	13.61	11.37	-	-	-	9.27	10.55	12.63	-	-	8.75	-	11.19
A.niger	9.50	9.76	10.36	10.67	8.37	-	-	8.23	7.65	8.14	7.14	-	6.18	10.88	-	-	-	-	-	9.56	13.64	9.29	10.49	9.65
Penicillium chrysogenum	13.88	15.47	18.56	23.47	24.75	23.69	20.65	18.69	18.07	17.48	16.1	16.13	14.23	21.24	22.48	23.77	24.08	26.16	24.38	22.91	20.83	20.93	17.8	15.59
P.citrinum	-	-	7.46	7.05	-	6.38	7.32	-	6.57	10.28	8.42	7.55	5.31	-	-	-	-	-	-	8.63	5.73	-	7.46	-
P.rubrum	4.29	7.94	-	5.75	8.62	9.63	-	8.45	7.94	-	7.23	7.09	6.43	-	-	-	6.31	-	-	-	6.03	7.77	-	-
Penicillium sp.	-	-	6.68	7.68	-	7.78	9.76	-	6.98	-	-	-	-	9.05	9.36	5.69	-	4.33	-	5.0	-	-	6.88	7.03
Trichoderma viride	28.36	29.99	27.43	24.37	22.08	19.54	20.69	21.08	19.69	22.58	23.79	26.89	28.64	26.72	24.89	24.01	21.17	18.46	20.33	22.82	24.16	26.36	26.94	29.83
T.harzianum	10.03	12.18	7.76	4.74	-	-	-	-	4.88	8.68	10.08	15.39	16.08	-	-	4.28	3.29	-	-	-	7.82	9.44	10.37	11.04
White sterile mycelium	6.07	1.17	-	2.01	8.68	4.05	2.88	7.14	-	7.33	6.21	8.92	-	-	3.44	5.41	7.05	8.69	9.83	-	8.32	-	2.29	3.43

Table: 7. Distribution of percentage relative abundance of fungal species of Alnus nepalensis leaf litter at higher altitude during 1988-89.

Species	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN
Mucor hiemalis	8.5	10.3	-	6.4	-	-	5.8	4.2	-	-	2.6	-	8.2	5.7	-	-	-
Rhizopus stolonifer	10.4	11.7	14.6	10.4	-		8.3	-	-	-	-	4.4	9.1	-	7.2	4.3	-
Absidia cylindrospora	6.1	7.0	-	5.8	4.4	6.7	-	-	5.4	4.0	-	-	-	-	8.5	6.4	-
Pythium sp.	-	-	6.6	7.2	9.8	11.2	-	8.5	-	-	-	-	-	2.5	2.3	-	9.3
Aspergillus flavus	5.7	-	8.2	5.2	8.4	-	11.3	15.3	15.5	13.3	-	-	8.2	12.5	-	10.9	-
A.niger	-	4.5	9.4	-	7.3	-	-	8.1	7.7	9.5	-	-	-	5.7	13.8	6.3	10.4
Cladosporium cladosporoides	-	-	7.1	5.3	-	3.6	4.0	-	-	8.3	9.9	6.2	8.3	-	-	-	5.3
Fusarium oxysporum	7.0	-	-	9.8	13.3	15.2	16.8	10.3	9.2	-	7.3	5.3	3.8	-	7.3	3.6	3.6
Penicillium chrysogenum	12.3	14.6	16.4	17.2	20.9	18.7	19.1	16.5	16.4	13.3	17.3	14.3	15.1	18.2	20.1	23.1	27.5
P.citrinum	5.6	5.8	-	-	7.5	9.3	8.6	-	-	-	10.6	12.6	-	9.3	5.4	-	-
Paecelomyces sp.	-	3.2	7.1	-	2.3	5.8	4.7	4.0	2.7	-	3.2	-	3.2	-	-	2.3	3.6
Trichoderma viride	29.3	27.5	24.2	23.8	20.6	21.8	18.3	22.2	23.1	27.9	30.7	33.9	29.1	28.5	25.5	21.6	19.6
T.harzianum	4.5	5.6	-	-	3.9	-	-	8.3	12.4	14.3	13.6	9.3	6.4	8.6	4.2	8.5	10.5
Geotrichum candidum	-	3.6	-	-	-	0.9	1.7	-	-	5.9	1.3	5.1	-	-	-	-	-
Verticillium sp.	5.9	-	6.5	7.0	2.1	2.0	-	2.9	3.5	3.6	3.5	6.2	8.5	5.2	2.6	7.0	8.3
White sterile mycelium	4.8	6.3	-	1.9	-	4.9	1.5	-	4.3	-	-	2.7	-	3.8	2.9	6.2	2.2

Table: 8 Distribution of percentage relative abundance of fungal species of Pinus kesiya (old) litter at higher altitude during 1988-89.

Species	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Jan
Mucor hiemalis	-	-	8.25	8.94	-	-	11.24	12.21	10.68	-	-	-	6.48	9.45	13.55	-	10.43	8.13	-	11.76	12.09	-	-	-
Rhizopus stolonifer	-	11.15	-	-	-	12.35	13.21	10.67	-	12.33	8.67	-	7.76	-	12.66	13.09	9.32	-	-	12.67	-	9.03	13.26	-
Pythium sp.	-	-	-	3.46	8.85	10.05	7.78	9.35	-	-	-	-	1.24	-	4.39	-	6.86	9.46	12.63	10.22	-	-	-	-
Cladosporium-Cladosporoides	8.89	-	10.08	-	9.44	-	-	-	-	10.46	-	11.19	-	-	-	9.73	-	-	14.25	-	10.83	6.39	-	5.12
Fusarium oxysporum	-	-	2.23	4.23	8.32	9.22	6.38	-	-	-	3.55	-	-	3.69	-	5.42	7.23	7.92	11.78	-	-	-	-	-
Aspergillus flavus	9.37	10.78	-	-	-	-	12.15	13.66	15.55	-	-	12.68	-	14.24	-	13.45	11.17	13.28	-	-	10.56	11.71	-	16.32
A.niger	10.14	10.85	12.35	11.25	9.64	11.34	-	-	11.68	13.48	10.43	-	9.56	12.38	-	-	-	11.85	-	-	9.48	12.44	12.53	10.05
Penicillium-chrysogenum	14.88	16.05	17.18	17.57	21.31	18.79	19.14	16.84	16.09	14.85	18.4	16.02	17.38	20.22	24.35	25.11	25.38	23.31	26.41	24.74	19.26	17.34	15.93	15.19
P.Citrinum	-	-	7.18	7.05	-	6.66	6.09	-	10.17	9.69	8.32	8.31	10.15	-	-	-	-	-	-	8.53	-	-	6.37	6.73
P.ruhrum	9.67	-	-	8.34	7.68	7.11	-	6.13	4.92	-	9.62	7.25	7.37	-	-	-	5.61	2.34	-	-	8.37	5.58	-	-
Penicillium sp.	-	8.5	6.26	-	-	0.97	3.4	-	-	1.21	-	-	-	6.59	8.3	5.62	-	4.15	-	6.97	-	-	7.64	5.47
Trichoderma viride	31.28	32.11	29.11	27.33	23.16	19.34	19.31	20.33	23.42	25.73	29.56	27.79	30.08	25.78	22.81	20.28	21.02	15.2	19.56	20.34	23.15	26.69	30.41	27.63
T.harzianum	12.35	10.56	7.36	8.36	-	-	-	-	7.49	8.88	8.74	10.58	7.66	-	-	5.17	2.98	-	7.19	-	6.26	7.49	10.08	13.49
White Sterile mycelium	3.42	-	-	-	6.48	4.17	1.30	4.78	-	3.37	2.71	6.18	-	-	8.42	2.13	-	4.36	5.77	-	-	3.33	3.78	-
Black Sterile mycelium	-	-	-	3.47	5.12	-	-	6.03	-	-	-	-	2.32	7.65	5.52	-	-	-	-	5.16	-	-	-	-

An apparent variation in the diversity of fungal species was observed among the different leaf litter species. Litter of weedy species, A.conizoides harboured more of phycomycetous fungi in the beginning of the decomposition compared to its woody counterparts where the primary colonizers included mainly the deuteromycetous forms followed by ascomycetous and a few phycomycetous species.

At higher altitude P.chrysogenum, T.viride, C.cladosporioides and Pythium sp. were observed as early colonizing species. F.oxysporum and Pythium sp. were frequently isolated towards the rainy season. The genera like Rhizopus, Cladosporium, Aspergillus, Fusarium, Mucor were isolated frequently during summer season while Alternaria, Verticillium, and Trichoderma were commonly isolated during winter. Some of the fungi like Geotrichum sp., Oidiodendron sp., and Paecilomyces sp., were isolated occasionally in low frequencies.

At higher altitude, the fungal population of all the litters showed a significant positive correlation with moisture content. Besides, a positive correlation was also established between fungal population and p^H of litter. However, the correlation was significant only in A. nepalensis litter fungi (Table 9a,9b,9c & 9d). At lower altitude too although a positive correlation was established between these traits but the correlations were not significant statistically (Table 10a,10b,10c & 10d).

DISCUSSION

The reduced fungal and bacterial population during the initial phase of litter decomposition may be due to the unavailability of soluble nutrients, which were still in a complex form. The distribution of microflora was therefore, dependent upon the availability of the specific substrate (Garrett, 1951 and Nikhra, 1981). The initial reduced mirobial population may be due to the

Table 9(a). Correlation coefficients (r) for p^H, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial populations of Pinus kesiya litter(old).

Sources of variation	D.F.	Fungal population	Bacterial population
p^H	22	0.261	0.558***
Moisture content	22	0.716***	0.877***
Total nitrogen	22	-0.102	-0.054
Phosphorus	22	0.076	-0.073
Cellulose	22	-0.356*	-0.324
Hemicellulose	22	-0.244	-0.333
Lignin	22	0.465**	0.394*
Total sugars	22	-0.408**	-0.366*
Total amino acids	22	-0.439**	-0.364*

*, **, ***, $P < 0.1$, 0.05 and 0.01 respectively.

Without asterisk statisticaly insignificant at these levels.

Table 9(b). Correlation coefficients (r) for p^H, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial populations of Alnus nepalensis litter.

Sources of variation	D.F.	Fungal population	Bacterial population
p^H	15	0.396	0.188
Moisture content	15	0.807***	0.776***
Total nitrogen	15	0.366	0.168
Phosphorus	15	0.181	-0.074
Cellulose	15	0.280	0.025
Hemicellulose	15	0.573*	0.0025
Lignin	15	0.134	0.051
Total sugars	15	0.126	-0.021
Total amino acids	15	0.0099	-0.095

*, **, ***, $P < 0.1$, 0.05 and 0.01 respectively.

Without asterisk statisticaly insignificant at these levels.

Table 9(c). Correlation coefficients (r) for p^H, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial populations of Pinus kesiya (young) litter.

Sources of variation	D.F.	Fungal population	Bacterial population
p^H	22	0.237	0.658***
Moisture content	22	0.740***	0.959***
Total nitrogen	22	0.299	0.04
Phosphorus	22	0.306	0.0053
Cellulose	22	-0.003	-0.222
Hemicellulose	22	-0.018	-0.289
Lignin	22	0.116	0.360*
Total sugars	22	-0.026	-0.116
Total amino acids	22	-0.108	-0.439**

*, **, ***, $P < 0.1$, 0.05 and 0.01 respectively.

Without asterisk statisticaly insignificant at these levels.

Table 9(d). Correlation coefficients (r) for p^H, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial populations of Myrica esculenta litter.

Sources of variation	D.F.	Fungal population	Bacterial population
p^H	19	0.254	0.491**
Moisture content	19	0.760***	0.867***
Total nitrogen	19	-0.183	-0.018
Phosphorus	19	-0.185	-0.054
Cellulose	19	-0.326	-0.104
Hemicellulose	19	-0.334	-0.114
Lignin	19	0.358	0.095
Total sugars	19	-0.312	-0.065
Total amino acids	19	-0.328	-0.062

*, **, ***, $P < 0.1$, 0.05 and 0.01 respectively.

Without asterisk statisticaly insignificant at these levels.

Table 10(a). Correlation coefficients (r) for p^H, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial populations of Ageratum conizoides litter.

Sources of variation	D.F.	Fungal population	Bacterial population
p^H	3	0.292	0.317
Moisture content	3	0.506	0.505
Total nitrogen	3	-0.183	-0.170
Phosphorus	3	-0.495	-0.512
Cellulose	3	-0.334	-0.355
Hemicellulose	3	-0.387	-0.401
Lignin	3	0.454	0.470
Total sugars	3	-0.538	-0.552
Total amino acids	3	-0.428	-0.447

Not significant at $P < 0.1$, 0.05 and 0.01 levels.

Table 10(b). Correlation coefficients (r) for p^H, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial populations of Mallotus philippinensis litter.

Sources of variation	D.F.	Fungal population	Bacterial population
p^H	5	0.282	0.439
Moisture content	5	0.737**	0.669*
Total nitrogen	5	0.387	0.338
Phosphorus	5	-0.161	0.068
Cellulose	5	-0.444	-0.142
Hemicellulose	5	-0.553	-0.181
Lignin	5	0.617	0.921***
Total sugars	5	-0.726**	-0.408
Total amino acids	5	-0.688*	-0.384

*, **, ***, $P<0.1$, 0.05 and 0.01 respectively.

Without asterisk statisticaly insignificant at these levels.

Table 10(c). Correlation coefficients (r) for p^H, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial populations of Holarrhena antidysenterica litter.

Sources of variation	D.F.	Fungal population	Bacterial population
p^H	7	0.344	0.604*
Moisture content	7	0.631*	0.702**
Total nitrogen	7	0.417	0.404
Phosphorus	7	-0.218	-0.431
Cellulose	7	-0.049	0.026
Hemicellulose	7	-0.100	-0.034
Lignin	7	0.739**	0.699**
Total sugars	7	-0.225	-0.178
Total amino acids	7	-0.212	-0.171

*, **, ***, $P < 0.1$, 0.05 and 0.01 respectively.

Without asterisk statisticaly insignificant at these levels.

Table 10(d). Correlation coefficients (r) for p^H, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial populations of Vitex glabrata litter.

Sources of variation	D.F.	Fungal population	Bacterial population
p^H	7	0.508	0.491
Moisture content	7	0.577	0.892***
Total nitrogen	7	0.499	0.100
Phosphorus	7	0.055	-0.183
Cellulose	7	0.129	-0.033
Hemicellulose	7	-0.031	-0.182
Lignin	7	0.844***	0.751**
Total sugars	7	-0.114	-0.291
Total amino acids	7	-0.134	-0.317

*, **, ***, $P<0.1$, 0.05 and 0.01 respectively.

Without asterisk statisticaly insignificant at these levels.

unconducive environmental conditions like low moisture and temperature encountered during that period. Witkamp (1963), Edmonds (1979) and Rai and Srivastava (1982) also attributed the low microbial count to moisture stress and low temperature.

The fungal and bacterial peaks observed in May-June at lower altitude and June-July at higher altitude may be correlated with the improved moisture content of the litters due to high rainfall and progressive rate of decomposition which must have caused the mobilization of the nutrients from the litter required for their growth (Das, 1980).

The seasonal fluctuation in the fungal and bacterial populations may be due to the climatic changes during the investigation. An increase in the microflora during rainy and summer seasons and subsequent decrease in the population during winter months was observed on the decomposing litters. Similar observations were made by Witkamp (1963), Holm and Jensen (1972), Das (1980) and Deka and Mishra (1982).

The high microbial population at lower altitude than at higher altitude may be due to the dominance of temperature influence over moisture effects (Shanks, 1954 and Witkamp, 1963). At lower altitude the maximum fungal and bacterial population on Ageratum conizoides as compared to its woody counterparts was due to the presence of more soluble sugars and high nitrogen in the plant tissues whereas their complexity might have acted as a limiting factor in case of woody litters (Witkamp, 1963; Martyniuk and Myskow, 1976 and Taylor et al, 1989). At higher altitude too the similar reasons have resulted in more microflora in Alnus nepalensis litter than others.

The reduced bacterial population on pine litter may be due to its acidic nature. However, Goodfellow et al (1968) suggested that p^H was not the limiting factor for microbial growth and their activities. While Whitehead et al (1983)

and Gannuarsson et al (1988) have suggested that the bacterial activitiy and growth were more related to the concentration of the leachates rather than the inhabitable surface area. At higher altitude the reduced fungal population during excess moisture content of litters may be due to the lack of aeration (Mikola, 1954; Witkamp, 1966 and Das, 1980).

Deuteromycetes and ascomycetes which constituted the primary colonizing community on pine and other woody leaf litters suggested that they could utilize locked up nutrients in litters while the appearance of phycomycetous later on during succession could only utilize the simpler nutrients either as fungal products or soluble forms. Millar (1974) and Mehrotra and Aneja (1979) also emphasised the dominance of deuteromycetes in the beginning of the decomposition and correlated it to their capability of cellulose utilization.

The limited fungal species diversity at higher altitude may be accounted to low nutrient availability for the microbial growth. This can also be due to the mycotoxic phenolic compounds released during the decomposition of pine litter (Lockwood and Lingappa 1963; Patrick 1971 and Tiwari, 1980). However certain phenolic compounds like ferulic acid can be easily used by some fungal species (Black and Dix, 1976). The difference in fungal species composition during different stages of decomposition of litters may be due to the fact that a variety of organic substances were released which favoured the growth of certain groups of fungi while adversely affected the others (Garrett, 1951 and Alexander, 1961).

Certain fungal genera like Paecilomyces, Oidiodendron, Geotrichum were rarely isolated while Penicillium, and Trichoderma, were isolated with high frequencies through out the sampling period. The discontinuity of occurrence may be governed by the dynamic change in the microbial balance, nutrient sta-

tus and their release from the decomposing litters and antagonistic factors (Lockwood, 1964). Brandsberg (1969) also reported that the fungal species which were constantly isolated were the most widely distributed species.

The results of the present investigation reveal that the altitude has a profound effect on the microbial population. The distribution of the microbial population on the litters is not only governed by the environmental conditions like temperature, rainfall etc. but was also influenced by the chemical composition of the plant species. The enhanced microbial population in turn can be used as a tool to faster the rate of decomposition and ultimately quick return of nutrients to the site.

CHAPTER II

MICROBIAL DECOMPOSITION OF LITTER

INTRODUCTION

Decomposition of chemically complex organic material is a combination of three major processes - Catabolism, comminution and leaching. In forest ecosystem nutrients locked up in plant detritus are mineralized during decomposition of organic residues and become available again for plant growth (Charley and Richards, 1983). Decomposition of plant litters also contributes to the formation of soil organic matter, an important component determining the chemical and physical characteristics of soil (Swift et al, 1979). Rate of decomposition and mineralization of essential plant nutrients are important regulating factors of ecosystem and primary production.

The microbial population especially fungi and bacteria are responsible for the universal decay process releasing the locked up nutrients. Fungi are considered as an important primary colonizers of lignified materials simply because they can degrade lignin which usually remains unattacked by other microbes.

Rate of decomposition is controlled by various factors i.e. the nature of decomposer community, the resource quality and the physico - chemical environments. The soil on which the litter decomposes may also influence the process of decomposition by acting as a reservoir of micro-organisms, which can colonize the litter. The substrate quality, which is an indices of chemical composition, vary. Litter from given species grown on sites with different soil types differs not only in mineral constituents but also in the amount and type of organic substances such as lignin, cellulose, hemicellulose, soluble carbohydrates etc., (Witkamp, 1960).

The slow rate of decomposition can result in the accumulation of large amount of locked up nutrients on soil surface thus limiting the nutrient supply for primary producers (Florence, 1965 and Lamb, 1971). The rate of decomposition is also influenced by a number of climatic factors like moisture, temperature and nature of soil microflora and fauna (Meentemeyer, 1978).

There are several studies available pertaining to the rate of decomposition and release of nutrients (Berg and Staaf, 1980; Berg et al, 1982; Parker et al, 1984; Taylor et al, 1989 and Upadhyay et al, 1989). However, studies on litter decomposition in relation to microbes (Howard and Howard, 1974; Jensen, 1974 and Rai and Srivastava, 1982) and different successional stages (Das, 1980; Deka, 1981 and Deka and Mishra, 1982) are meagre. The importance of litter decomposition in maintaining soil fertility is well established. Therefore, it was thought pertinent to study its importance in nutrient deficient soils. The present study aims to evaluate the rate of microbial decomposition of litters and changes in the organic and inorganic constituents at higher and lower altitudes under different successional stages of forest.

MATERIALS AND METHODS

(1) Rate of litter decomposition :

Nylon litter bag technique (Bocock et al, 1960) was followed to estimate the rate of litter decomposition (details are given in Chapter-1).

On each sampling period six litter bags were collected for each litter type; three bags were recovered to assess the loss in dry weight. The litter bags were opened and carefully separated and washed on 200 µm mesh sieve to clean the adhering soil particles. The materials were dried in a hot air oven at 60°C until a constant dry weight was obtained. Final dry weight of the samples were taken and percentage weight loss was calculated on the basis of oven dry weight. The decay constant (K) was calculated on the basis of Olsons

(1963) decay model for decay with no production, using the following formula:

$\frac{X_t}{X_o} = e^{-kt}$, where x_o = Initial weight.

X_t = weight after time 't'

K = the annual exponential (base e) decay coefficient.

(ii) Determination of moisture content and p^H of the litter:

The remaining bags were used for the determination of moisture content and p^H of the litters (details given in Chapter I).

(iii) Estimation of Organic constituents of litter:

Cellulose, hemicellulose, lignin, total soluble sugars and total amino acids of litters were estimated by following the method of Peach and Tracey (1955).

(a) Cellulose and Hemicellulose:

0.5 g of ground material was treated with 25% of aqueous KOH (w/v). The mixture was centrifuged at 3000 rpm for 15 minutes. The decant obtained was used for detection of hemicellulose. The residue left was washed with distilled water till the traces of KOH were removed. It was then oven dried at 105°C for 24 hours and cooled at room temperature in a desicator and weighed. The amount thus obtained was total cellulose.

The decant obtained was neutralized with equal amount of glacial acetic acid and ethanol. The precipitate was filtered, washed, dried and weighed as above for determination of total hemicellulose. Three replicates were taken

in each case.

(b) Lignin:

For the estimation of lignin, 0.5 g of dried litter powder was taken in a test tube and treated with 20 ml of 72% H_2SO_4 and kept in deep freeze for 24 hours. It was then centrifuged and the residue was collected and washed thoroughly to remove the traces of H_2SO_4 present. It was then oven dried and weighed. The amount so obtained gave the total lignin content in the litter.

The cellulose, hemicellulose and lignin content were ~~estimated~~ expressed on the initial dry weight of the litters.

(c) Total sugars and Amino acids:

100 mg of powdered sample was taken in a test-tube and treated with 80% ethanol. Occasionally, when any colour developed, it was treated with a small pinch of activated charcoal and centrifuged at 6000 rpm. The solution was filtered through a Whatman No. 1 filter paper. The clear filtrate was boiled on a hot water bath to remove the traces of ethanol. To it distilled water was added to make the volume upto 5 ml.

To 3 ml of above solution 6 ml of freshly prepared anthrone reagent (0.2% in H_2SO_4) was added slowly by the side of the test-tube and shaken gently. The solution was then kept for 3 minutes in the boiling water bath and cooled at room temperature. The O.D. due to the green colour of the filtrate was determined in a spectrophotometer at 610 nm. Standard curve was obtained from transmittance of varying concentration of glucose solution treated exactly as the samples. From the standard curve the values of the total sugars were expressed as µg/100 mg dry weight of the samples.

To the rest of the 2 ml solution, 2.5 ml of citrate buffer (p^H-5.5) and 2 ml ninhydrin solution were added. The mixture was kept in boiling water bath for 30 minutes and then cooled at room temperature. A light purple colour developed in the solution. The O.D. was determined at 540 nm in the Hitachi spectrophotometer. The total amino acids were calculated from the standard curve obtained from transmittance of different concentration of leucine solution treated exactly as samples. It was expressed as µg/100 mg dry weight of the samples.

(iv) Determination of inorganic constituents of the litter:

(a) Nitrogen:

Total nitrogen in the leaf litters was estimated by Micro-Kjeldahl method (Allen, 1974). 100 mg of powdered litter (sieved through 0.2 mm) was taken in a micro-kjeldahl flask with 2g of K_2SO_4 : HgO (20:1) mixture. To it 3ml of concentrated H_2SO_4 was added slowly down the neck while the flask was rotated. The flask was heated on a digestion rack. After the digest became colourless the heating was continued for another 15 minutes. After digestion the flask was allowed to cool down. The digested material was diluted with distilled water and filtered through Whatman No.1 filter paper. The blank digestion was prepared only with the mixture. Through digestion all the organic nitrogen converts into ammonia which was measured by Indo-phenol blue method. The nitrogen was calculated by using the following formula;

$$\text{Nitrogen (\%)} = \frac{\text{C(mg) X solution volume (ml)}}{\text{10 X aliquot (ml) X sample weight (g).}}$$

(b) Phosphorus:

Oven dried litter was ground and seived through a seive (0.2 mm) and used for phosphorus determination. 0.3g ground material was digested in a triacid mixture (HNO_3, H_2SO_4 and 60% $HClO_4$ in a ratio of 10:1:4) for the analysis of phosphorus. After digestion the volume was made to 50 ml and filtered through Whatman filter paper No. 42. Phosphorus in the digested sample was estimated following the molybdenum blue method of Jackson (1967).

$$\text{Phosphorus (\%)} = \frac{\text{C(mg) X solution volume (ml)}}{\text{10 X aliquot (ml) X sample weight (g)}}$$

RESULTS

1) Rate of Litter Decomposition:

The rate of decomposition of leaf litters was faster at lower altitude as compared to those at higher altitude.

At lower altitude the herbaceous litter decomposed very fast (K = 2.930) compared to other woody litters. In young forest stand the litters of Ageratum conizoides and Mallotus philippinensis (K = 1.587) decomposed much earlier than Holarrhena antidysenterica (K = 1.279)and Vitex glabrata (K = 1.139) litter of old stand (Fig.28).

At higher altitude the decomposition of Alnus nepalensis (K = 0.810) litter decomposed much faster than Myrica esculenta (K = 0.526) and Pinus kesiya (K = 0.372 & 0.398). Among the two pine litters kept in different forest stands, the litter of young forest stand decomposed faster than (Fig.29) its counterpart of old forest stand (Table 11). In case of pine litters the weight loss

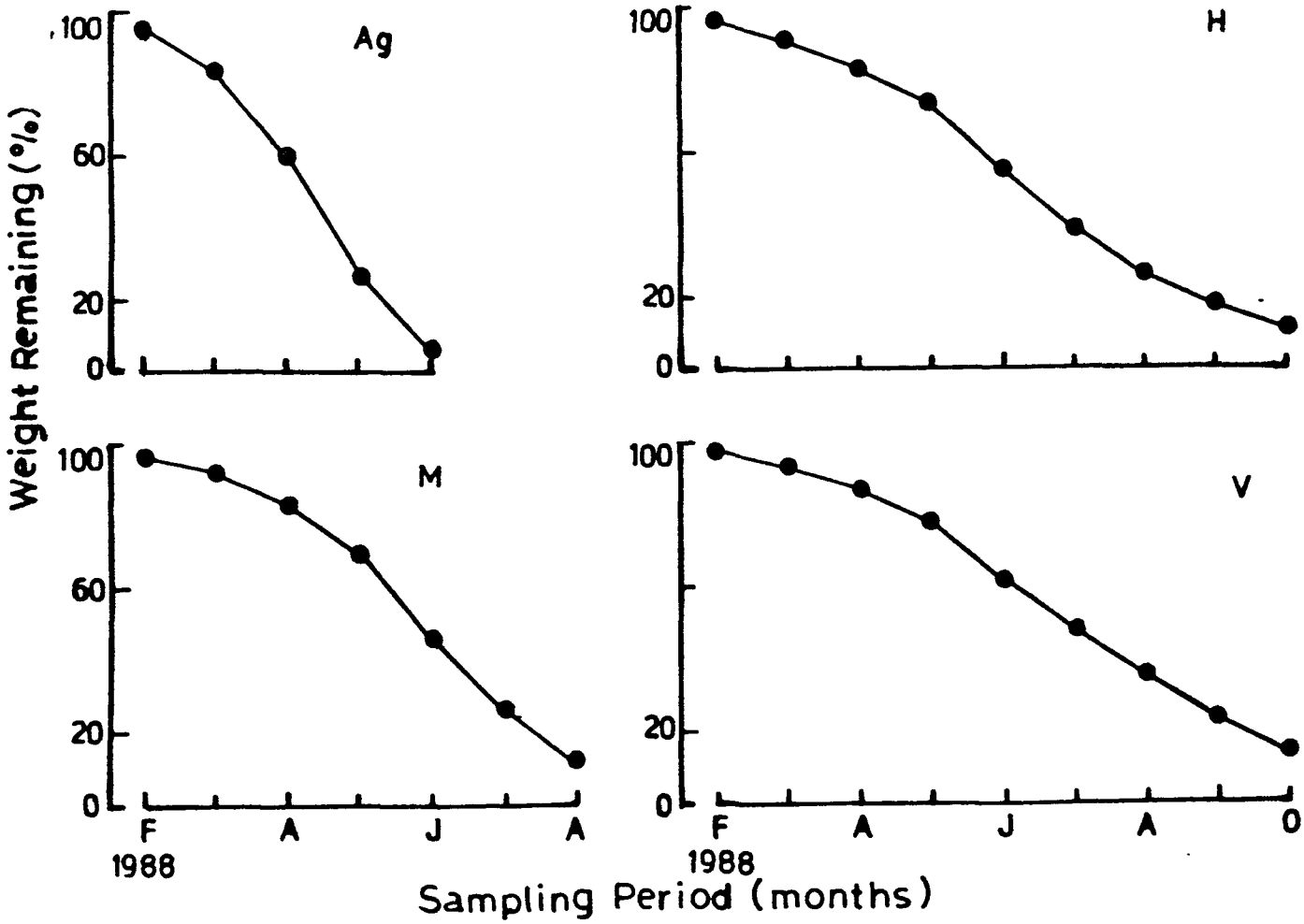


Fig. 28. Percentage of original dry weight of different leaf litters remaining after different time intervals at lower altitude. Ag-Ageratum conizoides M- Mallotus philippinensis, H- Holarrhena antidysenterica and V-Vitex glabrata.

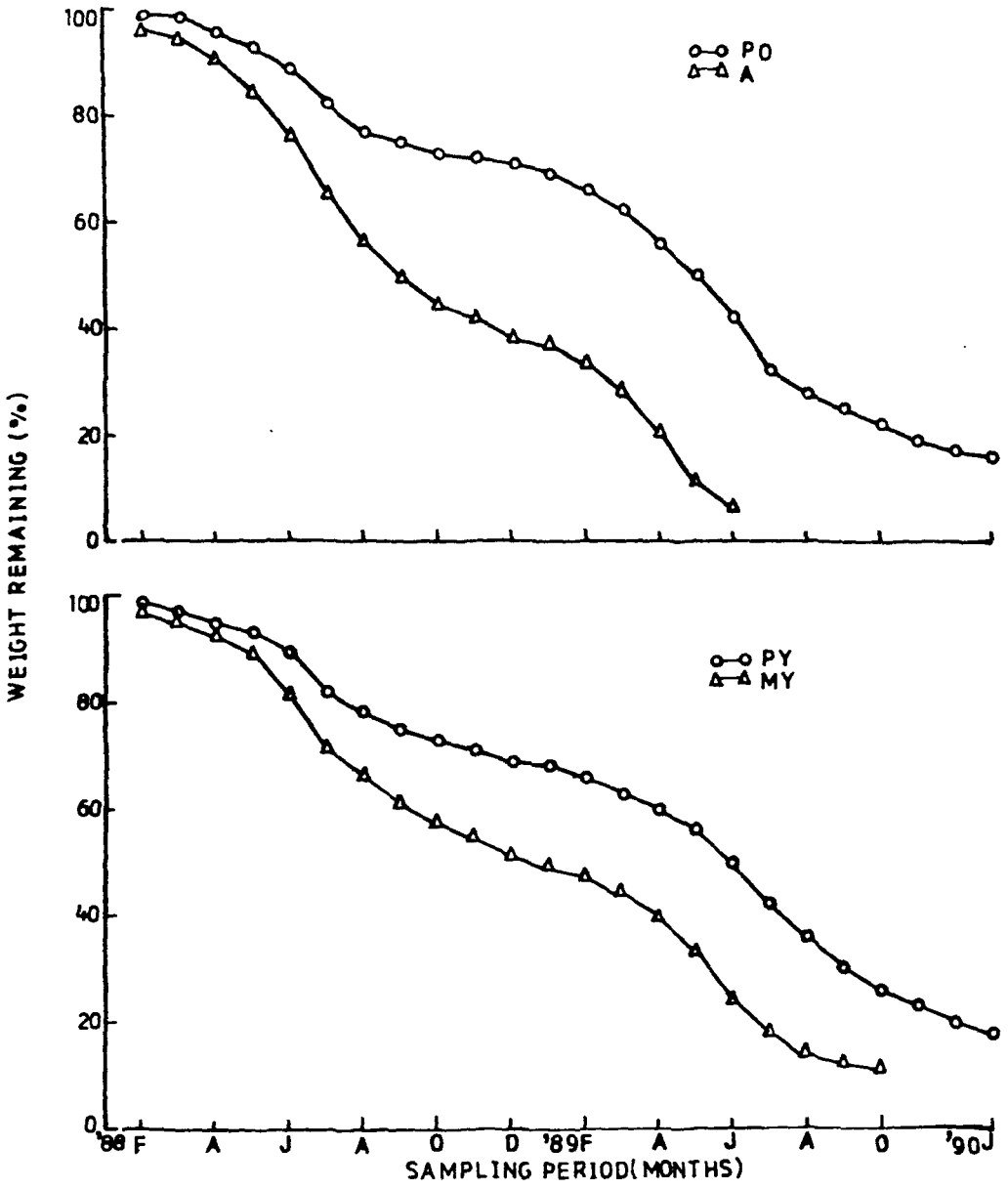


Fig. 29. Percentage of original dry weight of different leaf litters remaining after different time intervals at higher altitude. PO - Pinus kesiya (old), A - Alnus nepalensis, PY - Pinus kesiya (young) and MY - Myrica esculenta.

Table 11. Decay constant (K), half life and 95% life values for decomposition of different plant litters under field conditions.

Plant species	K (Per year)	Half life (year)	95% life (year)
Ageratum conizoides	2.93	0.237	1.03
Mallotus philippinensis	1.58	0.439	1.90
Holarrhena antidysenterica	1.28	0.541	2.34
Vitex glabrata	1.14	0.608	2.63
Pinus kesiya (old)	0.398	1.74	7.54
Alnus nepalensis	0.81	0.856	3.70
Pinus kesiya (young)	0.372	1.86	8.06
Myrica esculenta	0.526	1.32	5.70

was slow in the first year which improved in the second year. A marked seasonal variation was observed in all the leaf litters. Maximum weight loss was observed during rainy months while a repression in the litter decomposition rate was observed during winter months. At lower altitude, the relative weight loss of all the leaf litters correlated significantly with fungal population. The correlation with bacterial population, however, was significant in all the leaf litters except M.phillippinensis. At higher altitude both fungal and bacterial population of all the leaf litters correlated significantly with their respective relative weight loss. The bacterial and fungal population, however did not correlate significantly with absolute weight loss (Table 12 and 13).

p^H and moisture content of the litter:

The leaf litter at lower altitude were less acidic compared to those at higher altitude.

At lower altitude the herbaceous litter was highly basic than woody leaf litters. The p^H ranged from 5.95 to 8.6. Litters became more acidic towards the end of the decomposition (Fig.4).

At higher altitude pine litters were more acidic in comparison to broad leaf litters of A.nepalensis and M.esculenta. The p^H ranged from 4.45 to 6.45 (Fig.5). In the beginning the litters were acidic which decreased with the onset of rain. During winter period the acidity of litter again increased. Here too, the litters were more acidic towards the end of decomposition. The percentage of moisture content ranged from 17.8 to 66.0 at lower altitude. Among different leaf litters the moisture content was more in H.antidysenterica than V.glabrata, M.philippinensis & A.conizoides (Fig.4). The moisture content was minimum during dry winter months which increased with the onset of rain. The absolute weight loss was correlated significantly with moisture

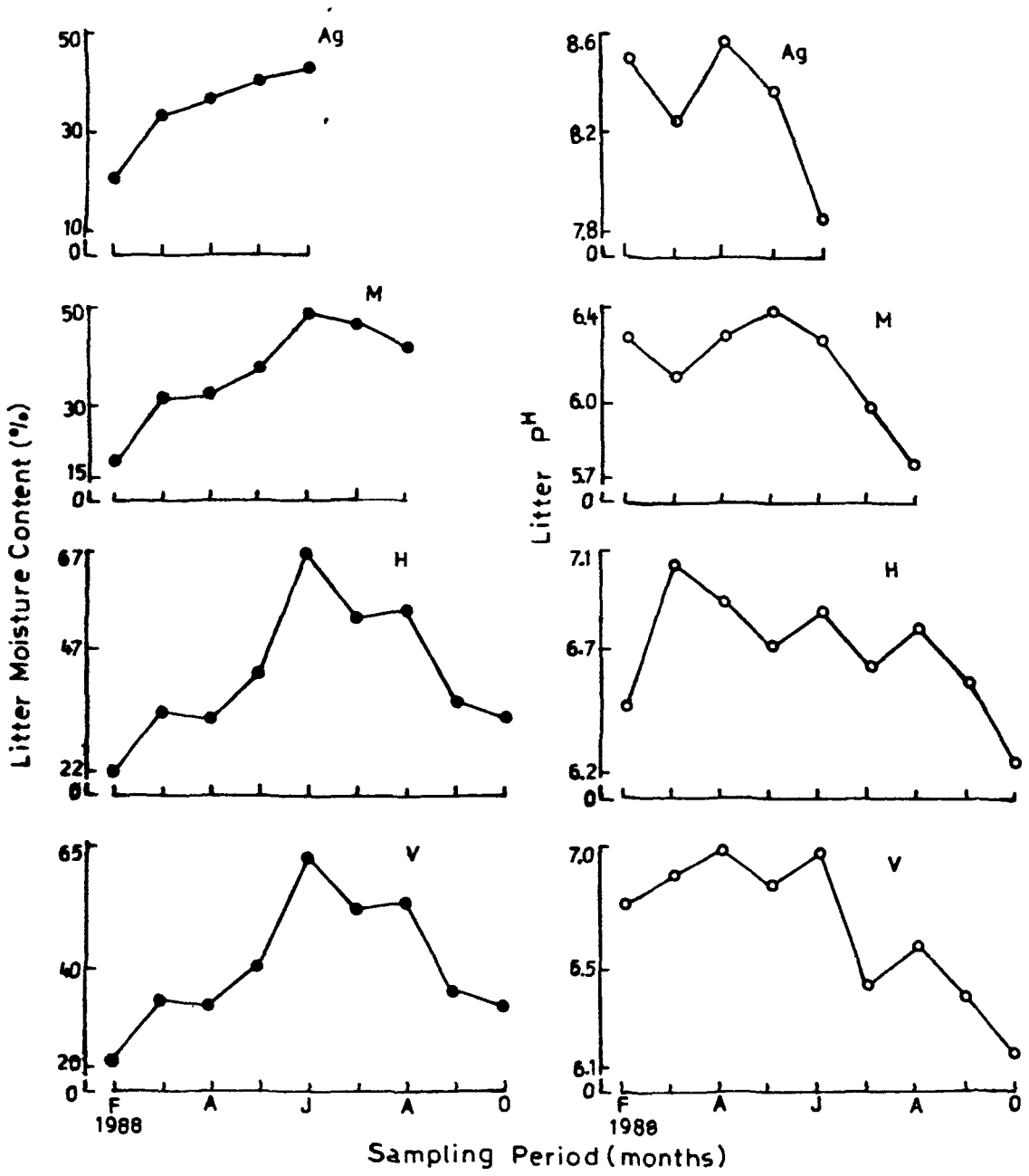


Fig. 4. Monthly variation in moisture content and p^H of different leaf litters at lower altitude. Ag - *Ageratum conizoides*, M- *Mallotus philippinensis*, H- *Holarrhena antidysenterica* and V - *Vitex glabrata*.

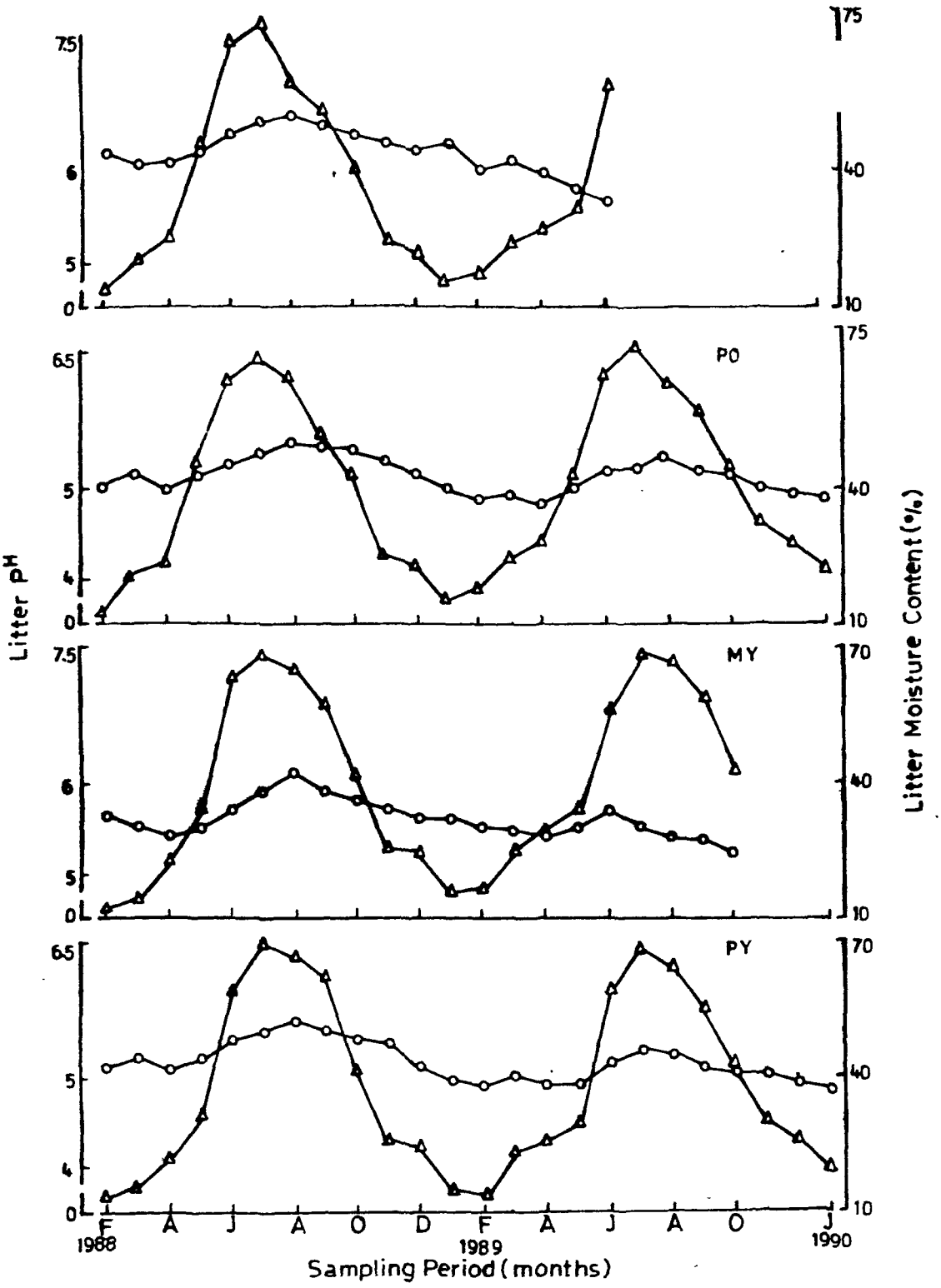


Fig. 5. Monthly variation in moisture content (Δ-Δ) and p^H (O-O) of different leaf litters at higher altitudes. PO - Pinus kesiya (old), A- Alnus nepalensis, PY- Pinus kesiya (young) and MY - Myrica esculenta.

Table: 12(a). Correlation coefficients (r) for cellulose, hemicellulose, lignin, total sugars, total amino acids, total nitrogen, phosphorus, fungal population, bacterial population, moisture content and absolute weight loss in young forest stand at lower altitude.

Sources of variation	Absolute weight loss			
	D.F.	Ageratum conizoides	D.F.	Mallotus phillipinensis
Cellulose	3	-0.996***	5	-0.998***
Hemicellulose	3	-0.989***	5	-0.970***
Lignin	3	-0.467	5	-0.082
Total Sugars	3	-0.926**	5	-0.846**
Total amino acids	3	-0.962***	5	-0.889***
Total nitrogen	3	-0.862*	5	-0.811**
Phosphorus	3	-0.996***	5	-0.847**
Fungal population	3	0.963***	5	0.380
Bacterial population	3	0.413	5	0.101
Moisture content	3	0.883**	5	0.781**

*,**,***, P< 0.1, 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 12b. Correlation coefficients (r) for cellulose, hemicellulose, lignin, total sugars, total amino acids, total nitrogen, phosphorus, fungal population, bacterial population and absolute weight loss in old forest stand at lower altitude.

Sources of variation	Absolute weight loss			
	D.F.	*Holarrhena antidysenterica*	D.F.	*Vitex glabrata*
Cellulose	7	-0.996***	7	-0.997***
Hemicellulose	7	-0.992***	7	-0.797***
Lignin	7	-0.621*	7	-0.521
Total Sugars	7	-0.939***	7	-0.930***
Total amino acids	7	-0.937***	7	-0.928***
Total nitrogen	7	-0.832***	7	-0.690**
Phosphorus	7	-0.880***	7	-0.160
Fungal population	7	-0.0029	7	0.119
Bacterial population	7	0.078	7	-0.055
Moisture content	7	0.414	7	0.397

*,**,***, $P < 0.1, 0.05$ and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

content of only A.conizoides and M.philippinensis litters (Table 12a and 12b).

However, the moisture content at higher altitude was high as compared to lower altitude. It ranged from 13.9 to 72% (Fig. 5). The moisture content was high for A.nepalensis followed by M.esculenta, P.kesiya (old) and P.kesiya (young). The seasonal variation was similar to that at lower altitude. The correlation of absolute weight loss with moisture content was not significant (Table 13a and 13b). highest in M esculenta (see table 13a) !

Organic constituents:

In general the organic constituents were maximum in the initial stage of decomposition and minimum at the final stage. The litters at lower altitude had more of cellulose and hemicellulose than lignin. The amount of cellulose and hemicellulose was observed to be more in A.conizoides than M.philippinensis, H.antidysenterica and V.glabrata. The lignin content was however, more in V.glabrata. The rate of decomposition of cellulose and hemicellulose was similar to that of weight loss of litter. The lignin content increased in the beginning. The degradation of lignin started towards later portion of litter decomposition. The decomposition of lignin was very slow as compared to that of cellulose and hemicellulose.

Total sugars and amino acids were more in A.conizoides and least in V.glabrata. Maximum loss of total sugars and amino acids was recorded in rainy months (Fig. 6).

At higher altitude, the lignin content was highest in pine litters followed by M.esculenta and A.nepalensis, which had more of cellulose and hemicellulose. Here too, the decomposition of cellulose and hemicellulose followed a pattern similar to that observed at lower altitude. However, the lignin content increased in the beginning and subsequently decreased towards the end

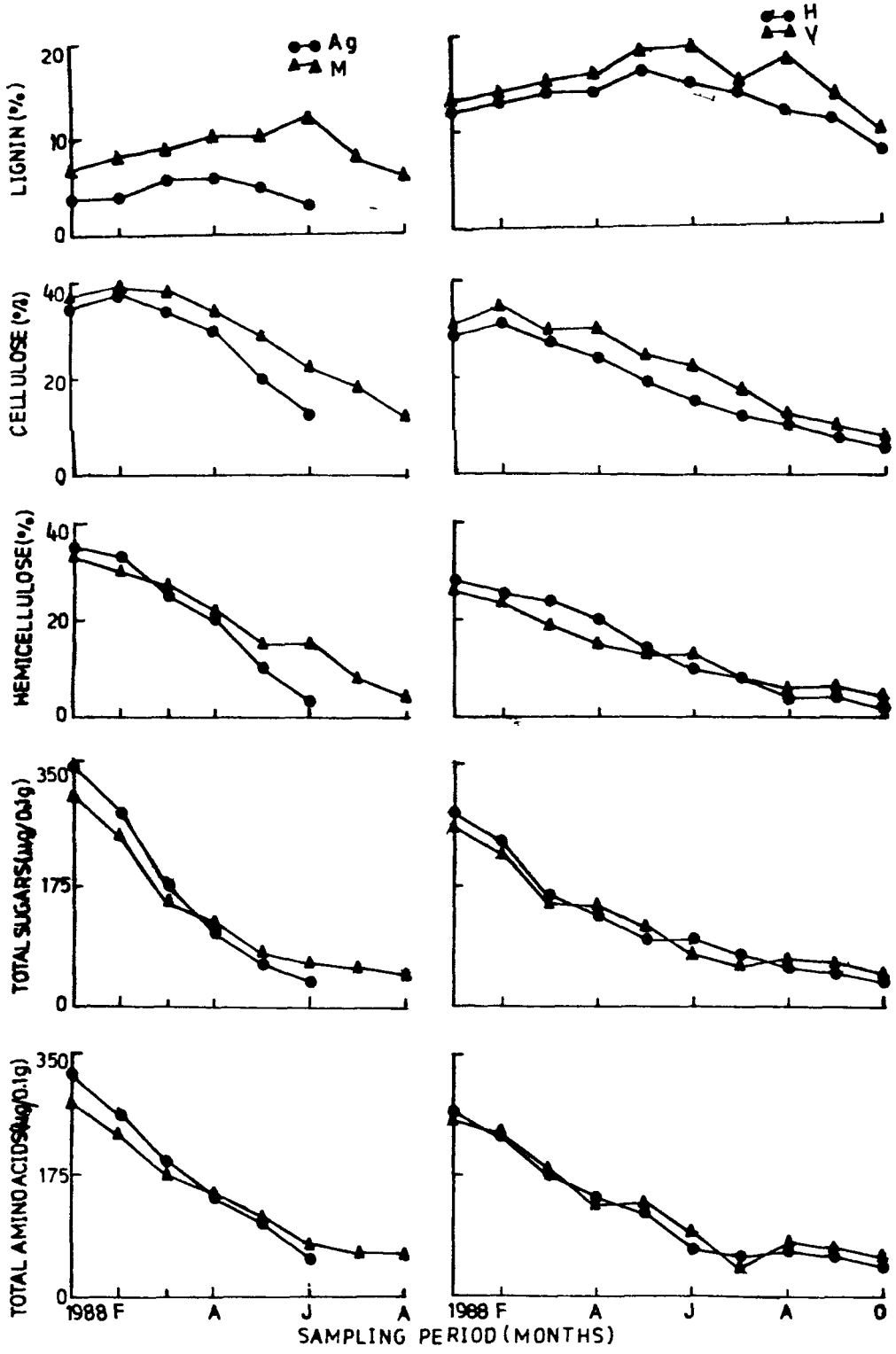


Fig. 6. Monthly variation in lignin, cellulose, hemicellulose, total sugars and amino acids content of different leaf litters at lower altitude. Ag- Ageratum conizoides, M- Mallotus philippinensis, H- Holarrhena antidysenterica and V- Vitex glabrata.

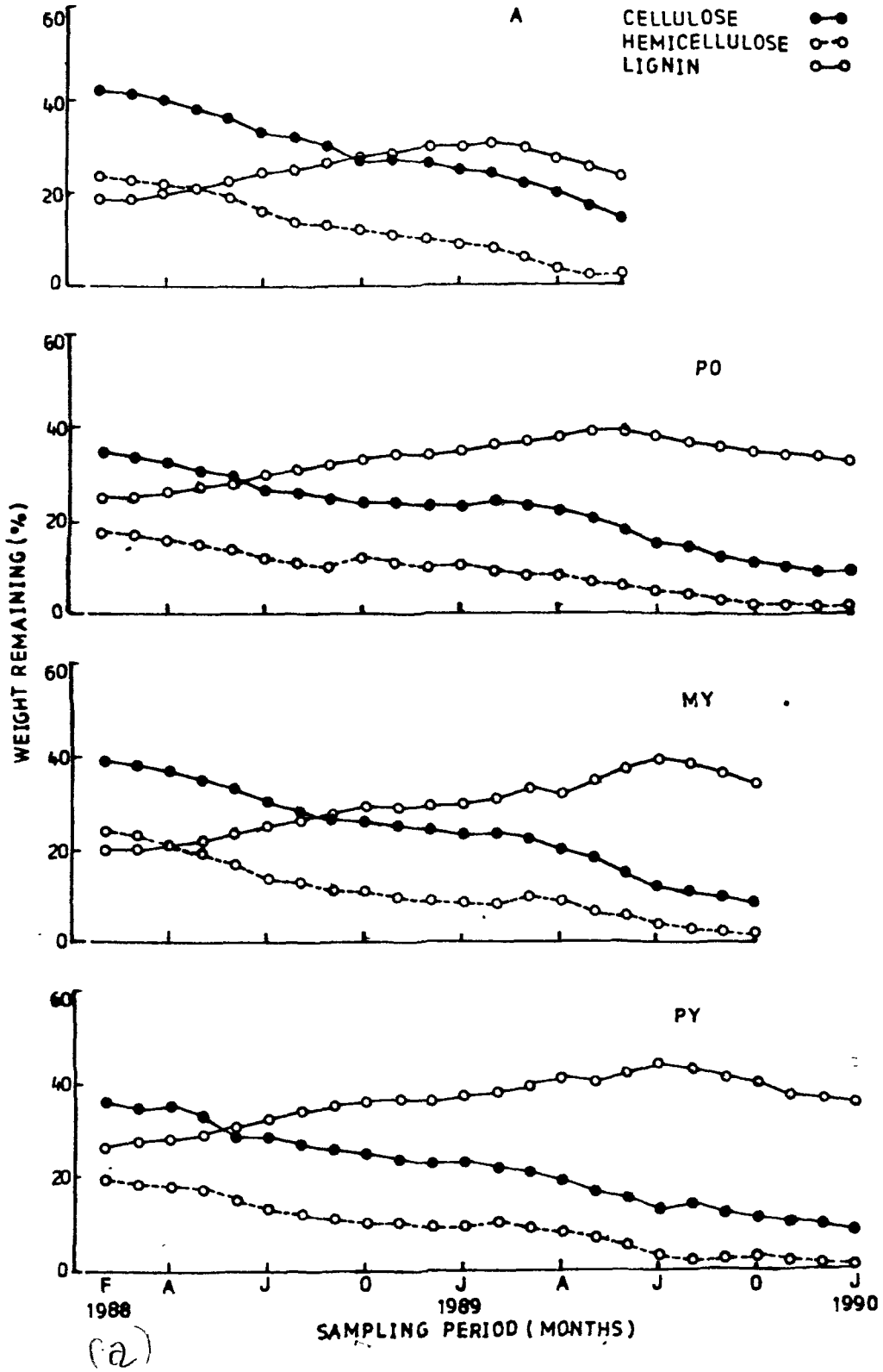
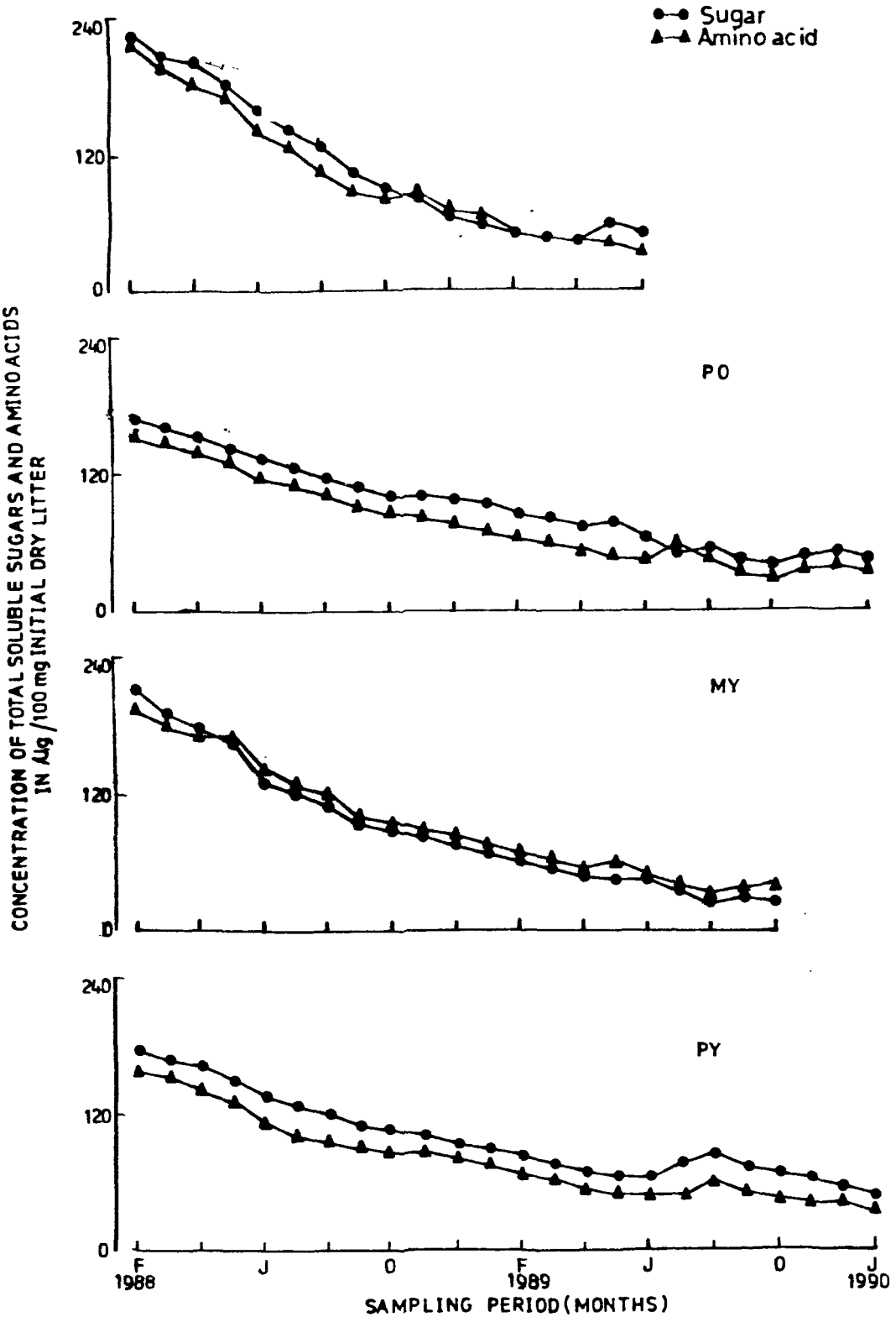


Fig. 7. Monthly variation in cellulose, hemicellulose and lignin content of different leaf litters at higher altitude. PO - Pinus kesiya (old), A- Alnus nepalensis, PY- Pinus kesiya (young) and MY - Myrica esculenta.



(b)

Fig. 7. Monthly variation in total sugars and amino acids of different leaf litters at higher altitudes. PO - Pinus kesiya (old), A- Alnus nepalensis, PY - Pinus kesiya (young) and MY- Myrica esculenta.

(Fig. 7a).

A.nepalensis litter contained more amount of soluble components than Myrica and Pine litters. The maximum change in total sugars and amino acids was observed during rainy months (Fig 7b)

It was observed that the rate of degradation of total sugars and amino acids was highest followed by hemicellulose, cellulose and lignin. At both the altitudes, the absolute weight loss of different litters showed a significant negative correlation with the weight remaining of different organic constituents like cellulose, hemicellulose, sugars and amino acids. However, lignin content was correlated positively with absolute weight loss only at higher altitude (Table 13a and 13b).

Inorganic constituents:

Nitrogen:

At lower altitude, A.conizoides litter had the maximum initial nitrogen followed by M.philippinensis, H.antidysenterica and V.glabrata. The initial nitrogen content ranged from 0.65 to 1.4%. The nitrogen content decreased in the beginning and again increased followed by a subsequent decrease till the end of the decomposition (Fig. 8).

At higher altitude the initial nitrogen content ranged from 0.56 to 1.9%. The maximum initial nitrogen content was recorded in A. nepalensis followed by M.esculenta, pine (old and young). The nitrogen content increased in the beginning and decreased afterwards. It remained immobilized for longer period at higher altitude (Fig.9).

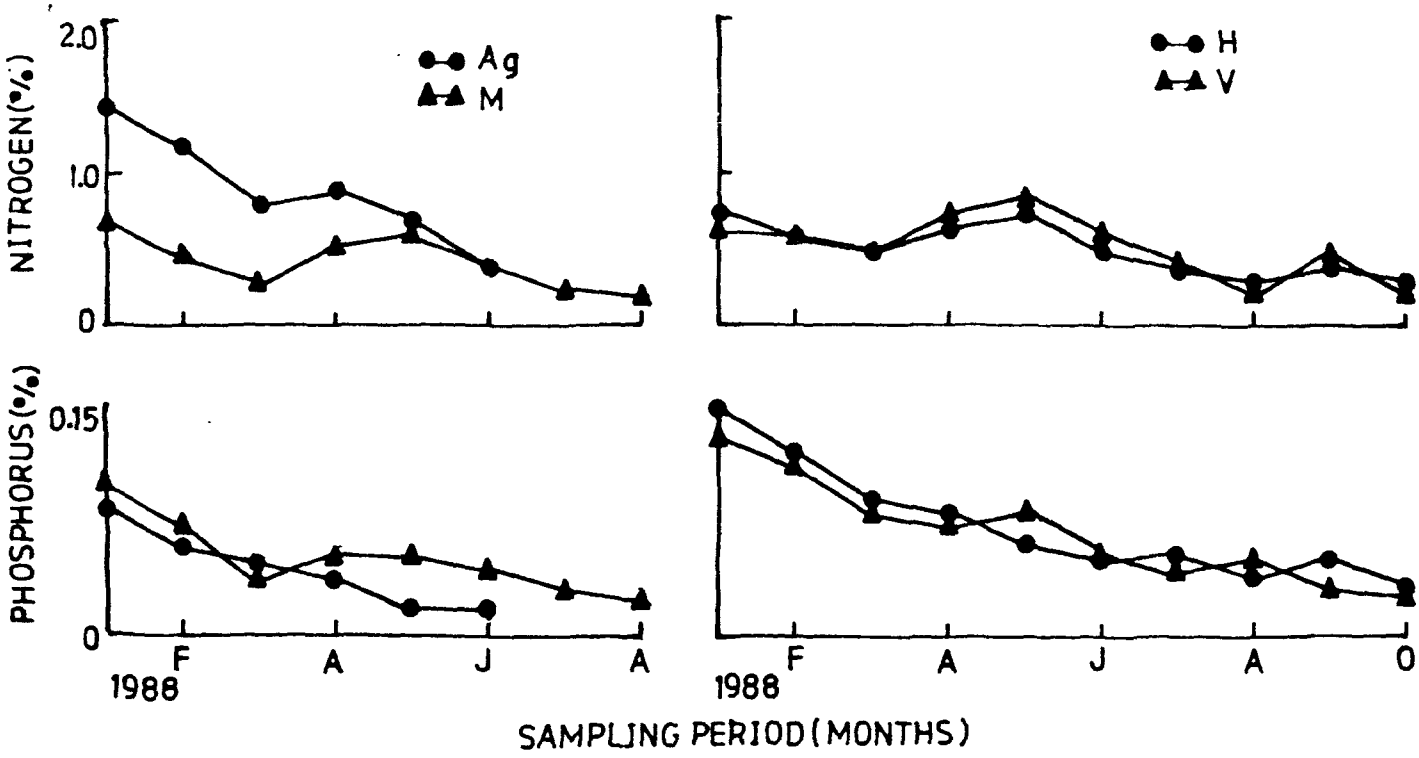


Fig. 8. Monthly variation in total nitrogen and phosphorus content of different leaf litters at lower altitude. Ag - Ageratum conizoides, M - Mallotus philippinensis, H- Holarrhena antidysenterica and V - Vitex glabrata.

Phosphorus:

At lower altitude the initial phosphorus content ranged from 0.08 to 0.15%. It was maximum in H.antidysenterica and minimum in A.conizoides whereas M.philippinensis and V.glabrata had intermediate concentrations. Phosphorus was not retained in the litter but released along with the decomposition of litter (Fig. 8).

The initial phosphorus content ranged from 0.046 to 0.073% at higher altitude. The maximum was observed in A.nepalensis followed by M.esculenta, P.kesiya (old) and P.kesiya (young). Unlike lower altitude, here the phosphorus was initially retained in the litters for few months and then released. (Fig 9) The absolute weight loss of different leaf litters was correlated negatively with the nitrogen and phosphorus content (Table13a and 13b).

DISCUSSION

The rate of decomposition was significantly faster for A.conizoides than those of M.philippinensis, H.antidysenterica and V.glabrata. This can be attributed to the high microbial population encountered in A.conizoides compared to other leaf litters. Berg et al (1982) and Taylor et al (1989) based on their results considered nitrogen as a limiting factor for microbial growth and activity. Edmonds (1980), McClaugherty et al (1985) and Upadhyay and Singh (1989) also concluded that litter poor in nitrogen content decomposed slowly than those with high N content. The high N content in A.conizoides must have been conducive for optimum microbial growth which ultimately affected the rate of decomposition.

At higher altitude the slow rate of decomposition of Pine litters can be assigned to low base status, high lignin content and impervious nature of

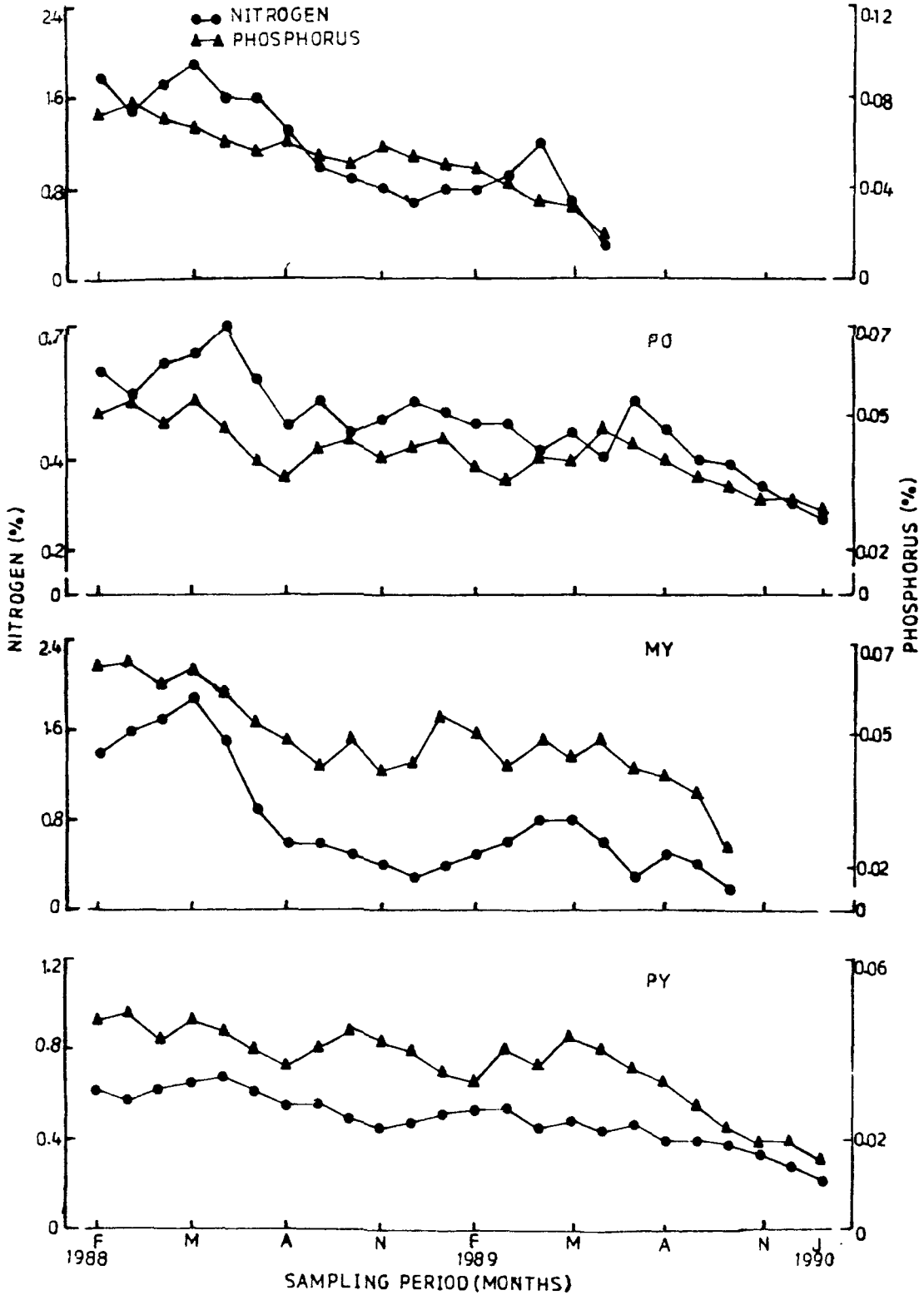


Fig. 9. Monthly variation in total nitrogen and phosphorus content of different leaf litters at higher altitude. PO- Pinus kesiya (old), A- Alnus nepalensis, PY - Pinus kesiya (young) and MY- Myrica esculenta.

Table: 13 (a). Correlation coefficients (r) for cellulose, hemicellulose, lignin, total sugars, total amino acids, total nitrogen, phosphorus, fungal population, bacterial population and absolute weight loss in young forest stand at higher altitude.

Sources of variation	Absolute weight loss			
	D.F.	*Pinus kesiya*	D.F.	*Myrica esculenta*
Cellulose	22	-0.993***	17	-0.997***
Hemicellulose	22	-0.983***	17	-0.999***
Lignin	22	0.940***	17	0.982***
Total sugars	22	-0.899***	17	-0.970***
Total amino acids	22	-0.918***	17	-0.979***
Total nitrogen	22	-0.876***	17	-0.809***
Phosphorus	22	-0.861***	17	-0.879***
Fungal population	22	-0.042	17	0.342
Bacterial population	22	0.205	17	0.096
Moisture content	22	0.138	17	0.406*

*,**,***, P< 0.1, 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table: 13(b). Correlation coefficients (r) for cellulose, hemicellulose, lignin, total sugars, total amino acids, total nitrogen, phosphorus, fungal population, bacterial population and absolute weight loss in old forest stand at higher altitude.

Sources of variation	Absolute weight loss			
	D.F.	Pinus kesiya	D.F.	Alnus nepalensis
Cellulose	22	-0.989***	15	-0.994***
Hemicellulose	22	-0.942***	15	-0.996***
Lignin	22	0.960***	15	0.828***
Total sugars	22	-0.973***	15	-0.965***
Total amino acids	22	-0.924***	15	-0.981***
Total nitrogen	22	-0.855***	15	-0.887***
Phosphorus	22	-0.792***	15	-0.918***
Fungal population	22	0.406*	15	-0.118
Bacterial population	22	0.341	15	0.035
Moisture content	22	0.164	15	-0.024

*,**,***, $P<0.1, 0.05$ and 0.05 respectively.

Without asterisk statistically insignificant at these levels.

cuticle (Millar, 1974 and Das, 1980). A.nepalensis decomposed faster than M.esculenta and P.kesiya. This may be due to its broader leaf nature with high initial nitrogen content compared to M.esculenta and Pine with smaller leaf size and comparatively less initial N content (Laishram and Yadava, 1988). The difference in decomposition rate can also be due to physical nature of plant material (Bhatt et al, 1985), difference in P^H, moisture content and substrate quality, which ultimately govern the distribution of microflora (Swift et al, 1979 and Howard and Howard, 1980)

The fast rate of decomposition during May - July may be as a result of high temperature and conducive water potentials (Stott et al, 1986 and Tiwari, 1988) which must have favoured the luxurious growth of microbes (Nagy and Macauley, 1982; Moore, 1986 and Tiwari, 1988).

The chemical nature of leaf litters changed considerably as they were decomposed. The amount of lignin increased in the beginning while that of hemicellulose and cellulose showed a decreasing trend. The initial absolute increase in lignin content can be attributed to the complexing of soluble polyphenols with proteins (Berg and Theander, 1984 and Schlesinger, 1985). Berg and Theander (1984) also discussed the formation of phenolic and other aromatic compounds which could be analysed as lignin from the degradation of carbohydrates. The initial decrease in cellulose and hemicellulose concentration may be due to their utilization by the microbes (Harper and Lynch, 1981).

The high rate of degradation of total sugars and amino acids during early phase of degradation may be due to their simpler chemical nature which must have ~~ameliorated~~ facilitated their utilization by the microbes. This can also be attributed to their highly soluble nature which must have enhanced their leaching along with rain water.

The decrease in N content at lower altitude may be attributed to the leaching of labile nutrients, thin cuticle and less sclerenchymatous tissues

(Upadhyay and Singh, 1989). The increase in the nitrogen content in the beginning at higher altitude may be due to a demand for nitrogen by heterotrophs in which the N gets immobilized during decomposition (Lousier and Parkinson, 1978).

The phosphorus content of litters at higher altitude was immobilized for slightly longer period while at lower altitude it was released instantly. This may be due to lower initial P concentration and higher C:P (Upadhyay and Singh, 1989). Staaf (1980) also found an increase in P concentration. They ascribed it to preferential retension of P and N while other nutrients are being rapidly lost during decomposition.

From the above results it can be inferred that the rate of decomposition is highly influenced by both abiotic and biotic factors. Besides, the chemical composition of litters, the successional stages of forest and the altitudinal difference also affected the rate of decomposition.

CHAPTER III

MICROBIAL ENZYMES RELATED TO THE DEGRADATION OF LITTER

INTRODUCTION

The plant litter decomposition on forest floor consists of two main stages, the first stage involves leaching and microbial utilization of labile compounds while the second stage brings about degradation of recalcitrant compounds mainly lignocellulose (Sinsabaugh and Linkins, 1987). The rate of plant litter decomposition is regulated by various enzymes which release readily assimilable substances from less available biopolymer (Spalding, 1980).

Cellulose and hemicellulose are the two major recalcitrant products added to soil through plant remains. The decomposition of cellulose, hemicellulose and other oligosaccharides can be brought about by different agents but the most important ones are the enzymes required for initial chemical breakdown (Ross and Speir, 1979). Cellulose, a homopolymer of glucose, is a major structural component of litter and therefore, is a vital energy source for the microbes (Sinsabaugh, et al 1981). Hydrolysis of cellulose into glucose is achieved by an enzyme complex cellulase. The cellulases include several functional groups which cleave the β-1,4 glycosyl bonds of cellulose at different points. These enzymes are primarily fungal in origin (Miele and Linkins, 1978).

Sucrose is the most ubiquitous and abundantly occurring disaccharide in the plant tissues. It is important to understand the mechanism of sucrose breakdown because it is the major soluble storage carbohydrate and major form from which carbon is translocated (Edelman, 1971). Sucrose is solubilized by invertase which can be either acidic or alkaline.

Starch is another commonly associated compound within most of the green plants, in practically every type of tissue i.e. leaves, stem, roots, seeds

etc. The amount of starch increases during active photosynthesis and decreases as it is enzymatically converted into sugars for translocation. Starch is hydrolysed by amylase which cleaves the β (1-4) bonds of starch.

The enzymes which mediates the hydrolysis of insoluble litter constituents (cellulases) are generally soluble while those mediating hydrolysis of more soluble constituents (amylase and invertase) are mostly insoluble. Due to the ubiquity of cellulose, cellulases are one of the most extensively studied enzyme system in plant litter (Eriksson and Wood, 1985 and Sinsabaugh and Linkins, 1989). Inspite of the important role played by invertase and amylase in hydrolysis of sugar and starch respectively, these enzymes are still not properly studied in plant litters. There are very few studies involving amylase and invertase in litter degradation (Spalding, 1980 and Ross, 1981).

The extra-cellular cellulase, amylase and invertase play an important role in microbial degradation of different carbohydrates in plant litter. Thus, making the locked up nutrients available for plant growth, they help to a great extent in recycling of different nutrients in different ecosystems. Therefore, the present study was taken up in order to correlate the efficiency of these enzymes in decomposition of different litters at higher and lower elevations under two successional stages of forest.

MATERIALS AND METHODS

The study was conducted at two altitudes (1500m and 100m MSL). At each altitude two forest stands of different secondary successional stages were selected. In each of the forest stands, two (dominant and codominant) plant species were considered for the present investigation. At lower altitude, leaf litters of Ageratum conizoides and Mallotus philippinensis were selected from young forest stand while, Holarrhena antidysenterica and Vitex glabrata

litters were selected in old forest stand. At higher altitude Pinus kesiya and Alnus nepalensis litters were taken in old forest stand and P.kesiya and Myrica esculenta litters in young forest stand. A known amount (10g) of each litter was weighed and kept in the nylon bags separately (mesh size - 1 mm, bag size -20 X 20 cm) (Bocock et al, 1960) on the forest floor of respective forest stands. Three bags of each leaf litter were collected at monthly intervals from each forest stand. The litter bags were brought to the laboratory in ice box for the estimation of cellulase, amylase and invertase enzymes to understand the microbial degradation of cellulose, starch and sucrose. Specific assays for the microbial metabolism were carried out as follows.

(i) Extraction of enzymes:

For extraction and assaying of these enzymes, Spalding's method (1977) was followed. The litter bags were opened and washed in 90 mm sieve with ice cold distilled water to remove the adhered soil particles. Extra water from the litter was absorbed by the filter paper. 10g of each litter was transferred separately into the waring blender and ground with 100 ml of buffer for 1 minute. For litters from higher altitude acetate buffer (p^H-5.5) was used while,for those from lower altitude phosphate buffer (p^H-6.5) was used. Homogenate was centrifuged at 9400g at 2°C for 20 minutes in a Sorvall RC 5 refrigerated centrifuge. The supernatents were filtered through Whatman filter paper (No.1). The extracts were stored at 2°C until assaying was completed.

(ii) Enzyme assay:

For enzyme assay, 1 ml of substrate solution was taken in a test tube along with 2 ml of enzyme extract. Substrates for cellulase -3% carboxymethyl

cellulose, Sodium salt (Sigma chemicals, USA), amylase-6% soluble starch (sigma) and invertase -6%, sucrose (Merck) were used. These substrates were prepared in the same buffer which was used for grinding the material. The test tubes with enzyme extract and substrate were incubated at 37 ± 1°C for 2 hours. After incubation, 3 ml of dinitrosalicylic acid was added to each tube and kept in boiling water bath for 5 minutes. To each tube 1 ml of sodium potassium tartarate (40%) was added. They were cooled at room temperature and the optical density of the solution was taken at 575 nm in a Hitachi 220 spectrophotometer. Microbial enzyme activities were expressed in terms of reducing sugars/g oven dried litter/hour.

RESULTS

(i) Cellulase activity:

At lower altitude the cellulase activity was more in Mallotus philippinensis followed by Holarrhena antidysenterica, Vitex glabrata and Ageratum conizoides. In all the leaf litters, the cellulase activity was less in the beginning which increased as the decomposition progressed. The cellulase enzyme showed a marked seasonal variation in all the litters. Maximum enzyme was assayed in M.philippinensis. The peak of cellulase was observed in May-June, thereafter it declined till the last sampling (Fig. 10). The cellulase enzyme in A.conizoides and V.glabrata was correlated significantly ($P<0.01$) with their fungal and bacterial population . Where as, a significant correlation was obtained only for fungal population of M.philippinensis (r=0.969) and H.antidysenterica (r=0.833). Cellulase activity in V.glabrata and H.antidysenterica litters also showed a significant correlation with moisture content. The percentage remaining cellulose of all the litters, however, showed a negative

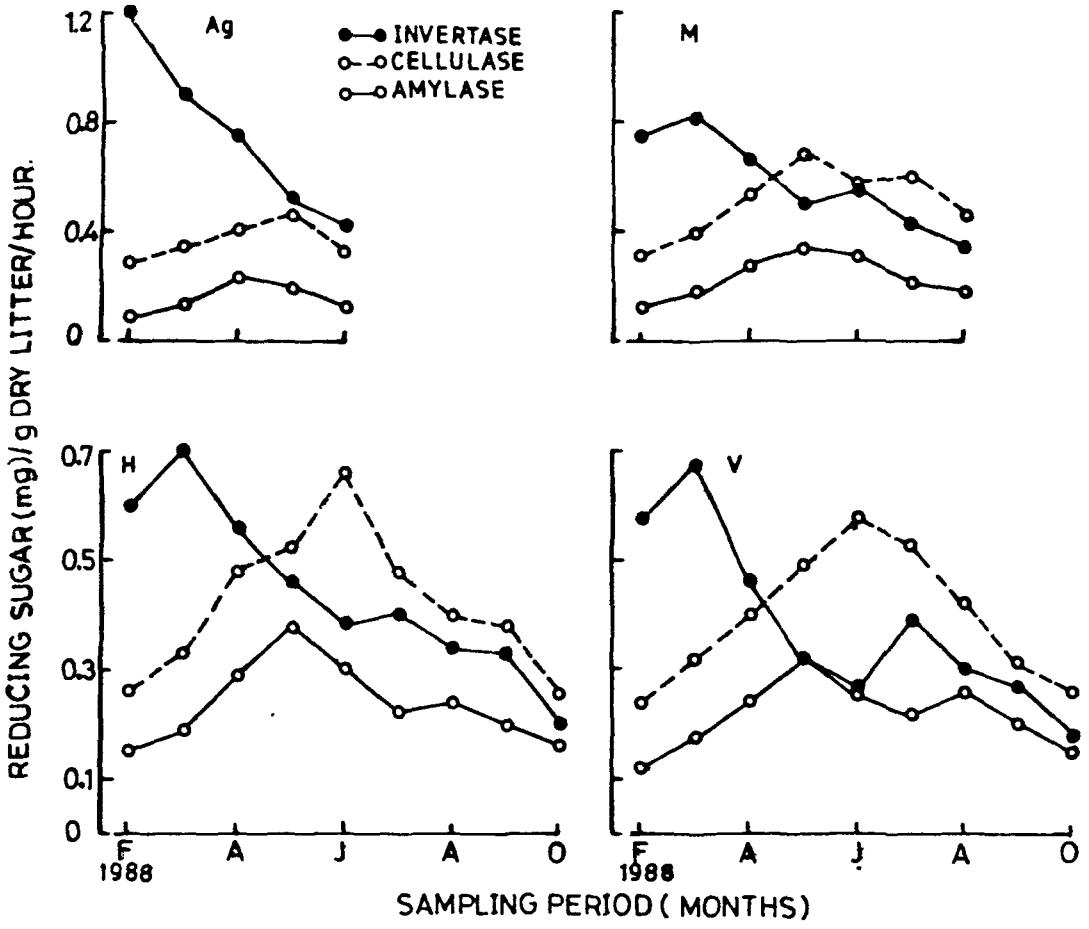


Fig. 10. Monthly variation in invertase, cellulase and amylase activities of different leaf litters at lower altitude. Ag-Ageratum conizoides, M- Mallotus philippinensis, H- Holarrhena antidysenterica and V- Vitex glabrata.

correlation with cellulase (Table 14a, 14b, 14c and 14d).

At higher altitude, the maximum cellulase was extracted from A.nepalensis followed by M.esculenta and P.kesiya. Like lower altitude, at higher altitude too the cellulase showed a marked seasonal variation. The maximum enzyme was extracted in June from all the litters. The activity decreased in July and again there was a slight increase in September (Fig. 11). In all the litters a significant positive correlation was established with their fungal and bacterial populations ($P < 0.05$). Except, A.nepalensis, the moisture content of M.esculenta and P.kesiya (young and old) litters was correlated significantly ($r=0.443$, 0.692 and 0.530 respectively) with their cellulase enzyme. Cellulase activity showed a negative correlation with cellulose of all the litters, however, the correlation was not significant (Table 15a, 15b, 15c and 15d).

(ii) Amylase activity:

Amylase activity was more in M.philippinensis and A.conizoides litters. The amylase activity, which was less in the beginning, increased with time. Like cellulase, amylase activity too showed a marked seasonal variation (Fig. 10). Unlike A.conizoides (April), the maximum amylase activity was observed in May in other litters. The amylase activity correlated positively with litter fungal population of H.antidysenterica and M.philippinensis while with litter bacterial population of V.glabrata and H.antidysenterica (Table 14a, 14b, 14c and 14d).

At higher altitude, amylase activity was highest in A.nepalensis and lowest in P.kesiya (young). The amylase activity of all the leaf litters showed a similar seasonal pattern. The highest activity was assayed in June. During winter months the activity was very less (Fig. 11). The amylase activity of all the litters showed a significant positive correlation with their moisture

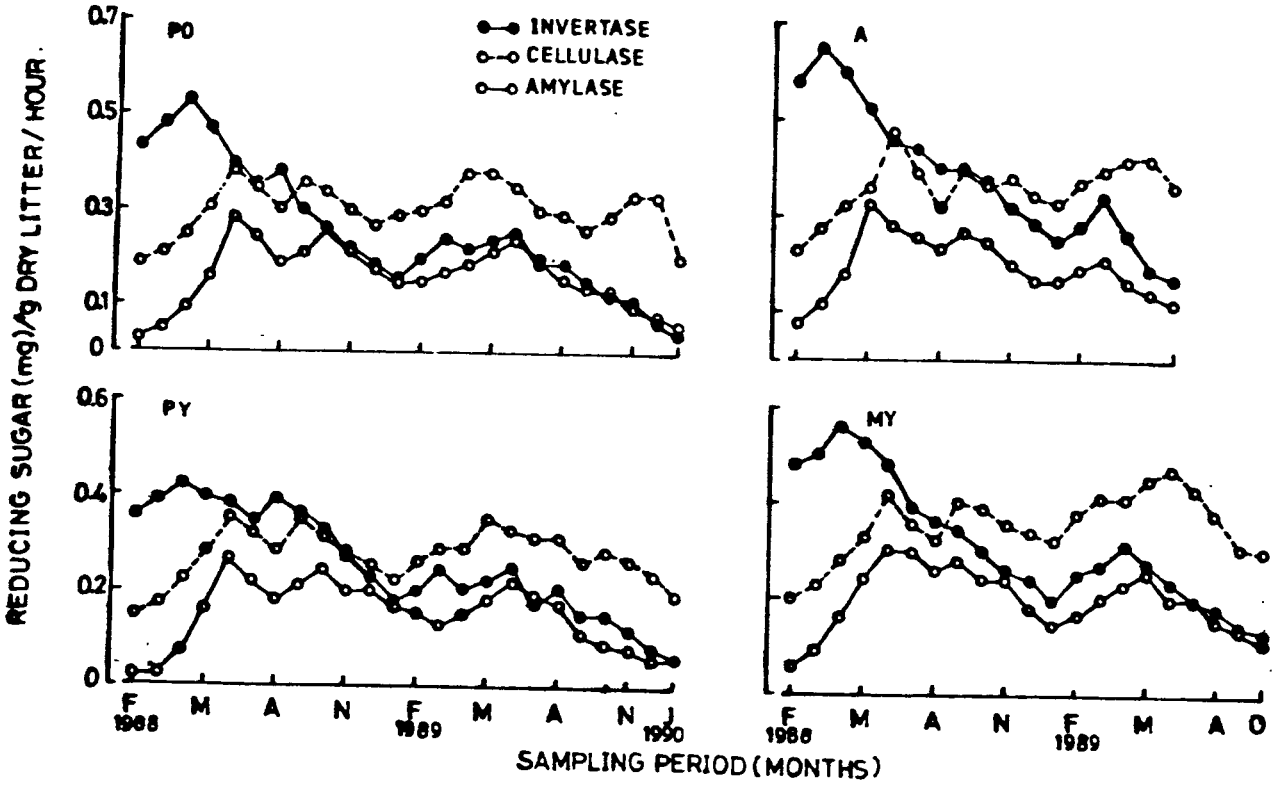


Fig. 11. Monthly variation in invertase, cellulase and amylase activities of different leaf litters at higher altitude. PO - Pinus kesiya (old), A- Alnus nepalensis, PY- Pinus kesiya (young) and MY- Myrica esculenta.

content and fungal and bacterial population . Except in A.nepalensis, the litter p^H of M.esculenta (r=0.579), P.kesiya (young) (r=0.586) and P.kesiya (old) (r=0.521) showed a significant positive correlation with their amylase activity (Table 15a, 15b, 15c and 15d).

(iii) Invertase activity:

Unlike cellulase and amylase, the invertase activity was more in the beginning and decreased as the decomposition progressed. High invertase activity was recorded in A.conizoides followed by M.philippinensis, H.antidysenterica and V.glabrata. At lower altitude, the invertase activity in all the litters showed an identical seasonal variation (Fig. 10). The amount of total sugars showed a significant positive correlation ($P < 0.05$) with invertase activity. However, invertase showed a significant negative correlation with weight loss ($P < 0.01$). It also correlated significantly with total nitrogen (except in V.glabrata) (Table 14a, 14b, 14c and 14d).

Like lower altitude, at higher altitude too the invertase activity was more in the beginning, which decreased with time. The activity was maximum in A.nepalensis followed by M.esculenta and P.kesiya (old and Young). In all the litters an identical seasonal pattern was observed. The invertase activity decreased till January which again increased in March-April (Fig. 11). The total sugars and total nitrogen showed a significant positive correlation with invertase activity ($P < 0.05$). However, a negative correlation ($P < 0.01$) was established with weight loss of litter (Table 15a, 15b, 15c and 15d).

The amylase, cellulase and invertase activities were more at lower altitude as compared to higher altitude.

Table 14(a). Correlation coefficients (r) for fungal population, bacterial population, moisture content, pH, total nitrogen, weight loss, cellulose, total sugars, amylase, cellulase and invertase activities of Ageratum conizoides litter.

Sources of variation	D.F.	Amylase	Cellulase	Invertase
Fungal population	3	0.746	0.988***	-0.452
Bacterial population	3	0.781	0.985***	-0.463
Moisture content	3	0.472	0.541	-0.978***
pH	3	0.435	0.266	0.643
Total nitrogen	3	-0.119	-0.179	0.926**
Weight loss	3	0.238	0.397	-0.96***
Cellulose	3	-	-0.344	-
Total sugars	3	-	-	0.989***

*, **, ***, P< 0.1, 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table14(b) . Correlation coefficients (r) for fungal population, bacterial population, moisture content, pH, total nitrogen, weight loss, cellulose, total sugars, amylase, cellulase and invertase activities of Mallotus philippinensis litter.

Sources of variation	D.F.	Amylase	Cellulase	Invertase
Fungal population	5	0.918***	0.969***	-0.732*
Bacterial population	5	0.752*	0.617	-0.176
Moisture content	5	0.584	0.716*	-0.940***
pH	5	0.541	0.226	-0.312
Total nitrogen	5	0.609	0.371	0.86**
Weight loss	5	0.194	0.456	-0.956***
Cellulose	5	-	-0.438	-
Total sugars	5	-	-	0.884***

*, **, ***, $P < 0.1$, 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 14(c). Correlation coefficients(r) for fungal population, bacterial population, moisture content, pH, total nitrogen, weight loss, cellulose, total sugars, amylase, cellulase and invertase activities of Holarrhena antidysenterica litter.

Sources of variation	D.F.	Amylase	Cellulase	Invertase
Fungal population	7	0.882***	0.833***	0.07
Bacterial population	7	0.887***	0.416	0.066
Moisture content	7	0.473	0.510	-0.368
pH	7	0.479	0.776**	0.644*
Total nitrogen	7	0.301	0.376	0.654*
Weight loss	7	-0.111	0.01	-0.936***
Cellulose	7	-	-0.065	-
Total sugars	7	-	-	0.876***

*,**,***, P< 0.1,0.5 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 14(d) . Correlation coefficients (r) for fungal population, bacterial population, moisture content, pH, total nitrogen, weight loss, cellulose, total sugars, amylase, cellulase and invertase activities of Vitex glabrata litter.

Sources of variation	D.F.	Amylase	Cellulase	Invertase
Fungal population	7	0.951***	0.850***	-0.207
Bacterial population	7	0.82***	0.915***	-0.293
Moisture content	7	0.600*	0.884***	-0.461
pH	7	0.403	0.387	0.222
Total nitrogen	7	0.233	0.170	0.326
Weight loss	7	0.078	0.067	-0.856***
Cellulose	7	-	0.037	-
Total sugars	7	-	-	0.819***

*, **, ***, $P < 0.1$, 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 15(a). Correlation coefficients (r) for fungal population, bacterial population, moisture content, pH, total nitrogen, weight loss, cellulose, total sugars, amylase, cellulase and invertase activities of Pinus kesiya (young) litter.

Sources of variation	D.F.	Amylase	Cellulase	Invertase
Fungal population	22	0.603***	0.722***	0.282
Bacterial population	22	0.546***	0.665***	0.066
Moisture content	22	0.576***	0.692***	0.155
pH	22	0.586***	0.430*	0.663***
Total nitrogen	22	0.284	0.160	0.868***
Weight loss	22	-0.135	0.098	-0.02
Cellulose	22	-	-0.162	-
Total sugars	22	-	-	0.886***

*, **, ***, P< 0.1, 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 15 (b). Correlation coefficient (r_H) fungal population, bacterial population, moisture content, p^H, total nitrogen, weight loss, cellulose, total sugars, amylase cellulase and invertase activities of Myrica esculenta litter.

Sources of variation	D.F.	Amylase	Cellulase	Invertase
Fungal population	22	0.638***	0.744***	-0.141
Bacterial population	22	0.680***	0.489**	0.063
Moisture content	22	0.518**	0.443**	-0.258
p^H	22	0.579***	0.303	0.760***
Total nitrogen	22	-0.147	-0.490**	0.939***
Weight loss	22	-0.013	0.479**	-0. 952***
Cellulose	22	-	-0.477**	-
Total sugars	22	-	-	0.935***

*,**,***, $P < 0.1$, 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 15(c). Correlation coefficients (r) for fungal population, bacterial population, moisture content, pH, total nitrogen, weight loss, cellulose, total sugars, amylase, cellulase and invertase activities of Alnus nepalensis litter.

Sources of variation	D.F.	Amylase	Cellulase	Invertase
Fungal population	15	0.815***	0.710***	0.123
Baterial population	15	0.615***	0.514**	0.006
Moisture content	15	0.620***	0.438*	0.028
pH	15	0.205	0.111	0.399
Total nitrogen	15	0.288	-0.287	0.869***
Weight loss	15	0.151	0.547**	-0.972***
Cellulose	15	-	-0.512**	-
Total sugars	15	-	-	0.492**

*,**,***, P< 0.1,0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 15(d). Correlation coefficients(r) for fungal population, bacterial population, moisture content, pH, total nitrogen, weight loss, cellulose, total sugars, amylase, cellulase and invertase activities of Pinus kesiya (old) litter.

Sources of variation	D.F.	Amylase	Cellulase	Invertase
Fungal population	22	0.432**	0.490**	0.259
Bacterial population	22	0.472**	0.478**	-0.047
Moisture content	22	0.613***	0.530**	0.093
pH	22	0.521**	0.295	0.282
Total nitrogen	22	0.261	0.545***	0.866***
Weight loss	22	-0.226	-0.330	-0.878***
Cellulose	22	-	-0.003	-
Total sugars	22	-	-	0.930***

*, **, ***, $P < 0.1$, 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

DISCUSSION

In order to explain how different types of leaf litters decompose at different rate under identical environmental conditions, it is essential to examine both the organic constituents of the litter, the nutritional requirement and preferences of the heterotrophs thriving on the litters (Sinsabaugh and Linkins, 1987). The negative correlation between cellulase activity and weight remaining of cellulose suggests that the amount of cellulose acts as a limiting factor for the activity of cellulase. The high cellulase activity in M.philippinensis litter was attributed to the high amount of cellulose present in the litter.

The improved cellulase activity in May-June may be due to the high microbial population encountered during that period. The correlation with moisture content in most of the litters is in confirmity with the results of Ross (1981) who also observed a correlation with cellulase activity and concluded that the moisture content favoured the synthesis of cellulase. The differential amount of cellulase, amylase and invertase extracted from different types of litters may be attributed to the difference in their chemical composition (Spalding, 1980). The low concentration of cellulase, amylase and invertase activities in Vitex glabrata and Pinus kesiya litters have been attributed to high phenolic content which may inhibit these enzymes (Benoit and Starkey, 1968). The low invertase activity towards the end of the decomposition may have resulted from comparatively its low synthesis by microbes either regulated by environmental changes or species composition (Ross, 1981). Ross (1976) and Spalding et al (1975) have suggested that soil enzymes responded to the type of vegetation. Ross (1981) has also observed more invertase in fresh leaves and least from organic horizon. The low enzyme concentration at higher

altitude suggested that the biochemical metabolism of plant remains would be slower at higher altitude than at lower altitude (Ross, 1981). The results of Ross (1981) supported the findings that the amylase activity which was less in the beginning of the litter degradation, increased with the time of decomposition of litter which was mainly related to the eukaryotic (fungi) population colonizing on the litter.

The results of the present investigation have suggested that the microbial enzyme activity mainly depends upon the age of the leaves (Ross, 1981), chemical nature of the litter, environmental conditions and the microbial population which utilizes different recalcitrant compounds for their metabolism.

CHAPTER IV

EFFECT OF TEMPERATURE AND LITTER QUALITY ON THE DECOMPOSITION OF DECIDUOUS AND CONIFEROUS LEAF LITTER IN LABORATORY MICROCOSMS

INTRODUCTION

The accumulated litter and humus layers on the forest floor are an important centres of organic material, nutrients and moisture in a forest ecosystem. The rate of decomposition, therefore, determines the nutrient availability to plants and microbes and thus regulate the nutrient cycling (Jorgensen, et al,1980). In deciduous forest ecosystem leaf fall constitute the bulk of the litter on the soil surface (Jensen, 1974). This litter decomposes on the soil - litter sub-system by a variety of microorganisms besides the lower arthropods and invertebrates. The slow rate of litter decomposition can result in the accumulation of large nutrient stocks on the soil surface thus limiting the nutrient availability to the primary producers (Melillo et al, 1982). The rate of litter decomposition is influenced by a number of factors including moisture, temperature, substrate quality and the nature of microorganisms and soil fauna engaged in the decomposition process (Witkamp, 1971). The chemical indices of substrate quality infer different elemental concentration besides, the concentration of different organic constituents. Plant species which differ consistently in their chemical and physical composition may play a different role in the control of decomposition process (Swift et al,1979).

There are various reports on the colonization of living plant materials as well as their dead remains by microbes (Dickinson and Pugh, 1974; Dickinson and Preece, 1976 and Aneja, 1981). Most of these studies, however, pertain to the role of vast arena of microbes which colonize the substrate in a definite successional pattern. But, there are very few studies on the role of individual fungi in the decomposition of litter (Ivarson, 1974; Aneja, 1981 and

Dkhar, 1983). In field, various factors affect the process of decomposition which makes it difficult to determine the importance of an individual variable independently of the others. However, the study under laboratory condition provides an opportunity to understand the role of individual factor which influences decomposition irrespective of other.

Therefore, the present experiment was undertaken to understand the importance of temperature and litter quality on microbial decomposition of broad leaved and coniferous litter.

MATERIALS AND METHODS

Holarrhena antidysenterica and Pinus kesiya were dominant plant species at lower and higher altitudes respectively. Therefore, the leaf litter of these two species were selected for the present study.

Two fungi, namely, Penicillium chrysogenum and Trichoderma viride which were dominant on these two litters and their mixture were also selected for the present investigation.

(i) Litter quality:

To study the effect of litter quality on microbial decomposition, different stages of litter i.e. fresh and partially decomposed (duff) were selected. In order to see the effect of fragmentation, two sets of fresh leaf litter, one with intact and another cut into 1cm segments were maintained. In each case three replicates were maintained. The experiment was continued for 180 days and the sampling was done every after 30 days.

(ii) Temperature:

In order to study the effect of temperature on microbial degradation of litter four different temperatures i.e. $5^{o}C$, $15^{o}C$, $25^{o}C$ and $35^{o}C$ were selected. This range of temperature was taken into account considering the minimum and maximum temperatures in the field conditions.

Inoculation of fungi to sterilized litter:

1 g air dried litter of P.kesiya and H.antidysenterica was taken in 250 ml conical flasks separately with 15 ml sterilized distilled water. Thereafter, the flasks were plugged nicely with cotton plugs and sterilized in an autoclave at 15 $lb/inch^2$ pressure for 30 minutes. The pure cultures of T.viride and P.chrysogenum were maintained on Czapex - Dox Agar medium (Raper and Thom, 1949). Each flask was inoculated with 6 blocks (1 cm, diameter) of the test fungi. The blocks were cut out with the help of a sterilized cork borer from the periphery of the ten days old pure culture of the test fungi. In case of mixture, 3 blocks each of P.chrysogenum and T.viride were used. While,the conical flasks with material and only the agar blocks (without fungi) served as controls. Three replicates were maintained in each set. The inoculation was carried out under aseptic conditions inside the laminar flow chamber. The flasks used for studying the effect of temperature were incubated at four different temperatures i.e. $5^{o}C$,$15^{o}C$,$25^{o}C$ and $35^{o}C \pm 1^{o}C$ in the B.O.D incubator. While, to study the effect of litter quality, the inoculated flasks were incubated at $25^{o}C \pm 1^{o}C$. Whenever, necessary the required amount of sterilized distilled water was poured in the flasks to maintain the proper moisture content of the litters. After each sampling period, the plant material was taken

out from the flasks and the fungal mycelium was removed by brushing or rubbing the litter gently. Litter of different treatments were then dried in a hot air oven at 60°C for 48 hours and dry weight was recorded. The percentage weight loss of the litter was calculated on the basis of oven dry weight of the samples. These samples were kept for the estimation of organic constituents.

(iii) Quantitative estimation of organic constituents from the Decomposing Litter:

Cellulose, hemicellulose, lignin, total sugars and total amino acids of different stages of decomposed litters were estimated by following the methods of Peach and Tracey (1955) (Details are given in Chapter II of the thesis).

RESULTS

(i) Microbial decomposition of litter:

(a) Temperature:

The leaf litter of Holarrhena antidysenterica decomposed more rapidly than Pinus kesiya. The rate of decomposition showed a negative correlation with temperature(Table 16). The decomposition was fastest at 25°C followed by 35°C, while very slow at 5°C (Fig. 12a). Even for P.kesiya litter, 25°C appeared as the most suitable temperature (Fig. 12b). The mixture of the two fungi, i.e. Trichoderma viride and Penicillium chrysogenum ameliorated the decomposition rate compared with their rate when used individually. P.chrysogenum was more efficient in terms of its decomposing ability than T.viride at high temperature and in H.antidysenterica litter. On the other hand T.viride decomposed pine litter more efficiently than H.antidysenterica litter.

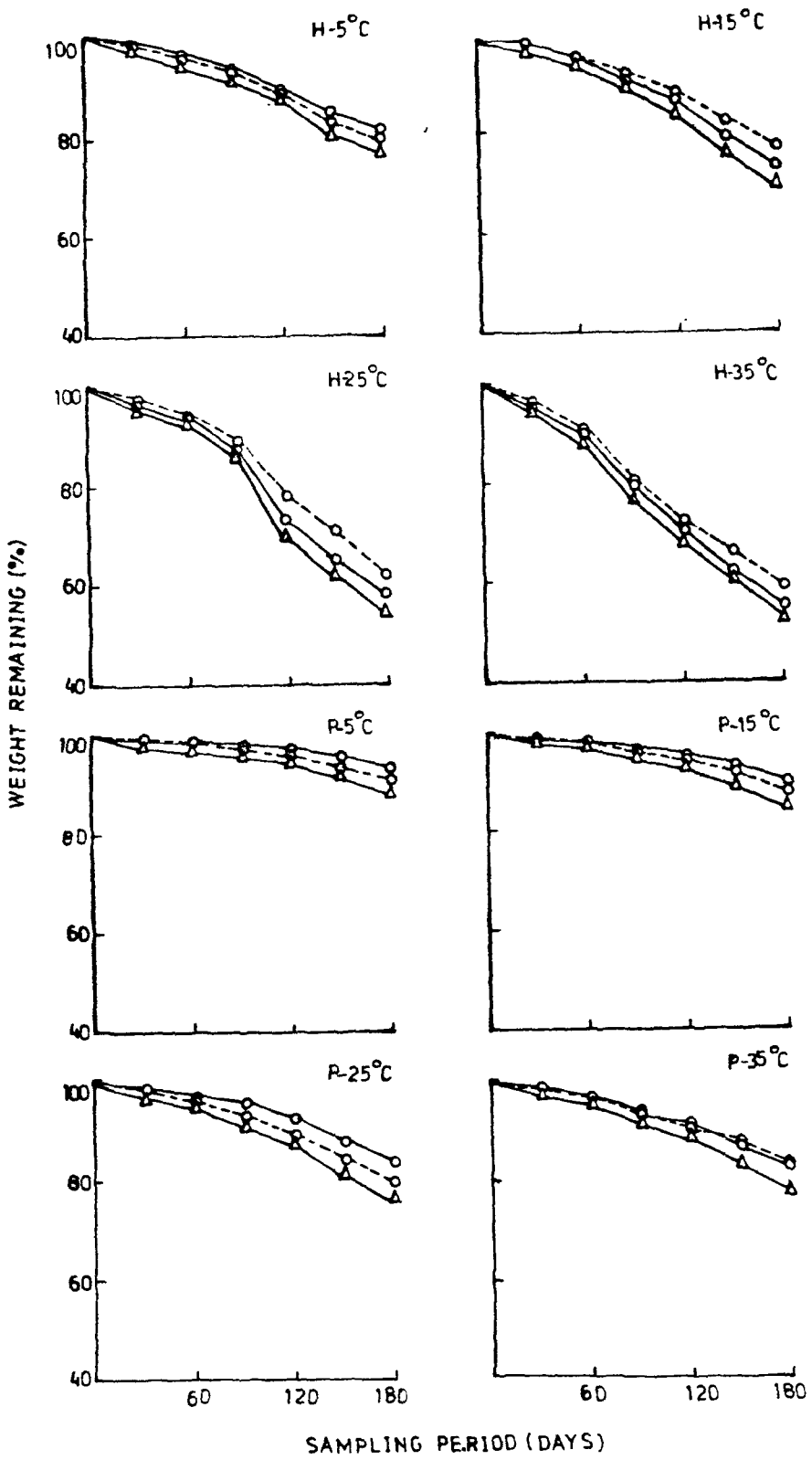


Fig. 12. (a&b) Percentage of original dry weight of Holarrhena antidysenterica (H) and Pinus kesiya (P) leaf litters remaining after different periods of decomposition at different temperatures by certain dominant fungal species. O-O- Penicillium chrysogenum, O-O- Trichoderma viride and Δ-Δ - Mixture of P.chrysogenum and T.viride.

Table 16. Correlation coefficients (r) for the weight remaining of *Pinus kesiya* and *Holarrhena antidysenterica* litter , temperature and test fungi.

Sources of variation	df	*P.kesiya*			df	*H. antidysenterica*		
		P	T	M		P	T	M
Temperature	2	-0.916*	-0.656	-0.855	2	-0.867	-0.813	-0.830

* $p < 0.1$

P = *Penicillium chrysogenum*

T = *Trichoderma viride*

M = Mixture of P and T

The decomposing ability of different fungal species varied significantly at different temperatures in both the litters (Table 17).

Out of the four temperatures maintained during the experiment, 5°C and 15°C didn't have an apparent effect on decomposition of the incubated litters, whereas 25°C and 35°C could affect the rate of decomposition. At 35°C, in control (without inoculum) sets around 7% and 2% litter was lost in case of H.antidysenterica and P.kesiya respectively. In all the treatments the weight loss was slow in the beginning which improved with time. However, the extent of improvement in the rate of decomposition varied with temperature and nature of litter. At 5°C and 15°C weight loss of litter was maximum after 120 days of incubation while at 25°C and 35°C it occurred after 90 days in H.antidysenterica. On the contrary in case of P.kesiya incubated at 5°C and 15°C it could only be achieved after 150 days while after 120 days at 25°C and 35°C (Fig. 12a and 12b).

The time taken for 95% decomposition decreased with increase in temperature. For H.antidysenterica at 5°C it took 12.6 years while at 25°C it was just 5.7 years, while for P.kesiya it was about 31.3 and 13.2 years respectively (Table 18).

(b) Litter quality:

In case of both H.antidysenterica and P.kesiya the partially decomposed litter decomposed much faster (K=0.754 and 0.348 respectively) than fresh fragmented (K=0.638 and 0.285 respectively) and fresh intact litters (K=0.551 and 0.250 respectively). The quality of litter had a marked effect on the rate of decomposition. In partially decomposed litter the maximum loss occurred after 60 days in H.antidysenterica and after 90 days in P.kesiya, while for

Table 17. Analysis of variance test for weight remaining of Pinus kesiya and Holarrhena antidysenterica litter as affected by temperature and test fungi.

Sources of variation	P.kesiya			H.antidysenterica		
	dF	MS	F	dF	MS	F
Temperature (T)	3	389.40	19.69**	3	1267.22	48.95**
Test fungi (F)	2	140.42	7.10**	2	166.25	6.42**
T X F	6	4.86	0.246	6	11.805	0.456
Error	48	19.77	-	48	25.89	-
Total	59	-	-	59	-	-

** $P < 0.01$.

Table 18(1). Decay constant (K), half life and 95% life values for Pinus kesiya and Holarrhena antidysenterica leaf litter decomposition by some test fungi at different temperatures under laboratory microcosms.

(A) Pinus kesiya

Temperature (°C)	Trichoderma viride			Penicillium chrysogenum			Mixture		
	K (Per year)	Half life (year)	95% life (year)	K (Per year)	Half life (year)	95% life (year)	K (per year)	Half life (year)	95% life (year)
5	0.077	9.0	38.96	0.058	11.95	51.72	0.096	7.22	31.25
15	0.111	6.24	27.03	0.092	7.53	32.61	0.141	4.91	21.28
25	0.194	3.57	15.46	0.151	4.59	19.87	0.227	3.05	13.22
35	0.131	5.29	22.90	0.141	4.99	21.28	0.194	3.57	15.46

(B) Holarrhena antidysenterica

Temperature (°C)	Trichoderma viride			Penicillium chrysogenum			Mixture		
	K (Per year)	Half life (year)	95% life (year)	K (per year)	Half life (year)	95% life (year)	K (per year)	Half life (year)	95% life (year)
5	0.205	3.38	14.63	0.184	3.77	16.3	0.238	2.91	12.6
15	0.227	3.05	13.21	0.273	2.54	10.99	0.310	2.24	9.68
25	0.415	1.67	7.23	0.473	1.47	6.34	0.519	1.34	5.78
35	0.348	1.99	8.62	0.401	1.73	7.48	0.429	1.62	6.99

Table 18 (2). Decay constant (K), half life and 95% life values for Pinus kesiya and Holarrhena antidysenterica leaf litter of different qualitiesby some test fungi under laboratory microcosms.

(A) Pinus kesiya

Litter quality	Trichoderma viride			Penicillium chrysogenum			Mixture		
	K (Per year)	Half life (year)	95% life (year)	K (per year)	Half life (year)	95% life (year)	K (per year)	Half life (year)	95% life (year)
PD	0.310	2.24	9.68	0.285	2.43	10.53	0.348	1.99	8.62
FF	0.238	2.91	12.6	0.194	3.57	15.46	0.285	2.43	10.53
FI	0.216	3.21	13.89	0.162	4.28	18.52	0.250	2.77	12.0

(B) Holarrhena antidysentrica

Litter quality	Trichoderma viride			Penicillium chrysogenum			Mixture		
	K (per year)	Half life (year)	95% life (year)	K (per year)	Half life (year)	95% life (year)	K (per year)	Half life (year)	95% life (year)
PD	0.656	1.06	4.57	0.694	0.999	4.32	0.754	0.919	3.98
FF	0.551	1.26	5.44	0.602	1.15	4.98	0.638	1.09	4.7
FI	0.504	1.38	5.95	0.519	1.34	5.78	0.551	1.26	5.44

PD = Partially decomposed litter

FF = Fresh fragmented litter

FI = Fresh Intact litter.

intact litter it took more than 90 days in case of former while more than 120 days in case of latter. The fragmentation of litter also sped up the rate of decomposition. In terms of different fungal inoculum, P.chrysogenum was more effective for H.antidysenterica while T.viride could decompose faster the P.kesiya litter. The mixture of these two, however, was still more efficient compared to the individual species (Fig. 13a and 13 b).

The time taken for 95% of decomposition according to Olson's (1963) model decreased with degradation of litter quality. It took more than three years (95% = 3.9) in case of partially decomposed H.antidysenterica litter for 95% degradation, while more than five years (95% = 5.95) for fresh intact litter. For P.kesiya it took 8.6 years for partially decomposed litter while 13.9 years for fresh intact litter (Table 18). The decomposition constant of partially decomposed, fresh fragmented and fresh intact litter varied significantly (Table 19).

(ii) Changes in organic constituents of litter:

(a) Temperature:

Both the soluble (total sugars and total amino acids) and insoluble (cellulose and hemicellulose) organic constituents of both the litters decomposed rapidly in the beginning of decomposition which either slowed down or remained constant towards the end of the decomposition. The decomposition of these constituents was fast at 25°C as compared to 5°C and 15°C. At 25°C, the decomposition of lignin started after 150 days in H.antidysenterica, while in P.kesiya it increased till the last sampling. The mixed inoculum was more efficient in decomposing the different organic constituents than P.chrysogenum and T.viride (Fig.14a and 14b).

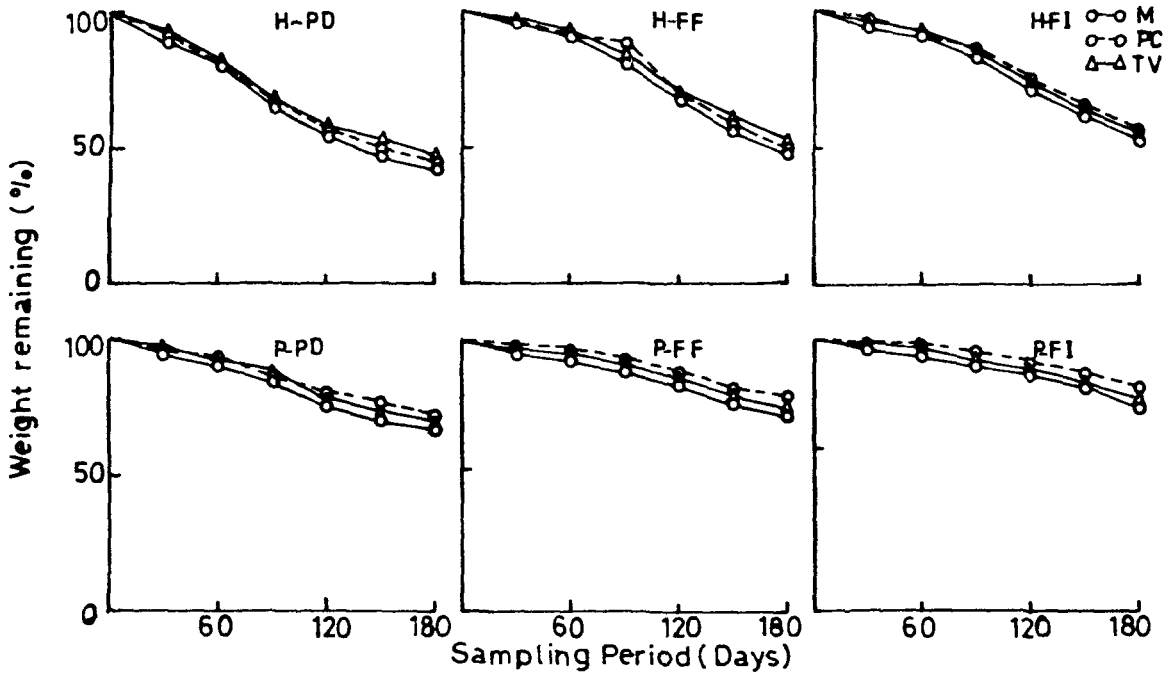


Fig. 13a & b. Percentage of original dry weight of Partially decomposed (PD), Fresh fragmented (FF) and Fresh intact (FI) leaf litters of Holarrhena antidysenterica (H) and Pinus kesiya (P) remaining after different periods of decomposition by certain dominant fungal species i.e. Penicillium chrysogenum (PC), Trichoderma viride (TV) and their mixture (M).

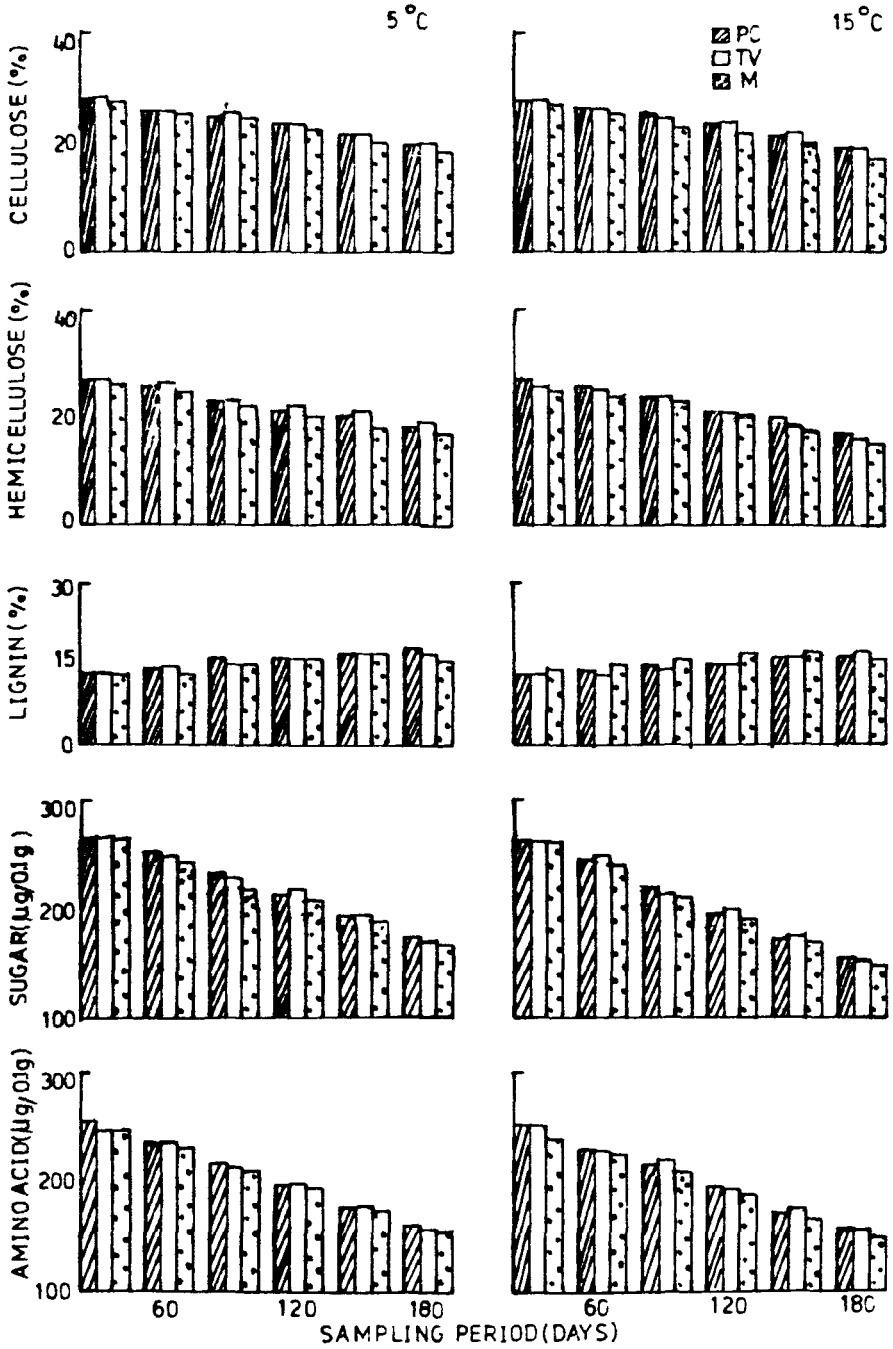


Fig. 14a. Variation in cellulose, hemicellulose, lignin, total sugars and amino acids of Holarrhena antidysenterica leaf litter at different temperatures (5^0C and 15^0C) and different periods of decomposition by test fungi. PC - Penicillium chrysogenum, TV - Trichoderma viride and M - Mixture of PC & TV.

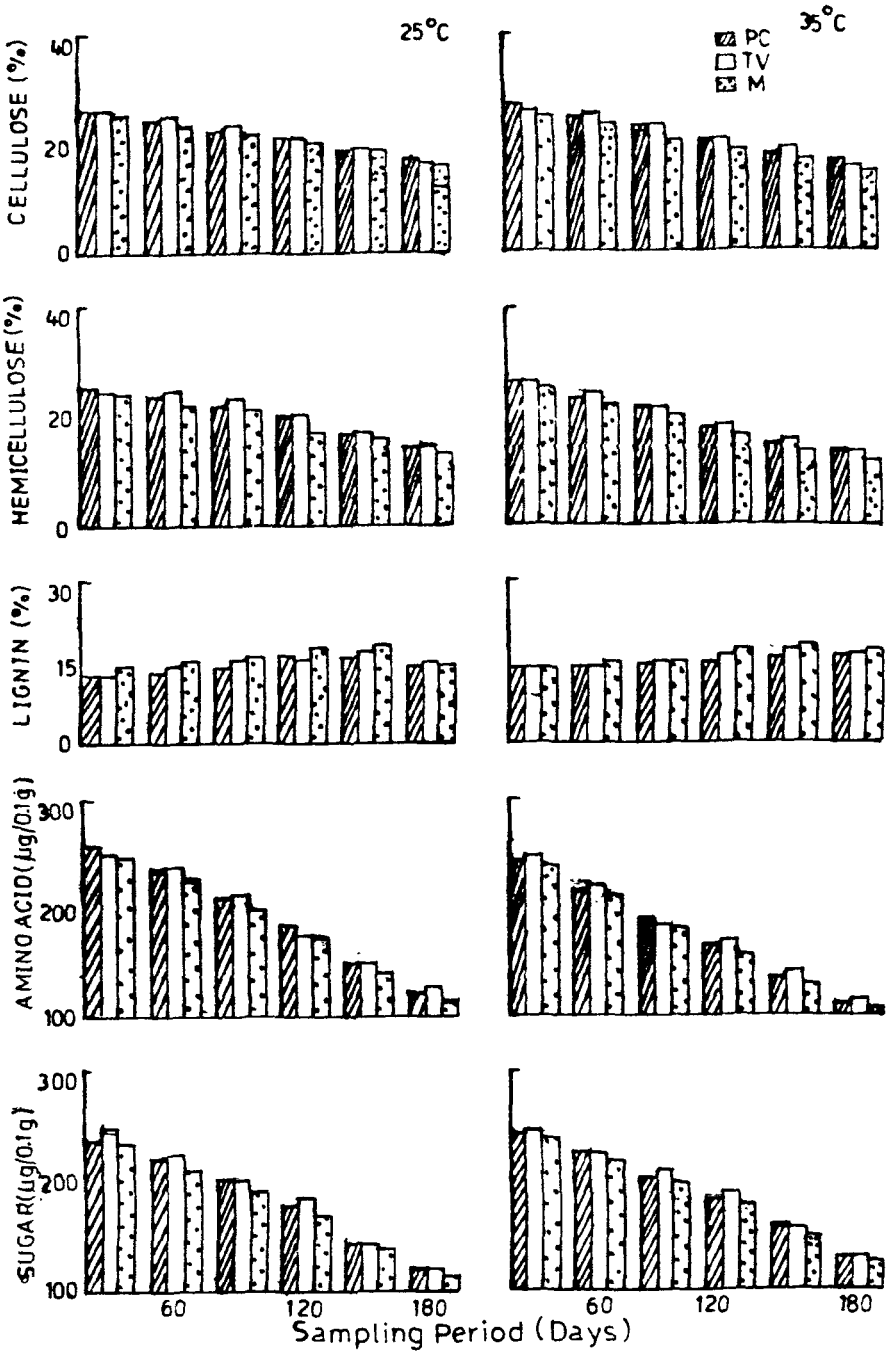


Fig. 14a. Variation in cellulose, hemicellulose, lignin, total sugars and amino acids of Holarrhena antidysenterica leaf litter at different temperatures. (25°C and 35°C) and different periods of decomposition by test fungi. PC - Penicillium chrysogenum, TV - Trichoderma viride and M - Mixture of PC and TV.

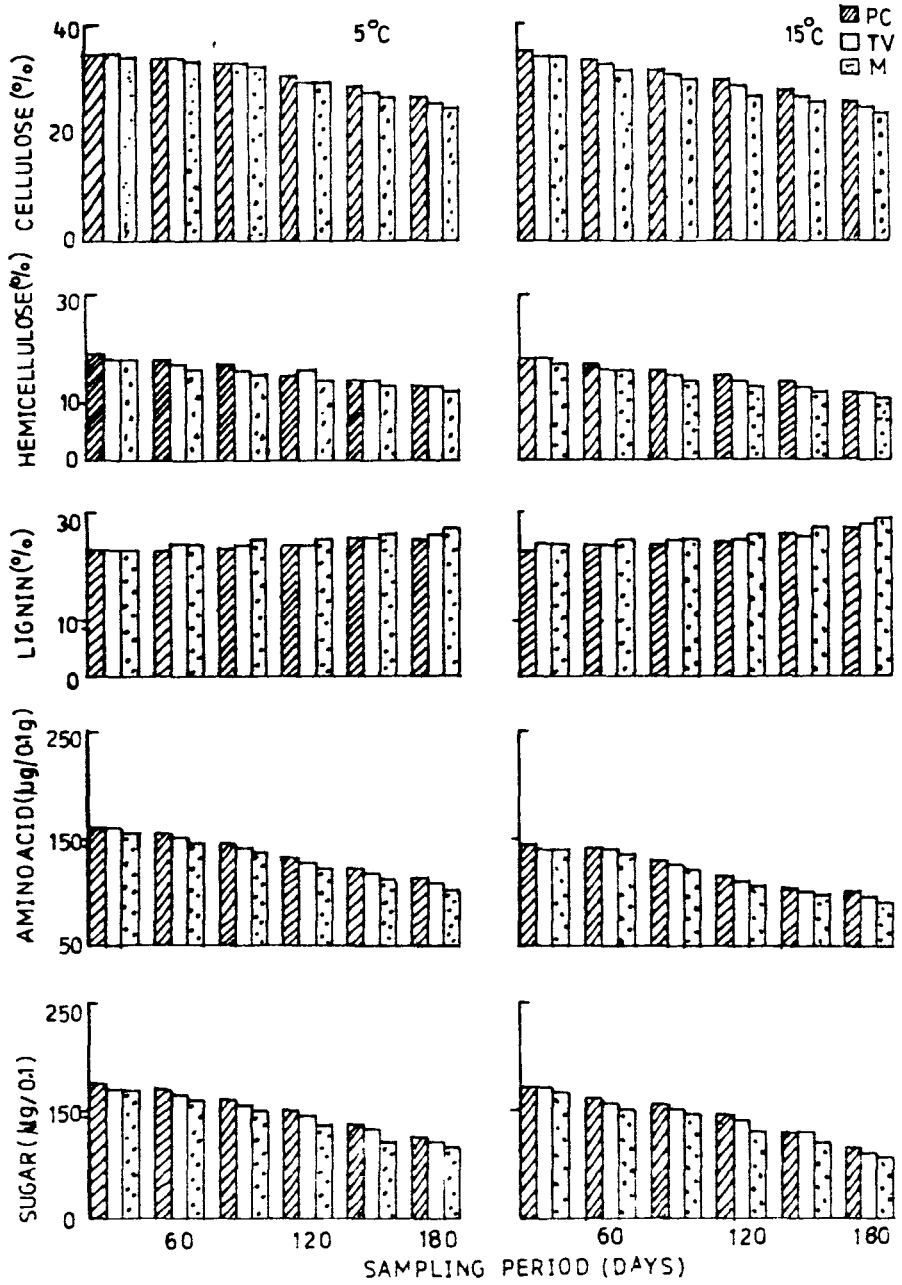


Fig. 14b. Variation in cellulose, hemicellulose, lignin, total sugars and amino acids of Pinus kesiya litter at different temperatures (5°C and 15°C) and at different periods of decomposition by test fungi. PC - Penicillium chrysogenum, TV - Trichoderma viride and M - Mixture of PC and TV.

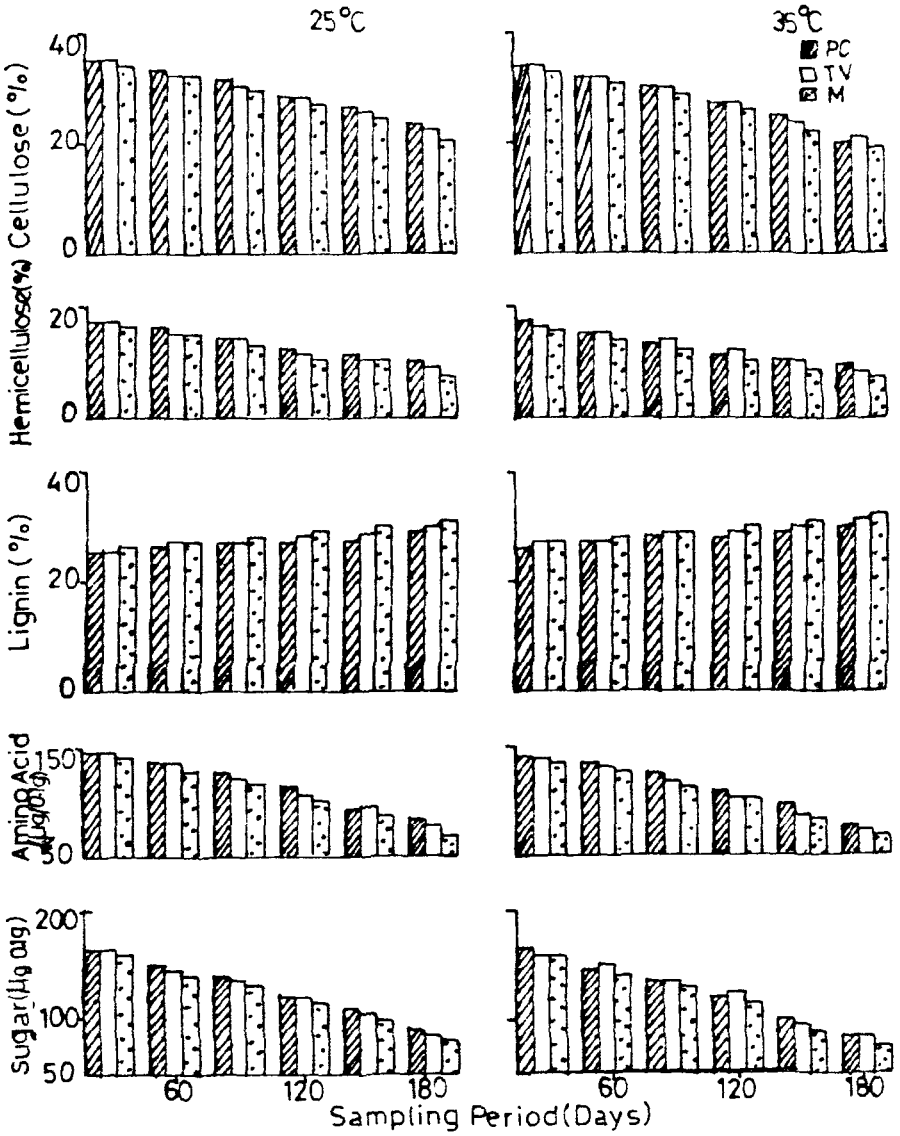


Fig. 14b. Variation in cellulose, hemicellulose, lignin, total sugars and amino acids of pinus kesiya litter at different temperatures (25°C and 35°C) and at different periods of decomposition by test fungi. PC - Penicillium chrysogenum, TV - Trichoderma viride and M - Mixture of PC and TV.

Table 19. Analysis of variance test for weight remaining of *Pinus kesiya* and *Holarrhena antidysenterica* litter as affected by their quality and the test fungi.

Sources of variation	*P.kesiya*			*H.antidysenterica*		
	dF	MS	F	dF	MS	F
Litter quality (L)	2	320.56	128.22**	2	277.22	188.98**
Test fungi (F)	2	183.89	73.56**	2	123.885	53.17**
L X F	4	4.723	1.89	4	5.56	2.39
Error	36	2.5	-	36	2.33	-
Total	44	-	-	44	-	-

** $P < 0.01$.

(b) Litter quality:

The litter quality also influenced the release of different organic constituents. In partially decomposed litter, initially less total sugars, amino acids, hemicellulose and cellulose were estimated compared to the fresh litter. However, the initial amount of lignin was more in the partially decomposed litter than in the fresh ones. The decomposition of lignin in partially decomposed litter started after 90 and 120 days respectively in H.antidysenterica and P.kesiya. The degradation of sugars, amino acids, cellulose and hemicellulose started earlier in fragmented litter than intact one. The degradation of lignin began after 120 days and 150 days in fragmented litters of H.antidysenterica and P.kesiya respectively. The decomposition of these organic constituents were more rapid in H.antidysenterica than P.kesiya (Fig. 15a and 15b).

DISCUSSION

The difference in weight loss of litters due to different fungal species under controlled conditions suggests that these species vary in their decomposing ability even under identical environmental conditions. Rai and Srivastava (1983) hypothesised that the colonization and decomposition potentiality of individual fungus were governed by their relative potential to decompose the dead tissues, the extent to which the litter has been subjected to microbial decomposition and prevalence of microbial antagonism. This can also be attributed to the prefrential use of different substrates by the microbes. The improved degradation of H.antidysenterica litter compared to P.kesiya might have been due to thick cuticle, highly suberised hypodermis and high lignin

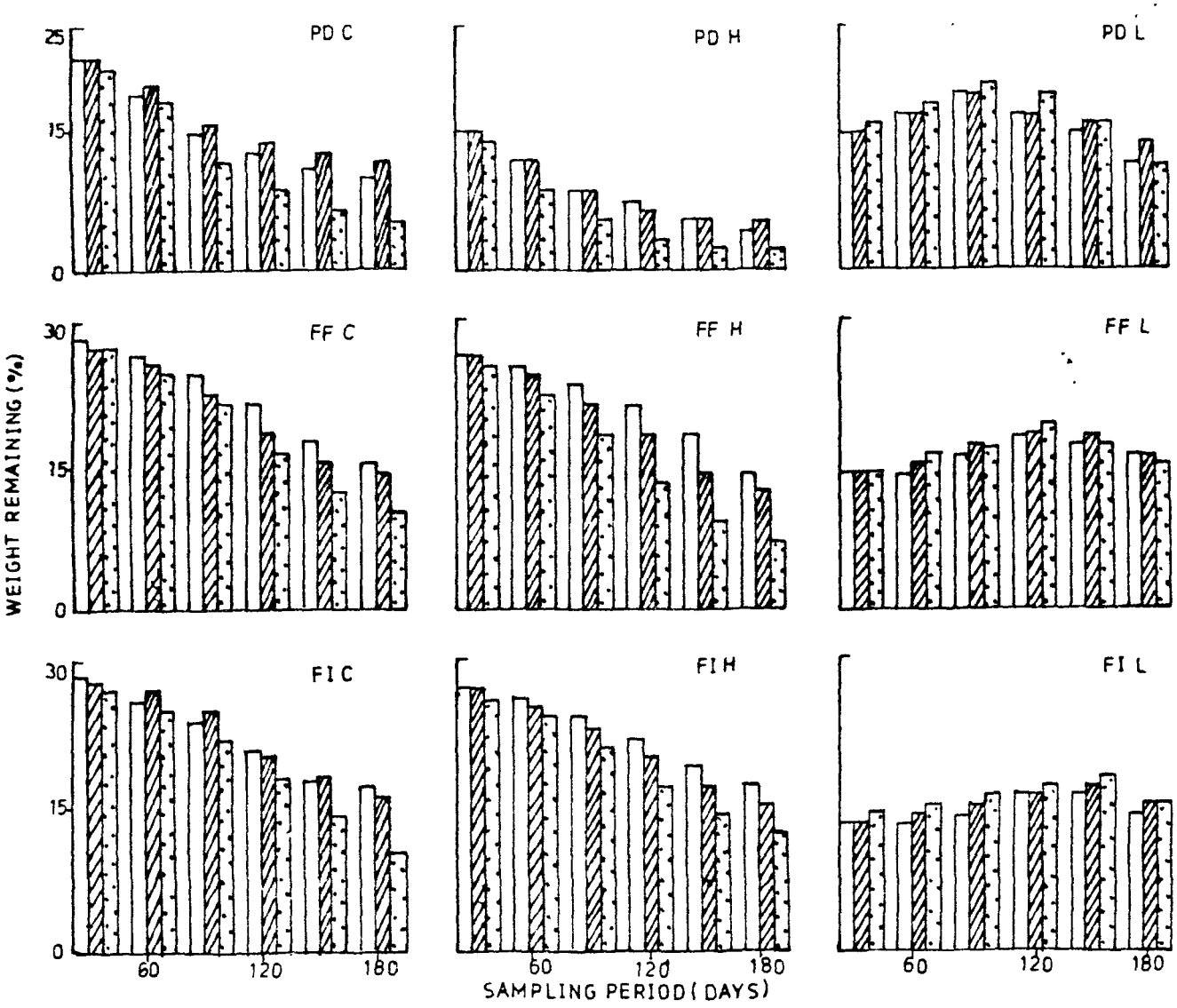


Fig. 15 a.Variation in cellulose, hemicellulose and lignin content of partially decomposed (PD), fresh fragmented (FF) and fresh intact (FI) Holarrhena antidysenterica leaf litter at different periods of decomposition.

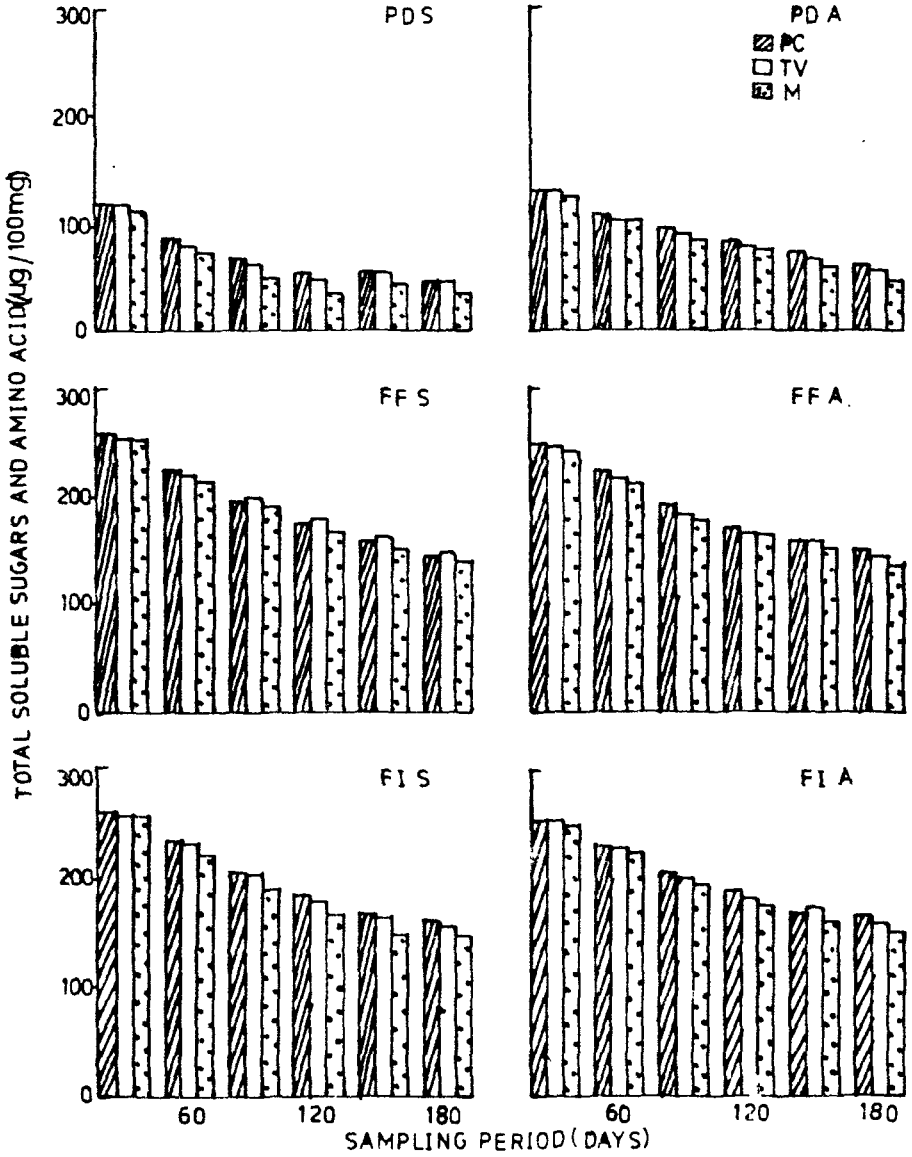


Fig. 15a. Variation in total sugars and amino acids of partially decomposed (PD), fresh fragmented (FF) and fresh intact (FI) leaf litter of Holarrhena antidysenterica by different test fungi. PC - Penicillium chrysogenum, TV - Trichoderma viride and M - Mixture of TV and PC.

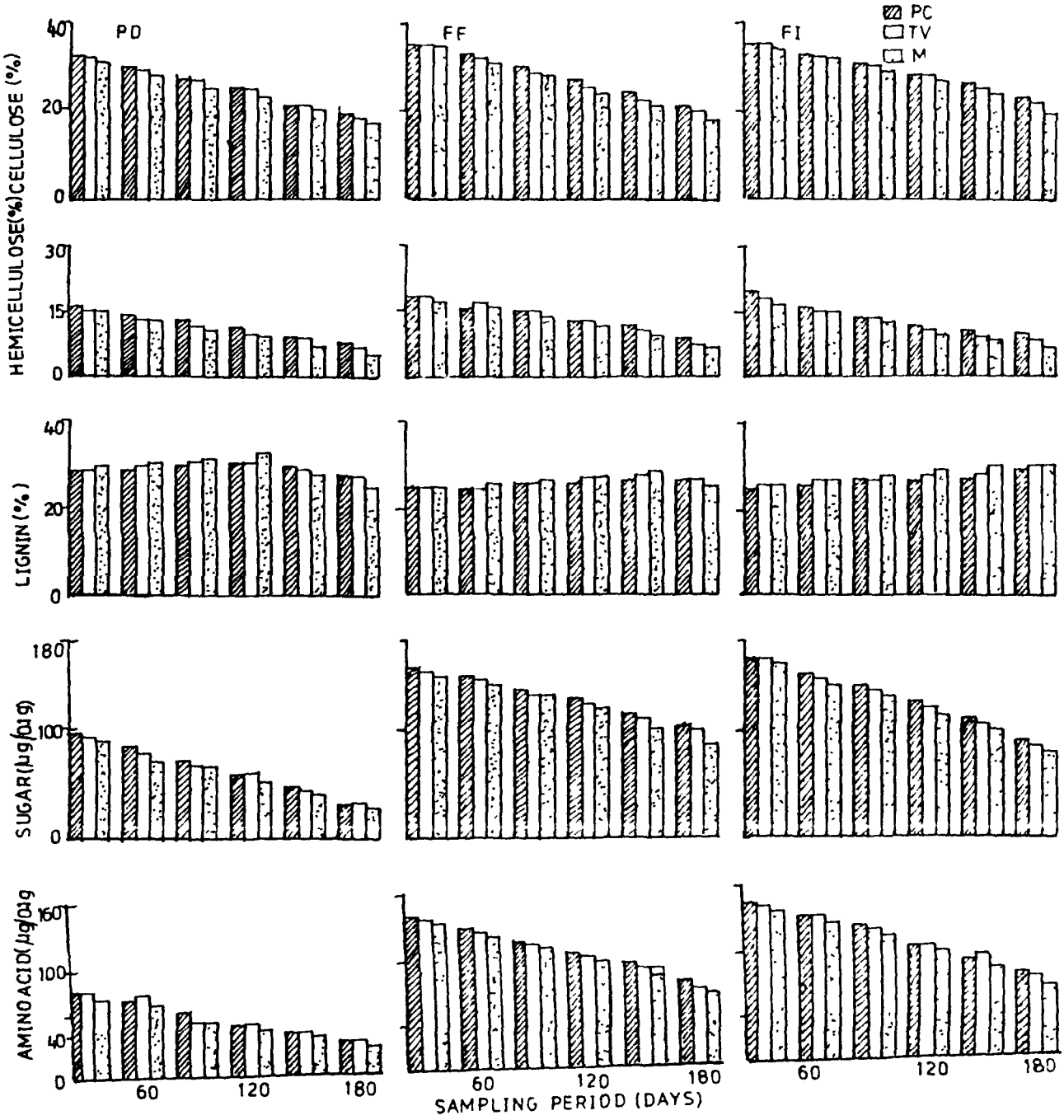


Fig. 15 b. Variation in cellulose, hemicellulose, lignin total sugars and amino acid contents of partially decomposed (PD), fresh fragmented (FF) and fresh intact (FI) Pinus kesiya leaf litter at different periods of decomposition.

content of pine litter (Das, 1980). Low concentration of soluble organic compounds in P.kesiya may be another factor for delayed decomposition.

The maximum decomposition at 25°C compared with low tempertures (5°C and 15°C) may be due to the optimum production of various enzymes by the microbes essential for the degradation of litter. The low decomposing ability at low temperatures might have arisen due to the inactivation of microbes by low temperatures. Stott et al (1986) and Summerell and Burgess (1989) also observed high weight loss at higher temperature.

The partially decomposed litter degraded faster than fragmented and intact litters. This can be assigned to the decomposed nature of former which must have caused removal of complex and resistant compounds or must have simplified their nature. Rai and Srivastava (1983) also concluded that fungi can utilize the partially decomposed substrate more readily than fresh ones. The rapid degradation of fragmented fresh litter,than intact ones could be related to exposure of more surface area for the microbial attack (Das, 1980).

The loss of lignin towards the later part of decomposition can be assigned to the active degradation of cellulose and hemicellulose and other simpler forms in the beginning which can be easily utilized by the microbes (Berg et al, 1984). This can also be due to the resistant nature of lignocellulosic complexes (Summerell and Burgess, 1989). The prefrential use of sugars, amino acids, cellulose and hemicellulose in the beginning indicates their role in the microbial growth (Harper and Lynch, 1981). Summerell and Burgess (1989) have also reported a decrease in the proportion of cellulose and hemicellulose during decomposition while increase in lignin concentration. These studies were in confirmity with the present investigation.

From the present investigation it may be concluded that the rate of litter decomposition is influenced by the temperature as well as the quality of

the litter. The differences in the decomposing ability between leaf litter fungi of different tree species and the concentration of readily soluble compounds also affected the rate of microbial decomposition.

CHAPTER V

IN VITRO PRODUCTION OF CELLULASES AND XYLANASE BY FUNGI: EFFECT OF TEMPERATURE AND P^H

INTRODUCTION

Cellulose, hemicellulose, pectin and starch are the major polysaccharides found in plant material. After cellulose, hemicellulose is the most abundant polysaccharide comprising 20 - 40% of total carbohydrate fraction (Hespell et al, 1987).

The microbial degradation of cellulose involves the secretion of extracellular enzymes called cellulase. Cellulase, a multienzyme system, catalyses the conversion of insoluble cellulose into simple water soluble mono and disaccharides. The cellulase complex consists of three major constituents i.e. endo-β-glucanase (C_x), exo-β-glucanase (C_1) and β-glucosidase (Fagerstam et al, 1984). The enzymatic degradation of cellulose involves the synergistic action of these three enzymes (Wachinger et al, 1989). The endoglucanase acts on amorphous cellulose attacking the internal glucosidic bonds, thereby increasing the number of free ends. The exoglucanase is characterized by its capability to release cellobiose units from non-reducing chain ends of micro-crystalline cellulose while β-glucosidase activates the hydrolysis of cellobiose to glucose.

The cellulolytic enzymes are produced by bacteria (Yamane et al, 1970 and Morales et al, 1984), actinomycetes (Stutzenberger, 1972) fungi (Clarke and Stone, 1965) and some invertebrates (Okada et al, 1966). There are various reports regarding cellulase production by different fungal species.

Besides, cellulose, hemicellulose is the second most abundant renewable polysaccharide in nature. Unlike cellulose, hemicellulose doesn't have a homogeneous chemical composition. The predominant polymer is a arabinose side chain (Hespell et al, 1987). Like cellulose, complete hydrolysis of xylan

too requires the synergistic activity of different enzymes (Bachman and McCarthy, 1989). Xylanase acts on a chain of xylose units where C_5 substituent is H. Constitutive xylanase production has been reported from a number of organisms (Lee et al, 1985).

The production of enzymes differ from organism to organism at different temperatures (Walsh and Stewart, 1971 and Trivedi and Rao, 1980), incubation length (Vilela et al, 1977) and p^H of the medium (Parley and Page, 1971). The temperature affects metabolic activities of fungi and the velocity of reaction by influencing the enzyme substrate complex. Solubility of ions and dissociation of molecules mediates the availability of nutrients for the organism by making their transport possible across the membrane (Berkley and Campbell, 1971). The production of extracellular enzymes by some fungi may occur over a wide range of p^H (4-8), while others have a narrow range (Batra, 1978). The extreme acidic and alkaline conditions influence the intracellular p^H which alleviates the intracellular enzymes. The extracellular enzymes, however, are directly affected by the environmental p^H.

Litter fungi vary in their nutritional requirements (Chang and Hudson, 1967). It was, therefore, aimed to evaluate the nutritional requirements of some common litter fungi especially for their ability to utilize cellulose and hemicellulose.

To study this, an investigation was carried out to investigate the optimum environmental conditions like temperature and p^H which facilitate large scale production of cellulasesand xylanase.

MATERIALS AND METHODS

(i) Selection of test fungi:

Three dominant litter fungal species Trichoderma viride, Penicillium chrysogenum, Fusarium oxysporum and their mixture were selected for the present study. The fungal species were maintained on basal agar medium supplemented with 1% carboxymethyl cellulose for cellulase activity and 1% xylan for xylanase activity (Pettersson's et al, 1963). The cultures were stored at $4^{o}C$.

(ii) Effect of temperature:

30 ml of Pettersson's liquid medium (p^{H} - 5.5) was taken in each 150 ml conical flasks. The flasks were plugged with cotton plugs and autoclaved at 15 $lb/inch^2$ pressure for 15 minutes. Each flask was inoculated with one agar disc (12 mm size) cut with sterilized corkborer of the test fungus obtained from 7 days old fungal colony growing on cellulose agar and xylan agar plates. For mixture 4mm agar block of each test fungus was used. The inoculated flasks were incubated at different temperatures i.e. $5^{o}C$, $15^{o}C$, $25^{o}C$ and $35^{o}C$ (these temperatures were in accordance with the field temperature range). Three replicates were maintained in each case.

(iii) Effect of p^{H}:

To study the effect of p^{H} on cellulases and xylanase activities, the basal medium was adjusted to different p^{H} i.e. 4.5, 5.0, 5.5, 6, 6.5 and 7 with the help of 0.01N NaOH and 0.01N HCl (keeping in view the p^{H} range

of different leaf litters). The basal medium was inoculated with different test fungal inoculum as mentioned above. Three replicates were maintained in each case. All the flasks were incubated at 25 ± 1°C. The inoculation was done under aseptic conditions inside the laminar flow chamber.

The flasks were taken out from the incubators after varying incubation periods. The culture medium was filtered through Whatman filter paper (No.1). The dry weight of the mycelium was determined by drying it at 80°C for 24 hours.

(iv) Enzyme assay:

(A) Cellulases assay:

(a) Endo-B-glucanase (EC 3.2.1.4);

It was assayed by determining the amount of reducing sugar released from the specific substrate, carboxymethyl cellulose (CMC), by following the method of Mahadevan and Sridhar (1986). 2 ml of CMC reagent (1%) was added to 1 ml of enzyme extracted. The sample was mixed thoroughly and incubated at 50°C for 30 minutes. Blank was prepared by adding boiled enzyme extract to the substrate. After incubation, 3ml of DNS reagent (Miller, 1972) was added in each tube and the mixture was kept in boiling water bath for 5 minutes. Thereafter, 1ml of 40% sodium potassium tartarate was added to the mixture. The tubes were cooled in running tap water. The optical density of the solution was recorded at 640 nm on Hitachi 220 spectrophotometer. Standard curve was prepared by using different concentrations of glucose.

(b) Exo-B-glucanase (EC 3.2.1.91):

For assaying Exo-B-glucanase, cotton fibres were used as substrate (Mandels and Weber, 1969). 50 mg of cotton fibres, 1 ml of citrate buffer (P^H-5.5) and 1 ml of enzyme were incubated at 45±1°C for 24 hours. After incubation 3 ml of DNS reagent was added to each tube and the reaction mixture was kept in a boiling water bath for 5 minutes after which 0.5 ml of sodium potassium tartarate was added to each tube. The optical density of the solution was read at 640 nm on Hitachi 220 spectrophotometer. Standard curve was prepared by using different concentrations of glucose.

(c) B-1-4, glucosidase (EC 3.2.1.21):

β-glucosidase activity was measured by using cellobiose (0.5% w/v) (Selby and Maitland, 1967) as substrate. 0.5 ml of substrate was added to 1 ml of enzyme extract and the mixture was incubated at 50±1°C. The reaction was stopped after 1 hour by adding 1.5 ml of DNS reagent. It was boiled for 5 minutes in a boiling waterbath. Then, to it 0.5 ml of 40% of sodium potassium tartarate was added. The tubes were cooled under running tap water and the absorbance colour intensity was recorded at 640 nm on Hitachi 220 spectrophotometer. The standard curve was plotted by using different concentrations of glucose.

(B) Xylanase assay:

The amount of reducing sugar produced due to xylanase activity was measured by using xylan (1%) as the substrate. To one ml of the enzyme, 0.5 ml of the substrate was added. The mixture was incubated at 40 ± 1°C for one hour. The reducing sugar thus formed was estimated by using Dinitrosalicylic

acid method (Miller, 1972).

The soluble protein was estimated as mentioned below using Lowry et al's (1951) method.

(C) Estimation of protein:

Extracellular protein was estimated by the method of Lowry et al (1951) using Folin-ciocalteau reagent (Phenol reagent). 1ml of sample was mixed with 5 ml of reagent A (mixture of 2% Na_2CO_3 in 0.1N NaOH and 0.3% $CuSO_4$. $5H_2O$ in 1% sodium potassium tartarate in a ratio of 50:1). The mixture was kept for 10 minutes and thereafter 0.5ml of phenol reagent (phenol:water ::1:1) was added to it. It was allowed to stand for 30 minutes. The optical density was read at 750 nm. The standard curve was prepared by taking different concentrations of Bovine serum albumin.

(D) Preparation of reagents:

(i) Carboxymethyl cellulose reagent:

Carboxymethyl cellulose, Na salt (Sigma)---- 1g

Citrate buffer, P^H-5.5, 100 mM ----------100ml

(ii)Cellobiose reagent:

Cellobiose (sigma) ----------0.6g

Citrate buffer, P^H-5.5, 100 mM ------100ml

(iii) Dinitrosalicylic acid reagent:

Dinitrosalicylic acid -------1g

Sodium hydroxide (1%) ---------100ml

Phenol ----------200mg

Sodium sulphite ----------50 mg

(iv) Solution I - 2% Na_2CO_3 in 0.1N NaOH

Solution II - 0.3% $CuSO_4$. $5H_2O$ in 1% sodium potassium tartarate.

Solution A - Solution I: Solution II: : 50 : 1

RESULTS

(i) Effect of incubation length on cellulases:

(a) Endo -β-glucanase activity (Cx):

Maximum Cx activity was recorded on the 15th day of incubation after which there was a gradual decrease in the activity. This optimum incubation period was constant for all the test fungi (Fig.16).

(b) Exo-β-glucanase (C_1):

Production of exo-β-glucanase was maximum on the 10th day in all the test fungi. Before and after the 10th day of incubation period the activity was very low (Fig. 17).

(c) β-glucosidase activity:

In all the test fungi the β-glucosidase activity increased with increase

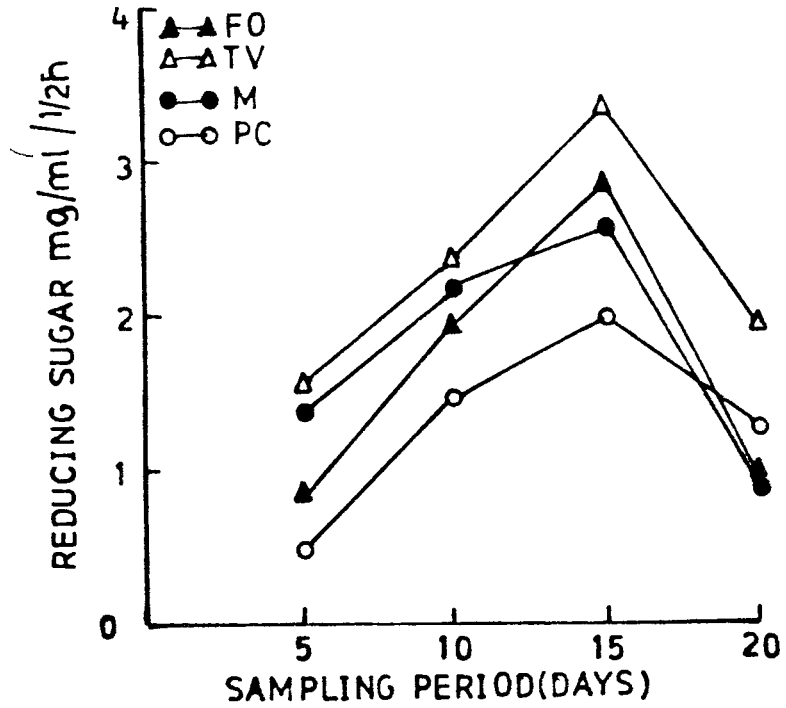


Fig.16. Effect of length of incubation period on the Endoglucanase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusarium oxysporum (FO) and their mixture (M).

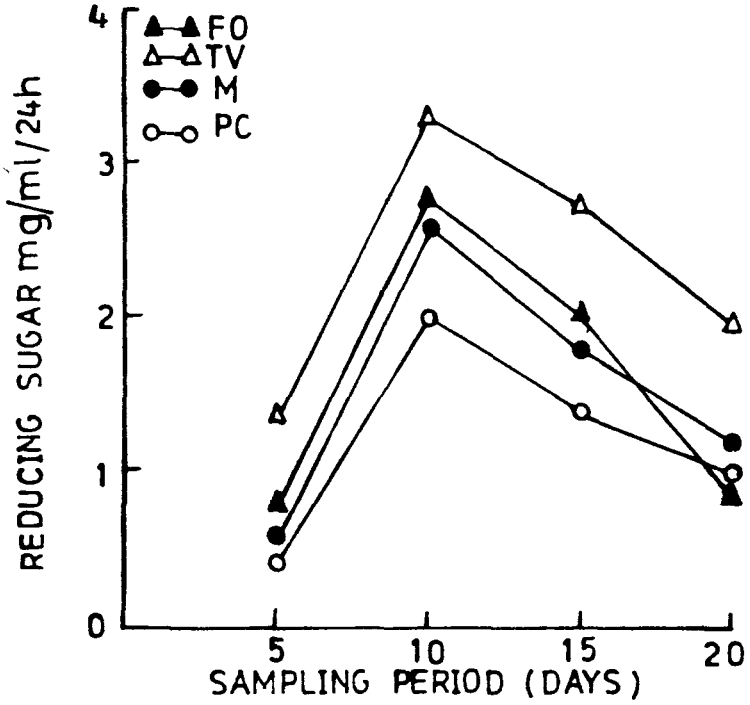


Fig. 17. Effect of length of incubation period on the exoglucanase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusarium oxysporum (FO) and their mixture (M).

in the incubation period till the 15th day, when maximum activity was recorded. The activity decreased with further increase in the incubation period (Fig. 18).

A sufficient amount of soluble protein was produced by all the test fungi. The highest amount of protein was estimated on the 15th day of incubation in all the cases (Table 20a).

In T.viride, F.oxysporum and mixture, the p^H of the medium shifted towards the acidic range where as in the medium inoculated with P.chrysogenum slight increase in the p^H was observed during the course of investigation (Table 20a).

The dry weight of mycelium increased with increase in the length of incubation period. On 5th day minimum dry weight of mycelium was obtained which increased till the 10th day of incubation after which it again decreased. The maximum mycelial dry weight was obtained in T.viride culture filtrate followed by mixture, P.chrysogenum and F.oxysporum (Table 20a).

On the basis of the results thus obtained, 15th day of incubation period was selected as the optimum incubation period for the production of different cellulases. Statistically, a significant ($P < 0.05$) variation was observed in the production of endoglucanase and cellobiase both due to length of incubation and test fungi. However, the production of exoglucanase varied significantly only due to the incubation length (Table 25).

((ii) Effect of length of incubation on the xylanase activity:

The maximum xylanase activity was recorded on the 10th day of incubation for all the test fungi. After that the activity declined with further increase in the incubation period (Fig. 27).

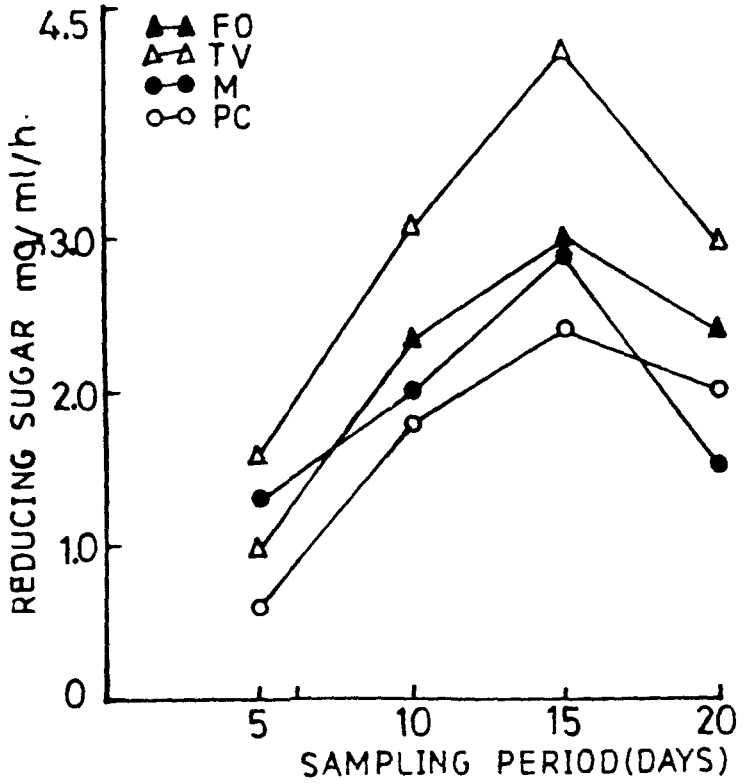


Fig. 18. Effect of length of incubation period on β-glucosidase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusarium oxysporum (FO) and their misture (M).

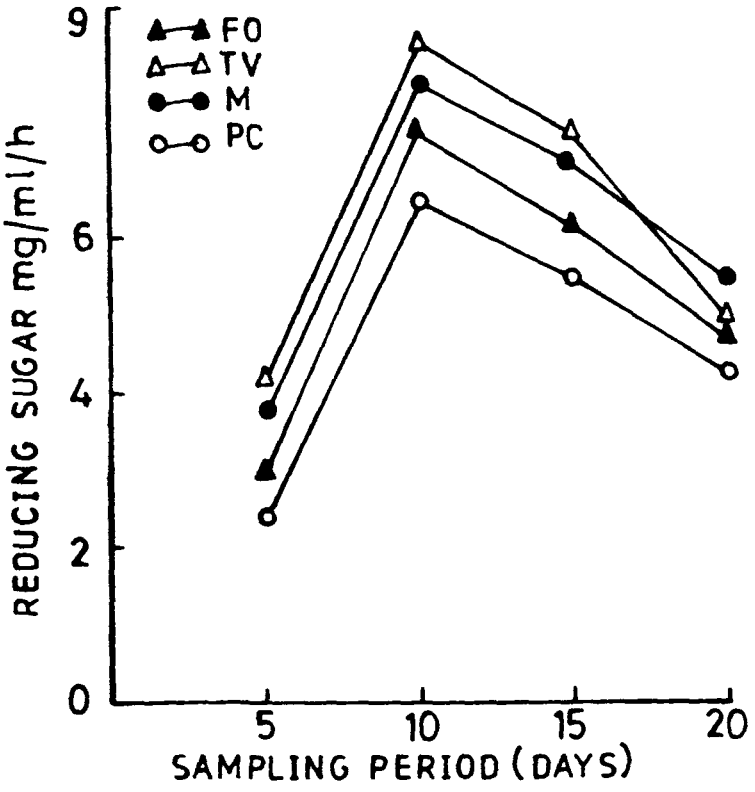


Fig. 27. Effect of length of incubation period on the xylanase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusarium oxysporum (FO) and their mixture (M).

Table 20a. Effect of incubation length on the production of protein (P = µg/ml), mycelial dry weight (DW = mg) and p^H of the medium by the test fungi.

Incubation Period (Days)	T.viride			P.chrysogenum			F.oxysporum			Mixture		
	P	DW	p^H	P	DW	p^H	P	DW	p^H	P	DW	p^H
5	92	38	5.3	80	35	5.6	84	28	5.4	112	35	5.7
10	126	56	5.2	105	49	5.8	109	50	5.2	120	52	5.3
15	174	78	5.0	154	69	5.3	155	60	5.0	143	75	5.1
20	120	96	4.9	126	78	4.7	94	70	4.6	100	84	4.9

Initial p^H of the medium = 5.5

Table 25. Analysis of variance test for cellulases (endoglucanase, exoglucanase and cellobiase) as affected by incubation length and test fungi.

Sources of Variation	Endoglucanase			Exoglucanase			Cellobiase		
	dF	MS	F	dF	MS	F	dF	MS	F
Incubation length (I)	3	16.55	31.46**	3	20.19	53.12**	3	24.12	56.42**
Test fungi (F)	3	4.15	7.89**	3	0.65	1.71	3	4.44	10.38**
I x F	9	2.31	4.39**	9	0.86	2.26	9	0.82	1.92
Error	64	0.526	-	64	0.38	-	64	0.428	-
Total	79	-	-	79	-	-	79	-	-

** $P < 0.01$

The incubation period for optimum production of soluble protein was also recorded on the 10th day. Unlike cellulases, the culture filtrate of all the test fungi became acidic with increase in incubation period (Table 20b).

(iii) Effect of p^H on cellulases:

Each of the fungal species tested for their cellulolytic activity under controlled conditions showed different p^H optima.

(a) Endo-β-glucanase activity:

The endo-β-glucanase activity was minimum at p^H - 4.5 for all the test fungi. Enzyme activity increased till p^H-5.5 after which it decreased with increase in p^H. F.oxysporum produced maximum reducing sugar (2.3 mg/ml) as a result of endo-glucanase activity followed by T.viride (2.1 mg/ml), mixture (2 mg/ml) and P.chrysogenum (1.6 mg/ml) (Fig.19).

(b) Exo-β-glucanase:

Unlike endo-β-gulcanase, the c_1 enzyme showed different p^H optima for different test fungi. However, at p^H-4.5 minimum activity was observed for all the test fungi. T.viride, which was most effecient in exo-β-glucanase production, produced maximum enzyme (3.9 mg/ml) at p^H - 6.0. F.oxysporum had slightly wider p^H range. In this fungi maximum enzyme was produced at p^H - 6.5 whereas, for mixture it was 5.5. P.chrysogenum was least effecient and produced the enzyme at P^H - 6.0 (Fig. 20).

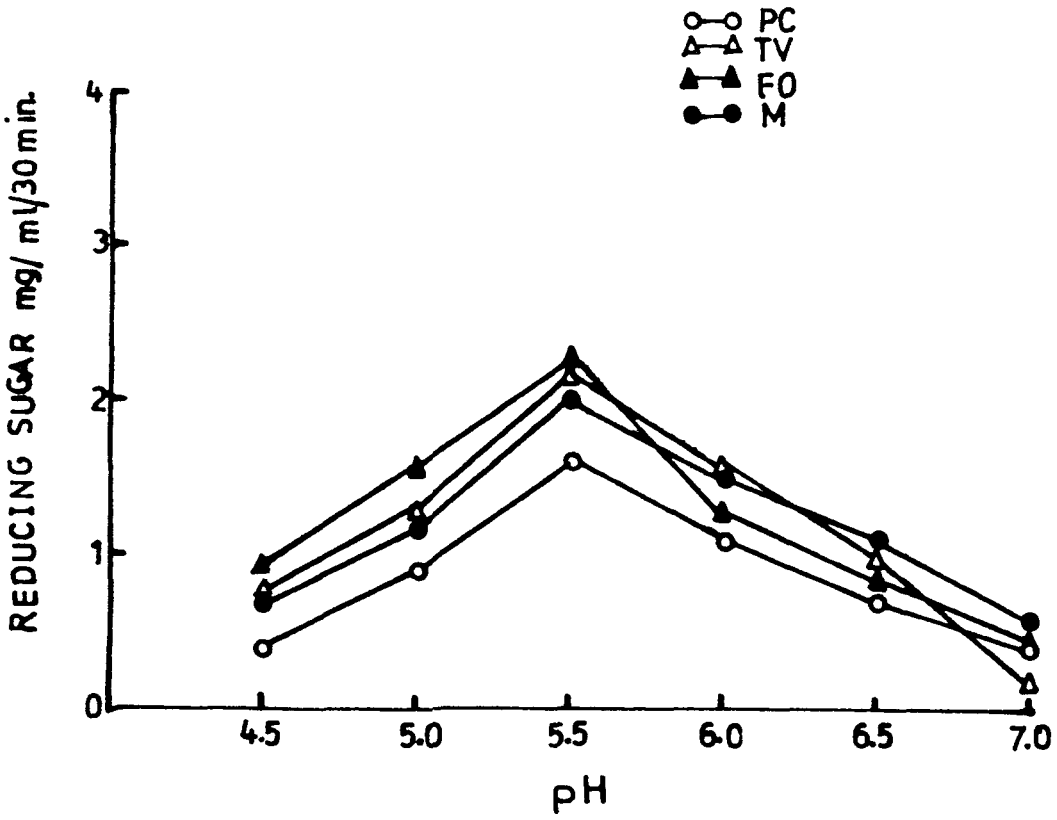


Fig. 19. Endoglucanase activity of Penicillum chrysogenum (PC), Trichoderma viride (TV), fusarium oxysporum (FO) and their mixture (M) at different p^H.

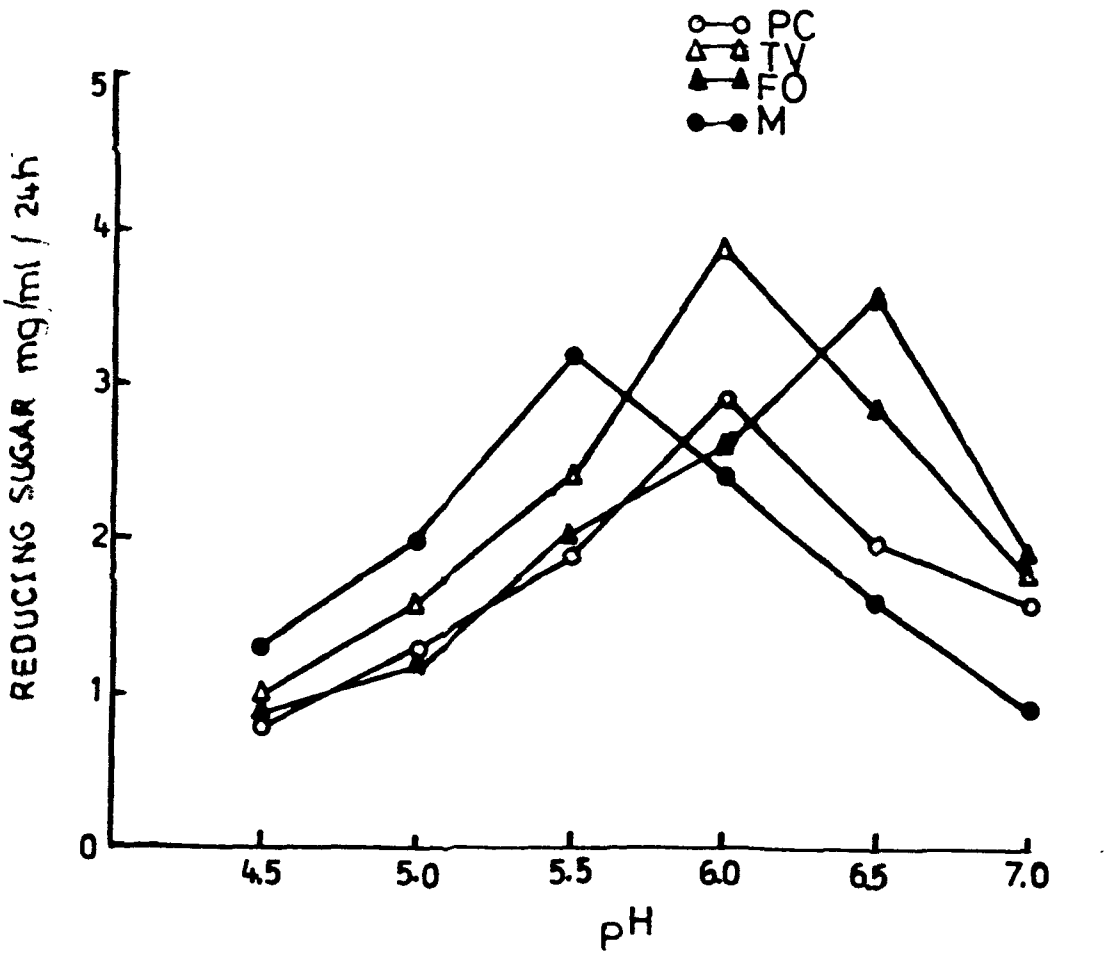


Fig. 20. Exoglucanase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusarium oxysporum (FO) and their mixture (M) at different p^H.

Table 20b. Effect of incubation length on the production of protein (P = µg/ml), mycelial dry weight (DW = mg) and p^H of the medium by the test fungi.

Incubation length (days)	T.viride			P.chrysogenum			F.oxysporum			Mixture		
	P	DW	p^H	P	DW	p^H	P	DW	p^H	P	DW	p^H
5	70	38	6.3	66	35	6.4	58	28	6.6	78	35	6.4
10	136	56	6.1	110	49	6.2	109	50	6.4	120	52	6.1
15	102	78	6.0	84	69	6.1	75	60	6.2	110	75	5.8
20	112	96	5.7	87	78	5.8	65	70	6.0	93	84	5.8

Initial p^H of the medium = 6.5

(c) β-glucosidase:

Both in T.viride and fungal mixture β-glucosidase activity reached the maximum values (4 mg/ml and 3.5 mg/ml respectively) at p^H - 5.0 whereas, for F.oxysporum highest β-glucosidase activity was observed at p^H - 5.5. It was observed that P.chrysogenum produced minimum enzyme in terms of reducing sugar (3.3 mg/ml) and peaked at p^H - 6.5 (Fig. 21). Both the test fungi and the test p^H significantly ($P < 0.05$) affected the production of different cellulases (Table 26).

(iv) Soluble Protein:

The optimum p^H for the release of soluble protein varied from test fungi to test fungi. In T.viride maximum protein was produced at p^H -5.5 and with further increase the amount of protein gradually decreased. Both for F.oxysporum and P.chrysogenum p^H -6.0 appeared as the optimum p^H for protein yield whereas, the mixture preferred slightly more acidic condition (P^H -5.5) for the optimum production of protein. In all the different test p^H, the p^H of the medium drifted towards acidity. The production of dry weight of mycelium varied along with the variation in p^H and test fungi used. Highest amount of mycelium was produced by T.viride at p^H -5.5 after which it decreased with further increase in p^H. For P.chrysogenum it was 5.5 while F.oxysporum produced the highest amount of dry mycelium at p^H -6.0 (Table 21).

(v) Effect of temperature on cellulases:

The optimum temperature for cellulolytic activity was 25°C in all the test fungi.

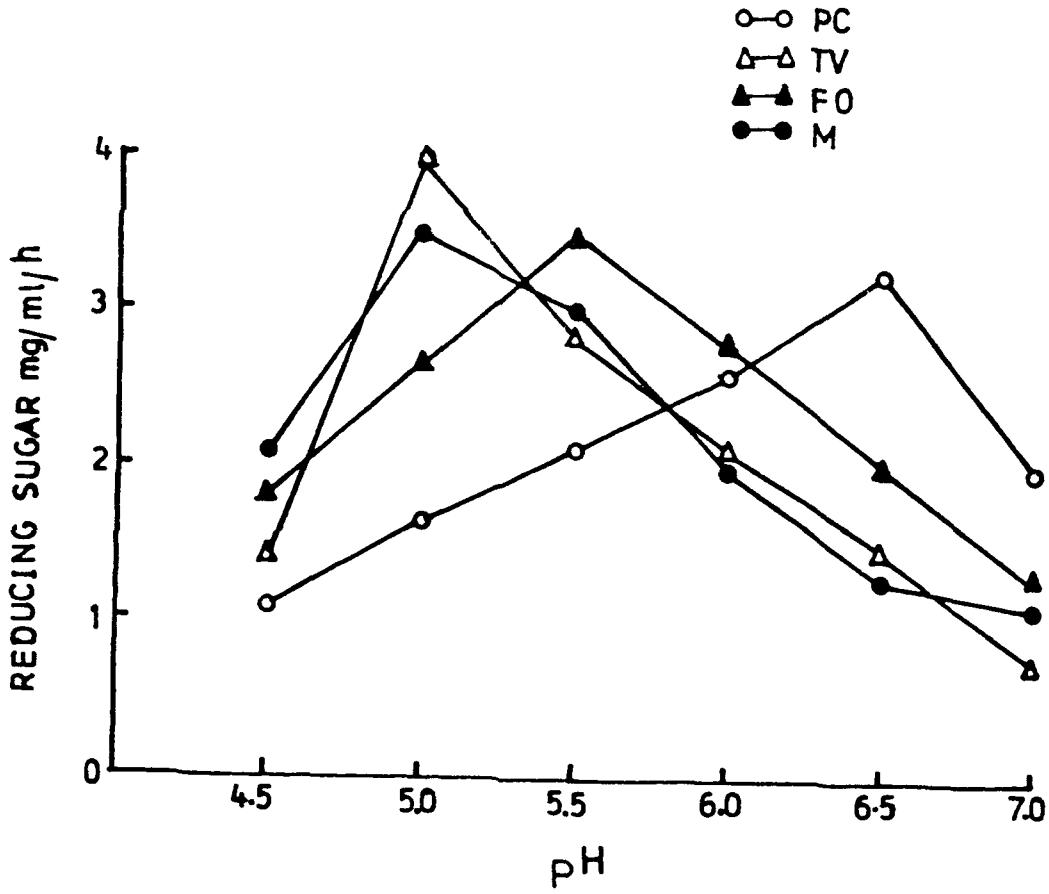


Fig. 21. β-glucosidase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusorium oxysporum (FO) and their mixture (M) at different pH

Table 21. Effect of p^H on the production of protein (P = µg/ml), mycelial dry weight (DW = mg) and p^H of the medium by the test fungi.

p^H	T.viride			P.chrysogenum			F.oxysporum			Mixture		
	P	DW	P^H	P	DW	p^H	P	DW	p^H	P	DW	p^H
4.5	85	47	4.47	70	52	4.45	73	43	4.44	90	58	4.44
5.0	100	56	4.94	92	58	5.01	98	60	4.80	115	68	4.94
5.5	212	88	5.21	106	75	5.13	131	76	5.18	168	69	5.20
6.0	184	75	5.84	132	69	5.72	165	89	5.76	140	68	5.60
6.5	143	61	6.4	128	70	6.33	89	74	6.27	129	77	6.26
7.0	90	63	6.8	102	65	6.97	72	80	6.7	80	70	6.85

Table 26. Analysis of variance test for cellulases (endoglucanase, exoglucanase and cellobiase) as affected by p^H and test fungi.

Sources of variation	Endoglucanase			Exoglucanase			Cellobiase		
	dF	MS	F	dF	MS	F	dF	MS	F
p^H (P)	5	6.198	79.46**	5	10.34	65.03**	5	8.93	35.16**
Test fungi (F)	3	1.197	15.35**	3	1.57	9.87**	3	0.437	1.72
P X F	15	0.225	2.88**	15	1.31	8.24**	15	2.38	9.37**
Error	96	0.078	-	96	0.159	-	96	0.254	-
Total	119	-	-	119	-	-	119	-	-

** $P < 0.01$.

(a) Endo- β -glucanase:

All the test fungi used in this experiment showed minimum Cx activity at 5°C. The activity increased with increase in temperature till 25°C where the optimum activity was recorded, which declined at 35°C. Among the test fungi T.viride produced maximum activity (2.9 mg/ml) followed by F.oxysporum (2.2 mg/ml), mixed inoculum (1.8 mg/ml) and P.chrysogenum (1.5 mg/ml) (Fig. 22).

(b) Exo-β-glucanase:

Like endo-β-glucanase, maximum reducing sugar due to C_1 enzyme was recorded at 25°C. Below and above this temperature, the exoglucanase activity was lowered. Among the four test fungi, T.viride produced highest amount of reducing sugar (3.8 mg/ml) and P.chrysogenum as least efficient species produced 2.5 mg/ml of reducing sugar (Fig. 23).

(c) β -glucosidase:

In all the test fungi minimum β-glucosidase activity was observed at 5°C. The activity got ameliorated with increase in temperature until it reached 25°C. At highest temperature (35°C) again the activity decreased. With reference to the reducing sugar produced by different test fungi the pattern was almost similar to that of exo-β-glucanase (Fig. 24). The production of endo-β-glucanase, exo-β-glucanase & β-glucosidase varied significantly ($p < 0.05$) due to temperature as well as test fungi (Table 27).

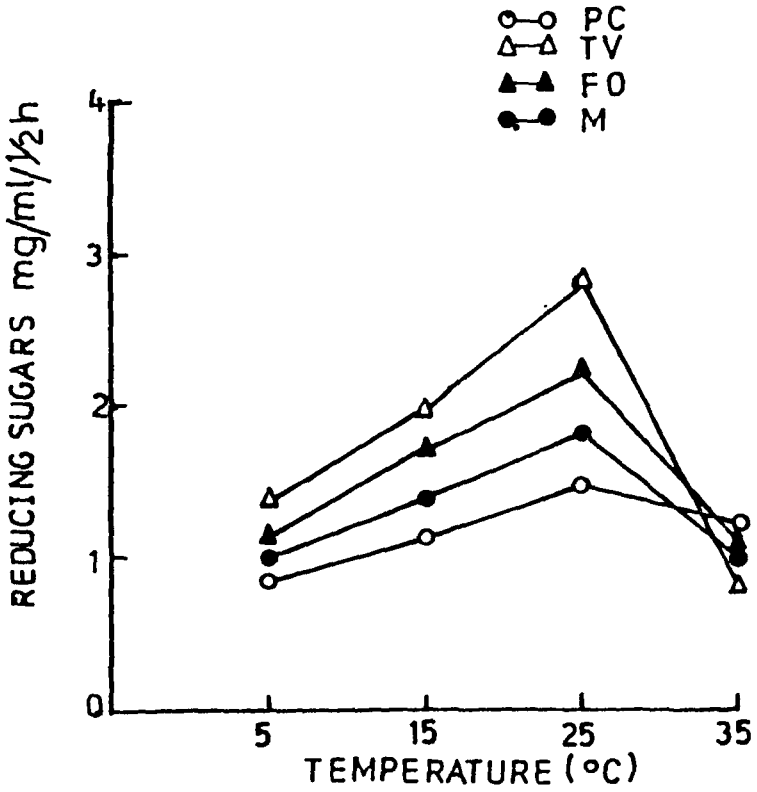


Fig. 22. Endoglucanase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusarium oxysporum (FO) and their mixture (M) at different temperatures.

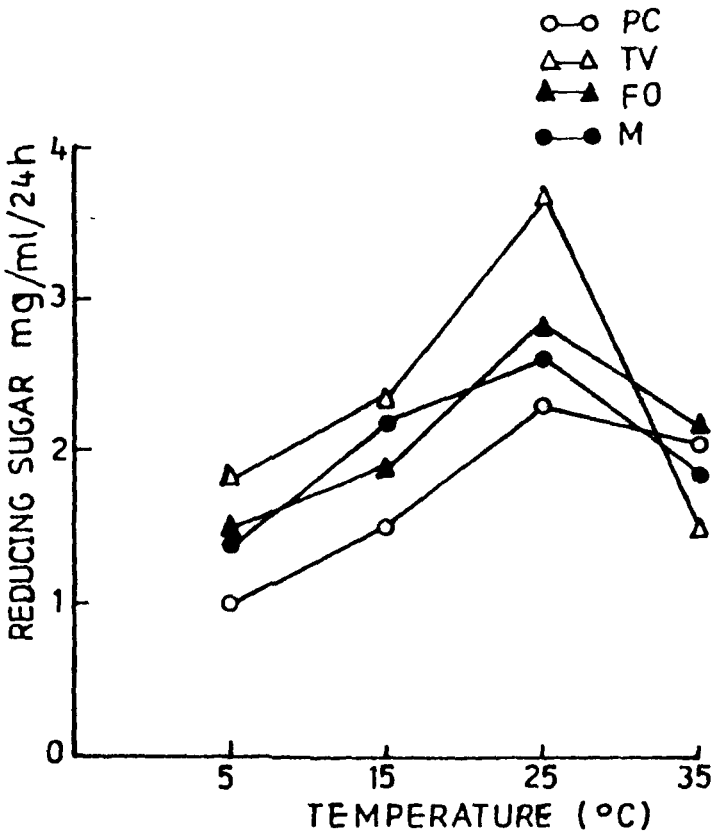


Fig. 23. Exoglucanase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusarium oxysporum (FO) and their mixture (M) at different temperatures.

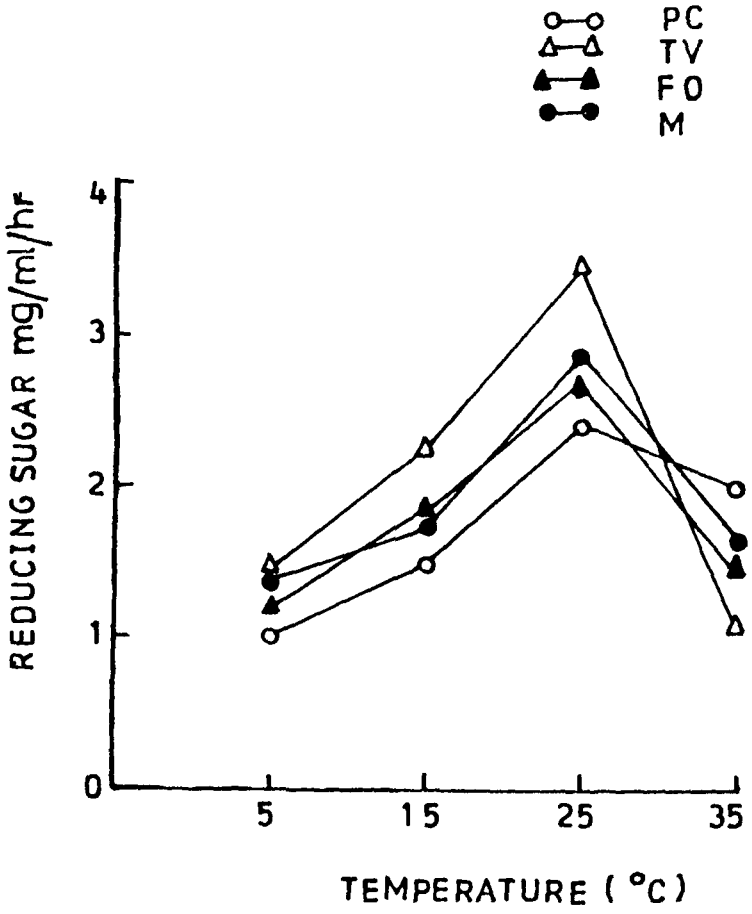


Fig. 24. β-glucosidase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusarium oxysporum (FO) and their mixture (M) at different temperatures.

Table 27. Analysis of variance test for cellulases (endoglucanase, exoglucanase and cellobiase) as affected by temperature and test fungi.

Sources of variation	Endoglucanase			Exoglucanase			Cellobiase		
	dF	MS	F	dF	MS	F	dF	MS	F
Temperature (T)	3	4.608	42.28**	3	6.973	69.04**	3	9.13	73.04**
Test fungi (F)	3	1.380	12.66**	3	1.853	18.34**	3	0.547	4.38**
T x F	9	0.590	5.41**	9	0.596	5.91**	9	0.719	5.75**
Error	64	0.109	-	64	0.101	-	64	0.125	-
Total	79	-	-	79	-	-	79	-	-

** P< 0.01.

(d) Soluble Protein:

Like cellulases, the temperature optima for the production of soluble protein was also found to be 25°C for all the test fungi. Like other treatments, at different temperatures too the p^H of the medium became acidic towards the end. The highest amount of mycelial dry weight was recorded at 25°C after which it decreased in case of most of the test fungi. The minimum amount of dry mycelium was obtained at 5°C (Table 22).

(vi) Effect of p^H on xylanase:

The different test fungi showed differential xylanase activity at different p^H. However, minimum reducing sugar was estimated at lowest p^H (4.5) due to xylanase activity in all the test fungi. T.viride produced maximum enzyme (8.3 mg/ml) at p^H -5.5 whereas P.chrysogenum and F.oxysporum had p^H - 6.5 optima for enzyme activity. In mixture the maximum xylanase activity was observed at p^H - 6.0. Among all the fungi, T.viride was most effecient in producing the xylanase enzyme. Like cellulases, even in case of xylanase the p^H of the medium became slightly more acidic towards the end of sampling period. Highest amount of dry mycelium was produced by T.viride and mixture growing at p^H - 5.5 while for P.chrysogenum it was at p^H - 6.0 and F.oxysporum p^H - 6.5. Highest amount of soluble protein was produced by T.viride followed by mixture F.oxysporum and P.chrysogenum (Table 23 and Fig. 25).

(vii) Effect of temperature on xylanase:

The xylanolytic activity in all the four test fungi was optimum at 25°C.

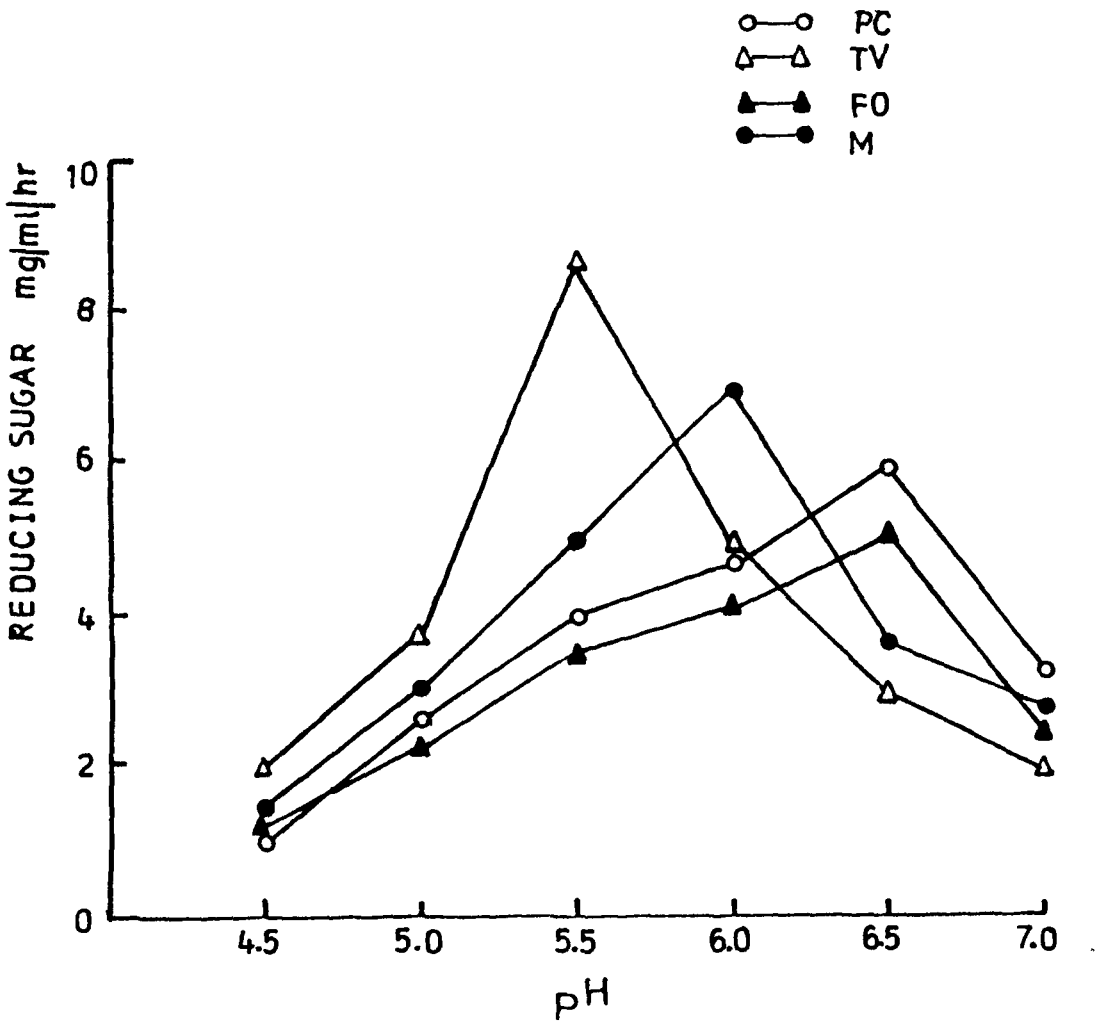


Fig. 25. Xylanase activity of Penicillium chrysogenum (PC) Trichoderma viride (TV), Fusarium oxysporum (FO) and their mixture (M) at different pH.

Table 22. Effect of temperature on the production of protein (P = µg/ml), mycelial dry weight (DW = mg) and p^H of the medium by the test fungi.

Temperature (°C)	*T.viride*			*P.chrysogenum*			*F.oxysporum*			Mixture		
	P	DW	p^H	P	DW	p^H	P	DW	p^H	P	DW	p^H
5	93	37	5.35	75	29	5.50	79	28	5.45	108	43	5.48
15	118	53	5.28	106	46	5.46	102	48	5.31	133	55	5.31
25	210	65	4.94	121	55	5.38	188	60	5.20	245	64	5.27
35	149	60	4.96	128	62	5.40	178	65	5.25	158	68	5.15

Initial p^H of the medium = 5.5

Table 23. Effect of p^H on the production of protein (P = µg/ml), mycelial dry weight (DW = mg) and p^H of the medium by the test fungi.

p^H	*T.viride*			*P.chrysogenum*			*F.oxysporum*			Mixture		
	P	DW	p^H	P	DW	p^H	P	DW	p^H	P	DW	p^H
4.5	85	55	4.3	60	40	4.8	65	48	4.2	78	50	4.4
5.0	108	68	4.8	93	56	4.7	87	62	4.5	90	67	4.7
5.5	155	104	5.4	118	79	5.2	112	69	5.2	136	99	5.3
6.0	134	85	5.7	138	89	5.4	125	79	5.4	148	90	5.8
6.5	120	68	5.9	148	80	6.2	98	88	6.3	128	90	5.9
7.0	96	46	6.7	120	76	6.5	85	80	6.6	110	78	6.5

Below and above this temperature the activity was markedly reduced. The highest amount of reducing sugar (9.8 mg/ml) was measured in T.viride. It was followed by mixture (7.5 mg/ml) P.chrysogenum (6mg/ml) and F.oxysporum (Fig. 26)

Maximum dry weight was obtained at 25°C by all the test fungi. T.viride produced maximum mycelial dry weight followed by mixture, F.oxysporum and P.chrysogenum. The p^H of the medium became acidic towards the end of the experiment (Table 24). The production of xylanase varied significantly ($P < 0.05$) due to temperature, p^H and the test fungi used (Table 28).

DISCUSSION

From the results obtained during this investigation it was apparent that the incubation period markedly affected the production of cellulases and xylanase. The results have also shown that the optimum period of incubation for cellulolytic and xylanolytic activities varied from organism to organism. The results were confirmed by other worker (Awasthy, 1987). Optimum amount of exo-B-glucanase (on 10th day of incubation) was estimated prior to endo-B-glucanase and cellobiase. This can be explained on the basis of the conclusions drawn by Reese et al (1950), who stated that exo-B-glucanase initiates the attack on cellulose by disaggregating the anhydroglucose chain while the endo-B-glucanase hydrolyse them to soluble sugars. This can also be due to the fact that exoglucanase acts more rapidly on longer chains and the rate falls off rapidly from tetramer to trimer to dimer (Reese, 1968). Appearance of xylanase before cellulases is in accordance with the results of Domsch and Gams (1969). Domsch and Gams (1969) screened various fungal species for their cellulolytic and xylanolytic activities and concluded that

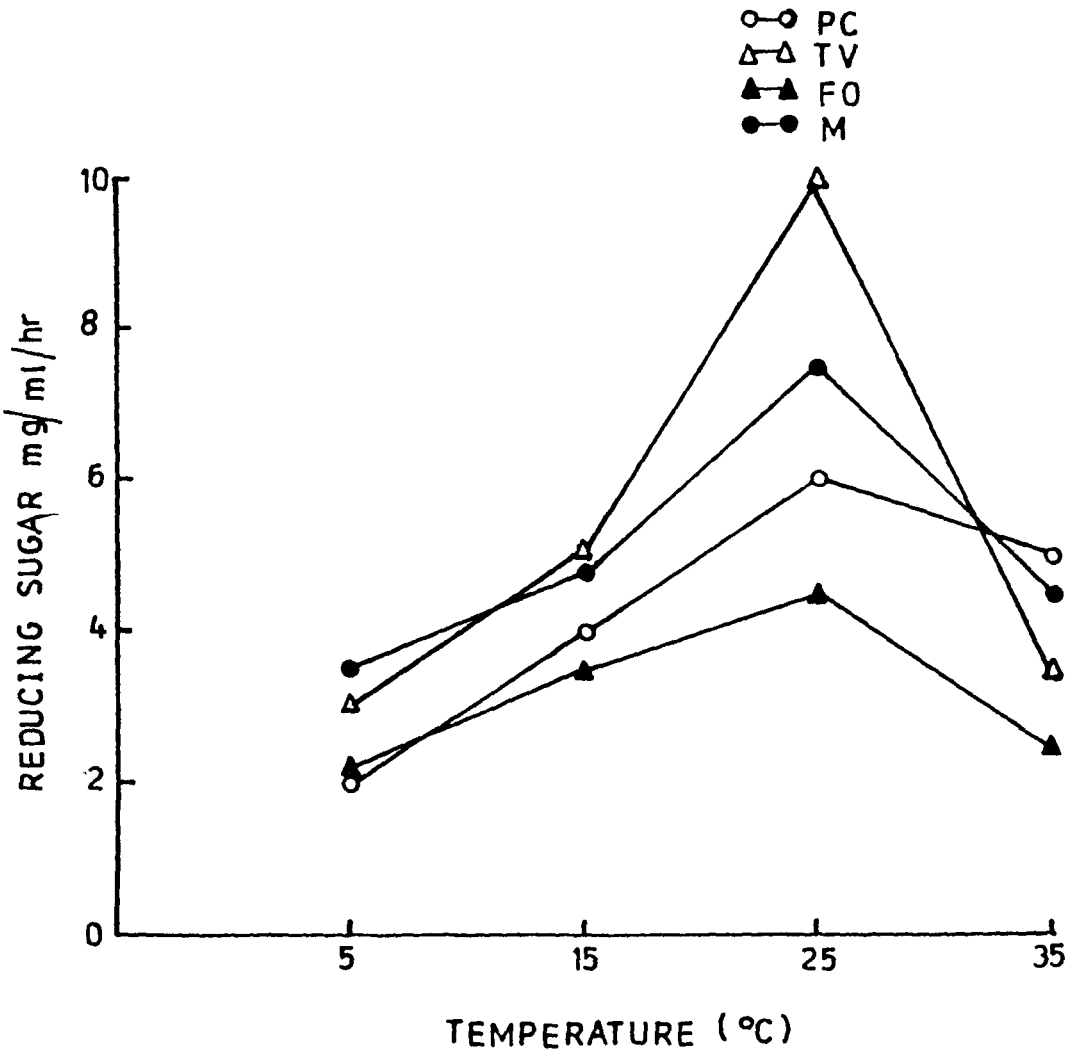


Fig. 26. Xylanase activity of Penicillium chrysogenum (PC), Trichoderma viride (TV), Fusarium oxysporum (FO) and their mixture (M) at different temperatures.

Table 24. Effect of temperature on the production of protein (P = μg/ml), mycelial dry weight (DW = mg) and p^H of the medium by the test fungi.

Temperature (°C)	T.viride			P.chrysogenum			F.oxysporum			Mixture		
	P	DW	p^H	P	DW	p^H	P	DW	p^H	P	DW	p^H
5	82	43	6.0	69	38	6.4	73	35	6.3	84	40	6.0
15	124	58	5.8	94	49	6.6	98	50	6.5	110	55	6.2
25	168	90	5.6	134	71	6.3	126	65	6.2	150	78	5.7
35	128	68	6.1	120	80	6.0	84	60	6.0	130	89	6.2

Initial p^H of the medium = 6.5

Table 28. Analysis of variance test for xylanase as affected by temperature, p^H, incubation length and test fungi.

Temperature				p^H				Incubation length			
Sources of variation	dF	MS	F	Sources of variation	dF	MS	F	Sources of variation	dF	MS	F
Temperature (T)	3	58.6	218.66**	p^H (P)	4	59.6	693.02**	Incubation length (I)	3	102.7	71.32
Test fungi (F)	3	16.23	60.56**	Test fungi (F)	3	5.19	60.35**	Test fungi (F)	3	12.033	8.356
T X F	9	4.39	16.38**	P X F	12	10.6	119.1**	I X F	9	3.45	2.396
Error	64	0.268	-	Error	96	0.086	-	Error	64	1.44	-
Total	79	-	-	Total	119	-	-	Total	79	-	-

** P< 0.01.

hemicellulose is more easily attacked than cellulose.

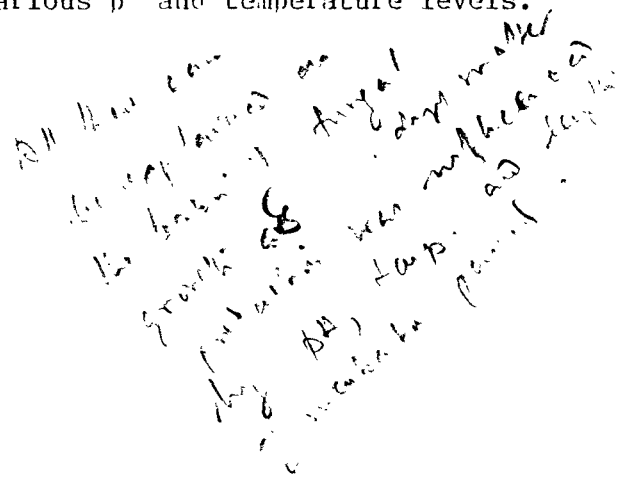
p^H of the culture filtrates shifted towards acidity with increase in incubation period except in Penicillium chrysogenum. This suggested that the isolates were acid producers as reported by Mandels (1975) in Trichoderma sp. The drop in p^H can be directly related to the substrate consumption (Gallo et al,1979).

The results suggest that both xylanase and cellulases were highly sensitive to p^H. Response of fungal species may differ greatly in enzyme producing capability in response to extracellular p^H. Endo-β-glucanase could tolerate a narrow range of p^H (optimum p^H - 5.5 for all the test fungi), while β-glucosidase possessed a wider range of p^H (5.0-6.5). Similar results were also obtained by Iwasaki et al (1965) and Awasthy (1987). The maximum exo-β-glucanase activity at p^H -6.0 in T.viride was similar to the results of Gritzali and Brown (1979) who also obtained very high activity in Trichoderma sp. at p^H - 6.0. Xylanase activity was also influenced by p^H which varied from species to species. The optimum p^H for different species ranged from 5.5 to 6.5. Bachmann and McCarthy (1989) obtained a wide p^H range of 5.0 to 9.0 for xylanase while working with actinomycetes.

Both xylanase and cellulases were markedly influenced by temperature. The maximum enzyme production was at 25°C. Chang (1967) on the basis of his results concluded that mesophilic fungi were more efficient in utilizing both cellulose and hemicellulose. With the degree of temperature the activity of fungi either decreased or increased. Generally, fungi were susceptible to high temperatures than low temperatures which explained the polysaccharase activity at 25°C (Nikhra,1981).

From the results it can be concluded that incubation period, temperature along with p^H constitute major factors which influence the production of

cellulases and xylanase by different fungi. Among the fungal species selected for the study, T.viride was most effecient both in terms of producing cellulases and xylanase. Therefore, T.viride can be exploited for improving the decomposition rate of litters at various pH and temperature levels.



GENERAL DISCUSSION

Population of fungi and bacteria was observed less in the beginning of litter decomposition (Fig.2). This was due to the unavailability of labile nutrients which were still in the complex form. Winter months also supported reduced microbial population on litter which was attributed to unfavourable environmental conditions especially low litter moisture and temperature in field conditions. Similar results have also been obtained by Rai and Srivastava (1982). At lower and higher altitudes the fungal and bacterial peaks were observed in May and June, and June and July respectively (Fig.2). Suitable moisture content and temperature favoured the rate of decomposition which resulted in the mobilization of nutrients from the litter required for microbial growth. At lower altitude total number of fungi and bacteria were higher compared to higher altitude. This was correlated to the dominance of temperature over moisture (Shanks, 1954 and Witkamp, 1963). Maximum number of fungi and bacteria on Ageratum conizoides litter (Fig. 2) was due to the availability of high amount of initial soluble sugars, amino acids and total nitrogen, where as their low concentration and complexity might have acted as a limiting factor in woody leaf litters.

The occurrence of more deuteromycetes and ascomycetes on woody litter at the early stage of decomposition was correlated to their ability to utilize locked up nutrients more efficiently. Dominance of phycomycetous forms on herbaceous litter suggested their greater affinity towards simple organic constituents like sugars and amino acids. Dominance of Trichoderma viride (Table 5-8) and Penicillium chrysogenum (Table 1-4) suggested their active role in the decomposition of various kinds of nutrients locked up in the litter. Their wide occurrence could also be explained in terms of their capability to uti-

lize different phytotoxic substances from decomposing litter (Black and Dix, 1976).

A.conizoides litter decomposed much faster than M.philippinensis, H.antidysenterica and V.glabrata litters (Fig.28). This was attributed to the significant positive correlation with fungal and bacterial populations (Table 12), which was mainly assigned to high initial nitrogen content of A.conizoides litter (Taylor et al, 1989 and Upadhyay and Singh, 1989). The slow rate of decomposition of pine litter (Fig.29) was correlated to its higher lignin content. Das (1980) has also attributed the slow rate of decomposition of pine litter to high lignin content and impervious nature of cuticle. The fast decomposition of A.nepalensis litter than M.esculenta and P.kesiya litters may be due to its broader leaf nature (Laishram and Yadava,1988) and significant positive correlation with total nitrogen content of the decomposing litter (Table 13). The differential decomposition rate might be due to different physical nature of different plant material (Bhatt et al, 1985), moisture content and substrate quality which governed the distribution of microflora (Swift et al, 1979)

In general, different types of organic constituents were more in the beginning of litter decomposition which decreased with time (Fig.6&7) This was attributed to their positive correlation with the weight remaining of decomposing litter (Table 12&13)However, lignin was correlated negatively with weight remaining. This may be due to the complexing of soluble phenols with proteins (Schlesinger, 1985) and formation of phenolic and other aromatic compounds which could be analysed as lignin (Berg and Theander, 1984). Sugars and amino acids degraded more rapidly than cellulose, hemicellulose and lignin (Fig. 6 and 7). This may be due to the preferential utilization of these compounds by microbes (Harper and Lynch, 1981).

An increase in the initial nitrogen content was observed in the beginning of decomposition at higher altitude (Fig. 7) which was attributed to the demand for nitrogen by the microbes in which the nitrogen gets immobilized. The initial increase in P content at higher altitude was also observed which was supported by the results obtained by Schlesinger and Hasey (1981). Fast rate of decomposition during May-July may be due to the negative correlation obtained between weight remaining and moisture content of the decomposing litter (Fig.4&5).Stott et al (1986) and Tiwari (1988) also ascribed the faster rate of decomposition to high temperature and conducive water potential.

The negative correlation obtained between cellulase and the amount of cellulose of decomposing litter (Table 14&15)suggests that the amount of cellulose acted as a limiting factor for cellulase activity. The correlation between cellulase and moisture content in most of the litter (Table 14) has also been confirmed by the results of Ross (1981). The differential amount of cellulase, amylase and invertase extracted from different litters may be attributed to their different chemical composition (Spalding, 1980). The significant positive correlation of amylase and cellulase with fungal and bacterial populations confirmed their microbial origin (Miele and Linkins, 1978). The low cellulase, amylase and invertase activities observed in V.glabrata and P.kesiya litters was attributed to high phenolic content which might have inhibited the enzymes (Benoit and Starkey, 1968). Low enzyme concentration recorded at higher altitude has suggested that biochemical metabolism of plant remains would be slower at higher altitude than at lower altitude (Ross, 1981). A significant positive correlation between invertase and remaining amount of sugars has suggested the substrate specific activity of the enzyme.

The difference in the rate of decomposition due to different fungal species under controlled conditions (Fig.12&14)has suggested that these species

vary in their decomposing abilities. This also explained the preferential use of different substrates by fungi. The rate of decomposition recorded at different test temperatures varied significantly. Maximum decomposition of litter was at 25°C, which was mainly due to the optimum production of various enzymes by the test fungi. Slow rate of decomposition at low temperatures might be due to the inactivation of fungi (Fig.12 a & b). Partially decomposed (duff) litters decomposed faster than fresh ones (Fig.13a&b) which explained that fungi could utilize the partially decomposed substrates more rapidly than fresh ones (Rai and Srivastava, 1983). An improvement in the decomposition rate of fragmented litter was observed over intact one, which was assigned to the exposure of increased surface area for microbial attack (Das, 1980). An increase in lignin content during last phase of decomposition was mainly due to the resistant nature of lignocellulosic complexes to microbes (Summerell and Burgess, 1989). Rapid degradation of sugars, amino acids, cellulose and hemicellulose in the beginning of decomposition process was attributed to their simple chemical nature and improved utilization by the microbes (Harper and Lynch, 1981). Initially less of sugars, amino acids, cellulose and hemicellulose were estimated from the partially decomposed litters than fresh ones. The low concentration of these organic constituents must have caused an improvement in their rate of decomposition over the fresh ones (Fig. 15a & b)

Production of cellulolytic and xylanolytic enzymes by microbes varied from organism to organism. Similar result was obtained by Awasthy (1987). Optimum level of exo-β-glucanase was obtained prior to endo-β-glucanase and β-glucosidase (Fig.16&18).This can be explained by the hypothesis of Reese et al (1950) who hypothesised that exo-β-glucanase initiated the attack on cellulose by disaggregating the anhydro glucose chain while endo-β-glucanase hydrolysed them to soluble sugars. The optimum production of xylanase occurred

before cellulases. This observation has been supported by Domsch and Gams (1969), who concluded that hemicellulose is easily attacked than cellulose. In general, a drift in p^H of culture media towards acidic range was observed with increase in incubation period (Table 20). This may be due to the acid producing ability of the fungal isolates (Mandels, 1975) or to the substrate consumption (Gallo et al, 1979).

Both cellulases and xylanase activities were influenced by the length of incubation, temperature and p^H of the medium. Maximum concentration of different enzymes assayed at 25°C, supported that mesophilic fungi were more efficient in utilizing hemicellulose or cellulose (Chang, 1967).

The results show that altitude has a marked effect on microbial population and fungal succession which ultimately govern the decomposition of various plant litters. The distribution of microbial population is not only influenced by various environmental factors but also by the chemical nature of litters. The differential rate of decomposition in different forest stands shows that the vegetational cover which has influenced microbial population has also affected the litter decomposition.

SUMMARY

The study was conducted in order to find out the effect of altitudinal variation and vegetational cover on microbial succession, decomposition and enzymes under forest ecosystem. The study sites were situated at two altitudes i.e. at higher altitude (1500m MSL) at Shillong and at lower altitude (100m MSL) at Byrnihat. At each altitude two forest stands of different regenerational stages were selected. Two plant species, dominant and codominant, were selected in each forest stand. At lower altitude, Ageratum conizoides and Mallotus philippinensis in young stand and Holarrhena antidysenterica and Vitex glabrata in old stand were selected. At higher altitude, Alnus nepalensis and Pinus kesiya were selected in old forest stand whereas, Myrica esculenta and Pinus kesiya were taken from young stand.

Quantitatively the bacterial population was more than the fungal population at both the altitudes. Bacterial and fungal populations were less in the beginning of litter decomposition but increased with the progress of decomposition. Microbial population was minimum at the end of litter decomposition. At lower altitude, A.conizoides litter harboured maximum fungal and bacterial populations followed by M.philippinensis, V.glabrata and H.antidysenterica. In young and old stands the microbial (fungal and bacterial) population attained its peak in May-June. The litters at lower altitude harboured more microbial population than at higher altitude.

At higher altitude, litter of A.nepalensis supported more fungal and bacterial population compared to M.esculenta and P.kesiya. A marked seasonality in microbial population was observed on all the litters. Bacterial population exhibited peaks in July and September, while the fungal population showed its

peaks in June and September. During winter months, the litter harboured minimum microbial population.

Eighteen fungal species were isolated from litter at higher altitude and twenty five species at lower altitude. Penicillium chrysogenum was dominant at lower altitude, while Trichoderma viride at higher altitude. The weedy leaf litter harboured more of phycomycetous fungi compared to its woody counterparts where the primary colonizers were mainly deuteromycetes followed by ascomycetes and few phycomycetes.

Fungal and bacterial populations showed significant positive correlation with the moisture content of litter at both the altitudes.

The herbaceous litter decomposed faster (K=2.930) at lower altitude compared to woody litters of M.philippinensis (K=1.587), H.antidysenterica (K=1.279) and V.glabrata (K=1.139). The decomposition of plant litter at higher altitude was slow than at lower altitude. Litter of A.nepalensis (K=0.810) decomposed faster than M.esculenta (K=0.526) and P.kesiya (K(old)= 0.398 and K (young)= 0.372). The relative weight loss of all the litters except M.philippinensis was correlated significantly with fungal population and bacterial population.

The litter at lower altitude were less acidic than at higher altitude which became more acidic towards the end of decomposition. The moisture content of litters ranged from 17.8 to 66% at lower altitude and 13.9 to 72% at higher altitude.

Quantitatively organic constituents were maximum in the early stage of decomposition than its final stage. The amount of cellulose, hemicellulose, total soluble sugars and amino acids was more in A.conizoides than M.philippinensis, H.antidysenterica and V.glabrata litters. The lignin content was, however, more in V.glabrata. Lignin degradation started towards the later part of litter decomposition. At higher altitude the lignin content was maximum

in pine litter and minimum in A.nepalensis. The latter had more cellulose, hemicellulose, total soluble sugars and amino acids. At both the altitudes the absolute weight loss of different litters showed a significant negative correlation with the weight remaining of different organic constituents. While lignin content was correlated positively with absolute weight loss.

The initial nitrogen content was more in A.conizoides followed by M.philippinensis, H.antidysenterica and V.glabrata. The phosphorus content, however, was maximum in H.antidysenterica and minimum in A.conizoides. At higher altitude A.nepalensis had maximum initial nitrogen and phosphorus contents followed by M.esculenta and P.kesiya. Unlike nitrogen, which was retained for some time in the litter, phosphorus was released along with the decomposition of litter at lower altitude. At higher altitude, however, the retention of nitrogen and phosphorus was for longer period. The absolute weight loss of different litters showed negative correlation with their nitrogen and phosphorus contents.

Both amylase and cellulase activities were less in the beginning of decomposition and increased with time, while a reverse pattern was observed for invertase. At lower altitude, the cellulase activity was more in M.philippinensis followed by H.antidysenterica, V.glabrata and A.conizoides. Amylase activity was more in M.philippinensis and H.antidysenterica followed by V.glabrata and A.conizoides. Unlike amylase and cellulase, the invertase activity was more in A.conizoides and less in V.glabrata. All the enzymes showed a marked seasonal variation. Cellulase and amylase showed a significant positive correlation with fungal and bacterial populations of litter. A positive correlation was established between cellulase, amylase and absolute weight loss of different litters. The invertase activity, however, showed a negative correlation with fungal and bacterial populations and absolute weight loss. Maximum cellulase, amylase and invertase enzymes were extracted from the litter of A.nepa-

lensis followed by M.esculenta and P.kesiya at higher altitude. The seasonal variation in microbial enzymes was similar to lower altitude. Cellulase and amylase activities showed a significant correlation with fungal and bacterial populations. However, absolute weight loss showed a positive correlation with cellulase and negative correlation with amylase activity. A negative correlation was also observed between weight remaining of cellulose and cellulase. The invertase activity showed a positive correlation with fungal and bacterial populations. The weight remaining of total soluble sugars was correlated significantly with invertase activity of all the litters.

Litter of H.antidysenterica decomposed faster than P.kesiya under laboratory conditions. The rate of decomposition showed a negative correlation with temperature. The optimum temperature for decomposition was observed at 25°C. The mixture of the two test fungi i.e. T.viride and P.chrysogenum ameliorated the decomposition compared to their individual rate. The decomposing ability of different fungal species varied significantly ($P < 0.05$) at different temperatures. Maximum weight loss in litter of H.antidysenterica occurred after 120 days at 5°C and 15°C, while after 90 days at 25° and 35°C. On the contrary, it could be achieved after 150 days at 5° and 15°C and after 120 days at 25° and 35°C respectively in P.kesiya litter.

Litter quality had a marked effect on the rate of decomposition. The duff decomposed much faster than either fresh fragmented or intact litter. Maximum loss of duff (partially decomposed litter) occurred after 60 days in H.antidysenterica and 90 days in P.kesiya. Intact litter of H.antidysenterica and P.kesiya took more than 90 and 120 days respectively. The decomposition constant (K) of duff, fresh fragmented and intact litters varied significantly. The test fungi differed in their decomposing ability. Sugars and amino acids along with cellulose and hemicellulose were microbially degraded rapidly in the beginning of decomposition. The decomposition of different organic con-

stituents was faster at 25°C compared to 5°C,15°C and 35°C. Lignin decomposition took place only towards the end of the biodegradation process.

Total sugars and amino acids, hemicellulose and cellulose were estimated less in duff compared to fresh litter. However, lignin was more in partially decomposed litter than fresh ones. The decomposition of lignin in partially decomposed H.antidysenterica litter started after 90 days while in case of P.kesiya litter it began after 120 days.

Maximum endo-β-glucanase and β-glucosidase were produced on 15th day of microbial inoculation after which it decreased gradually. Production of exo- B-glucanase was, however, maximum on 10th day of fungal inoculation. The p^H of the medium inoculated with T.viride, F.oxysporum and mixture became more acidic while in P.chrysogenum inoculated medium slight increase in p^H was observed. The dry weight of mycelium of all the test fungi increased with increase in the length of incubation. It was maximum for T.viride. The maximum xylanase activity was recorded on 10th day of incubation in all the test fungi. Mycelial dry weight was minimum on 5th day which increased till 10th day and declined again. The maximum mycelial dry weight was obtained in culture filtratesof T.viride and F.oxysporum.

Different test fungi showed different p^H optima for the production of cellulases. Endo- β-glucanase activity was maximum at p^H - 5.5 for all the test fungi, while the production of exo- β-glucanase varied with p^H. T.viride and P.chrysogenum produced maximum exo-β-glucanase at p^H 6.0, while F.oxysporum and the mixture respectively at p^H 6.5 and 5.5. T.viride, F.oxysporum, P.chrysogenum and their mixture produced optimum β-glucosidase respectively at p^H 5.0, 5.5, 6.5 and 5.0. Maximum soluble protein was produced at p^H - 5.5 by T.viride and at p^H - 6 by F.oxysporum and P.chrysogenum. The variation in cellulase production at different test p^H was statistically significant. The mixture of these fungi preferred slightly more acidic condition (p^H -5.5) for

the optimum production of protein. At all the different test p^H, the p^H of the medium drifted towards acidity. Mycelial dry weight varied from p^H to p^H and the specific test fungi.

Fungi showed differential xylanase activity at different p^H. T.viride produced maximum xylanase at p^H -5.5 whereas, P.chrysogenum and F.oxysporum at p^H -6.5 and their mixture at p^H -6. Like cellulases, even in xylanase the p^H of the test medium became acidic towards the end of the sampling period. The mycelial dry weight, produced by different fungal species growing on a medium supplemented with xylan as a carbon source, varied with p^H and fungal species.

All the test fungi produced minimum cellulases and xylanase at 5°C. Cellulases and xylanase activities increased with increase in temperature till 25°C. At the highest temperature i.e. 35°C, the activities again declined. T.viride produced maximum cellulases, followed by mixed inoculum, F.oxysporum and P.chrysogenum. Besides, T.viride also produced maximum xylanase as compared to other test fungi. Optimum amount of soluble protein and mycelial dry weight was also obtained at 25°C. A significant variation was observed in the xylanase and cellulase activities, both due to different temperatures and different test fungi.

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*Original not seen.