# BIOCHEMICAL AND BIOTECHNOLOGICAL INVESTIGATIONS ON THE WATER - FERN *SALVINIA MOLESTA* MITCHELL.

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**COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY**

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**DOCTOR OF PHILOSOPHY**

in

PLANT BIOCHEMISTRY AND BIOTECHNOLOGY

UNDER THE FACULTY OF MARINE SCIENCES.

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*Dedicated to the Almighty, My Parents, Teachers and Well Wishers*

## DECLARATION

I hereby declare that the thesis entitled **"Biochemical and Biotechnological investigations on the water-fern *Salvinia molesta* Mitchell"** is an authentic record of research work carried out by me under the supervision and guidance of Prof. Babu Philip, in partial fulfilment of the requirements for the award of the Ph. D degree under the faculty of Marine Sciences, Cochin University of Science and Technology and that no part of it has previously formed the basis for the award of any degree, diploma or associateship in any University.

Kochi. - 16
Date : 5/11/1998

**Peter K. Mani**

## CERTIFICATE

This is to certify that the thesis entitled "**Biochemical and Biotechnological investigations on the water-fern *Salvinia molesta* Mitchell**" submitted herewith by Mr. Peter K. Mani is an authentic record of the research work carried out by him in the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Kochi-16, under my supervision and guidance in partial fulfilment of the requirements for the award of Ph.D degree of Cochin University of Science and Technology and that no part thereof has been presented before, for any other degree or diploma in any University.

**Dr. Babu Philip,**
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**Kochi- 16,**
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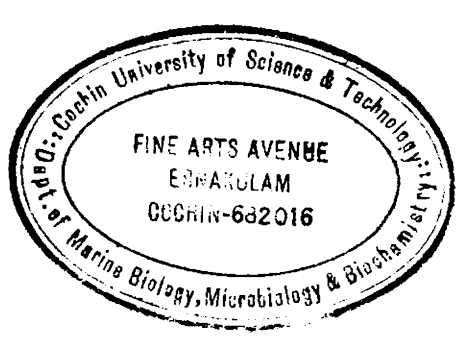

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## Abbreviations used in the thesis.

| | |
|---|---|
| % | Percentage |
| $^0$C | Degree centigrade |
| 2,4-D | 2,4- Dichloro Phenoxy Acetic Acid |
| ADF | Acid Detergent Fibre |
| AOAC | Association of Official Analytical Chemists |
| BOD | Biological Oxygen Demand |
| CEC | Cation exchange capacity |
| cm | Centimetre |
| CNT | Control |
| COD | Chemical Oxygen Demand |
| Conc. | Concentration |
| DNA | Deoxy Ribo Nucleic acid |
| DS | Dried *Salvinia* |
| EC | *Eichhornia crassipes* , |
| EDTA | Ethylene Diamine Tetra Acetate |
| Fig. | Figure |
| g & gm | gram |
| h | Hour (s) |
| ha | Hectare |
| IAA | Indole 3- acetic acid |
| ICBN | International Code for Botanical Nomenclature |
| K cal | Kilo calorie |
| Kg | Kilo gram |
| LAR | Leaf Area Ratio |
| M | Molar (Moles per litre) |
| MCPA | Methyl Chloro- Phenoxy Acetic acid |
| meq / gm | Milliequivalents per gram |
| mg | Milligram |
| min | Minutes |
| ml | Milli litre |
| N | Normal |
| NAD$^+$ | Nicotinamide Adenine Dinucleotide |
| NADP$^+$ | Nicotinamide Adenine Dinucleotide Phosphate |
| NASA | National Aeronautics & Space Administration |

| | |
|---|---|
| nm | Nano metre |
| NPM | Normal potting mixture |
| OC | Organic carbon |
| PC | *Pleurotus citrinopileatus* |
| PF | *Pleurotus florida* |
| pH | Potentia hydrogenii |
| ppm | Parts per million |
| PS | Paddy straw |
| PS | *Pleurotus sajor-caju* |
| RBD | Randomized block design |
| RNA | Ribo Nucleic Acid |
| rpm | Revolutions per minute |
| SB | Salvinia before mushroom cultivation |
| SEM | Standard Error of Mean |
| SH | Sulfhydril group |
| SM | *Salvinia molesta* |
| TCA | Tricarboxylic acid cycle |
| wt | weight |

## CONTENTS

# CHAPTER I

## GENERAL INTRODUCTION

### Aquatic weeds and their impact on Ecosystem

Scientifically, a weed can be defined as any undesired, uncultivated plant especially one growing in profusion and crowding out a desired crop, spoiling a crop field, lawn etc or altering the homeostasis of a particular ecosystem in a declining dimension. Of all weeds, water fern (*Salvinia molesta* Mitchell) and water hyacinth ( *Eichhornia crassipes* Mart Solms ) are the most widespread and problematic weeds the world over. (Holm , et al. 1969, Gupta 1979, Finlayson & Mitchell , 1983).

Aquatic weeds are those unwanted plants which grow in water and complete at least a part of their life-cycle in water. Many aquatic plants are desirable since they may play temporarily a beneficial role in reducing , agricultural , domestic and industrial pollution. However, letting a particular type of plant to grow and killing it over a period of time, will consequently release nutrients back into the water, which may help in fish-production by producing a continuous supply of phytoplankton . Many aquatic plants are considered as weeds as they deprive the humans of all facets of efficient use of water and cause harmful effects (Rao, 1992, Mitchell , 1973).

Besides water fern & water hyacinth, other major types of aquatic weeds include, *Pistia stratiotes, Typha spp, Hydrilla verticillata, Lemnoids, Ipomea spp. Vallisneria spp, Nymphaea stellata, Potamogeton spp* etc. All these weed forms cause large scale depletion of water resources and harm the water quality and also bring about major impediment and losses in agricultural production and in the long run lead to deterioration in the environmental health.(Abbasi & Nipaney, 1986).

Distribution

*Salvinia molesta* is cosmopolitan in distribution and it is abundantly seen in Africa, South America, Australia, India, Indonesia, Bengladesh, Burma, Srilanka, Combodia, Mexico, New Zealand, Phillippines, Papua New Guinea and Thailand.

## Aquatic weed infestation in India

There is wide variation in the estimates of infestation by different aquatic weeds in India. (Varshney & Singh, 1976) . It is however estimated that 20 to 25% of the total cultivable waters in India is currently infested with water hyacinth and water fern, while in the states of West Bengal, Orrisa, Bihar and Assam it is about 40%. A detailed district wise survey by Biswas, (1978), brought into light the occurrence of a spontaneous increase in aquatic weed problem between 1965 & 1975. Again in the 19 districts surveyed in Andhra Pradesh, the weed infestation ranges

between 11 to 60%. In Rajasthan, the long stretch of the Chambal irrigation canal area is heavily infested with aquatic weeds like water hyacinth, Typha and many submerged weeds. In Madhya Pradesh most of the 25 tanks in the city of Jabalpur were infested by aquatic weeds (Maley & Takur 1973, Mani, et .al 1976, Philpose, 1976). Unni, (1973), observed that *Salvinia* forms the most predominant weed infesting the wet land area used for rice cultivation in Kerala, while water hyacinth was abundantly infesting the wastelands and neglected ponds.(Cook and Gut, 1976, Thomas , 1976). In this state, *Salvinia* is a serious threat to her (i) hydroelectric projects (ii) pisciculture (iii) navigation and (iv) low land paddy fields. The fern was introduced into Kakki reservoir of Sabarigiri hydroelectric scheme in 1967 as packing material for a boat (Gupta, 1987). Within a short time, *Salvinia* covered as much as 1900 ha of paddy fields and now it is luxuriantly growing in almost all districts especially in Alappuzha, Kottayam (Plate I), Ernakulam and Trichur. Its young sporophytes adhere to crop seedlings and grow rapidly into thick mats.

The major impacts of aquatic weeds on ecosystem include :-

( i ) Depletion of water resources and hindrance to water use.

As a result of evapotranspiration, the water-loss from a reservoir, infested with floating weeds is 30 to 40% more than that from a weed-free area . Weeds also impede the flow of water in water bodies by 20 to 95% , which may result in (a) forced seepage and silting, leading to water-logging and salinity problems (b) floods caused by the restriction of the flow in flood- control channels (c) silting processes and gradual collection of weed debris cause

problems (b) floods caused by the restriction of the flow in flood- control channels (c) silting processes and gradual collection of weed debris cause reduction in the width and storing capacity of the channels. (Abbasi & Nipaney, 1985). Aquatic weeds out compete other field crops like paddy for mineral nutrients, sunlight and available space and may cause eutrophication. (Varshney & Rzoska, 1976). Again, aquatic weeds wind around the propellers of boats, thus seriously hampering transportation. (Thomas and Room, 1986, Kannan, 1979). Thus they form the prime factor in thwarting major irrigation projects.

(ii) Environmental impact of aquatic weeds.

Water pollution problems :- Aquatic weeds consume nutrients and oxygen from water and fertilizers applied to the field crops thus rendering large areas out of production. During decay they release substances toxic to paddy and also affect the soil reaction , resulting in unfavourable pH for plant growth . Their spreading (mat formation), reduces the area available to the aquatic fauna and other mobile forms and hinder their mobility and adversely affect the ecosystem itself. (Mitchell, et al 1980). Mat formation (Plate II, fig 4) also prevents sunlight from reaching the submerged fauna and flora and thus cut off their energy source. Decaying processes of weeds adds to the depletion of dissolved oxygen and spoils the water quality by increasing the Biological Oxygen Demand (BOD ), and Chemical Oxygen Demand (COD), and

the content of pathogenic organisms . During tidal movements especially in the backwater areas and estuaries, large quantities of *Salvinia* are transported to the inshore regions of the sea and they pollute the beaches and intertidal zones, thus affecting the littoral fauna. Their profuse growth breaks natural water currents. The water becomes stagnant favouring the conditions ideal for the breeding of mosquitoes and other disease vectors. (Sharma, et al., 1978).

(iii) Impact on Agriculture and Fisheries

Aquatic weeds cause direct interference in the agricultural production by cohabiting with the useful crop and consuming major shares of nutrients and water intended for the target crop and also compete for available space and sunlight (Plate I) and cause massive wastage of human labour and energy in de-weeding (Steward , 1970). In West Bengal alone the loss is at the rate of Rs. 110 milllion / year. Aquatic weeds are also known to carry plant pathogens which infect several crops. (Mukhopadhyaya & Taraphdar, 1976). Experimentally, water extracts of fresh and decaying leaves and rhizomes of water hycainth were found to be phytotoxic (Ahmed, et al., 1982 ) and also found to inhibit germination and seedling growth in radish .

Mc Vea and Boyd (1975) have reported that fish production reduced from 905 Kg /ha at 0 % weed cover to 281 kg / ha at 25 % cover, which is possibly owing to the reduction in phytoplankton from shading and removal of phosphates

by water hyacinth . The impact of *Salvinia* infestation on fisheries is typified by the case study of Sepik river in Papua New Guinea where it resulted in a decline in saltfish yield from 30,000 tonnes / year to nil (Thorp, 1978, Mitchell, et al 1980). In India , of about 8 lakh ha .of fresh waters available for pisciculture, about 40% is rendered unsuitable for fish production by these weeds . Now a days , even in man-made fish -farms and other aquaculture projects invasion of weeds creates much nuisance .

(iv) Impact on environmental health

The stagnation of water bodies caused by the weed mats , and the niche available ( on the leaves , stems and roots ) to the harmful microbes and other vectors of diseases are the two factors which combine to make weed - infested water bodies ideal breeding grounds for the carriers of diseases such as malaria, yellow fever, river blindness and encephallitis . Floating weeds especially water hyacinth and water fern have been reported to promote growth of all species of mosquitoes ( *Aedes sp, Anopheles sps , Mansonia spp & Culex spp.*) (Gopal, 1976 &1987, Rady, 1979)

The weeds also provide ideal habitat for the growth of molluscs, which in turn choke water supply and impart undesirable taste and odour to water. (Krishnamoorthy & Rajagopalan, 1970). These molluscs also act as intermediate hosts of blood and liver flukes, and debilitating disease such as schistosomiasis which may spread as the mobile weed carries the snails to a

new location. Water hyacinth plays an important role in triggering off cholera epidemics in the tropical countries as it concentrates *Vibrio cholerae* around its roots. (Spira, et. al. 1981).

Apart from these hazards, aquatic weeds reduce the recreational values of ponds,lakes, tanks, streams etc, as the water is made turbid or dirty with an undesirable odour.

(a) Distribution & Systematic position

Experimental material :-

*Salvinia* is a free-floating fresh-water fern, named after the Greek scholar Antonio Maria Salvinia (1653-1729). According to International Code for Botanical Nomenclature (ICBN) its systematic position is as follows. (Vasishta, 1987).

Division - Fillicophyta or Pterophyta

Class - Leptosporangiopsida

Order - Salviniales

Family - Salviniaceae.

The Genus is native to South America and there are about 13 species, mostly seen in South America and African Countries. (Hattingh, 1961, Boughey, 1963, Edwards and Thomas, 1977). *Salvinia molesta*, *S. natans* & *S. auriculata* are common in India. Of these, *Salvinia molesta* Mitchell is the most widespread species in Kerala. (Joy, 1978, Dixit, 1984).

(b) External Characters :-

The plant body of this perennial root-less fern consists of a branched, horizontal, spongy stem of about 2mm thickness with nodes and internodes beset with whorls of leaves. The leaves are formed in clusters of three from the nodes. Two leaves of the cluster are arranged in opposite pairs above the surface of water forming normal floating leaves, and the third one is submerged. They differ in their morphology. The floating leaves are photosynthetic, and are soft herbaceous in texture, conduplicate in young condition. These leaves are erect, sessile, obovate to oblong about 1.5 to 2cm in size, entire, lower surfaces glaborous, upper surface spongy with dense hairs in the intervenal areas. These hairs are stiff and erect with a common stalk and divided into 4-septate hooked branches. Veins are slightly distinct below, anastomosing to form parallel elongated areoles. The submerged leaves are dark brown in colour long and filiform modified into root-like organs, about 7 to 10cm in length, densely clothed with septate hairs. They appear like roots and probably serve in maintaining buoyancy and also provide protection for the reproductive structures namely sporocarps. Sporocarps are borne in cluster on submerged leaves, brown in colour ovoid, apiculate up to 2mm in diameter, sessile, densely hairy. Microsporangia borne on the branched receptacle in cluster in sympodial manner in microsporocarps, while megasporangia are limited in number borne in megasporocarps. (Manickam & Irudayaraj, 1991). With

regard to the sporocarp production, *Salvinia* exhibits clearcut photoperiodic responses i.e. long nights and short days promote sporocarp production in *Salvinia* (Hendricks, 1956, Naylor, 1961).

(c) The Unique features of *Salvinia molesta* Mitchell are

(i) The presence of four uniseriate hairs on the apices of the papillae on the upper surface of the leaves, that are united at their distal ends.

(ii) Presence of long straight chains of sessile or sub-sessile male sporocarps (microsporocarps) upto 2mm in diameter. (Forno, 1983, Harley & Mitchell, 1981).

(d) Cytology :- According to Mitchell (1973), the plant is a pentaploid hybrid between *Salvinia auriculata*. Aublet and *Salvinia biloba* having 45 chromosomes. (Kuriachan, 1967, 1979, Loyal & Grewal, 1964).

(e) Growth stages

During the life cycle plant shows four prominent Growth stages.

(i) Primary (juvenile) stage, Plate II (Fig 1) (ii) Intermediary stage, Plate II (Fig 2) (iii) Secondary stage Plate II (Fig 3) & (Fig 4) and (iv) sporocarp stage (sketch I) which differ from each other slightly in morphology (Madhusoodhanan, 1987, 1989). In the primary juvenile phase, fronds are flat on the water surface which are about 10 mm in diameter when juvenile plants float free. However when new fronds are added,

the upper surfaces of paired fronds are folded inward in pairs and the entire structure become perpendicular (rotated about 90° ) to the water surface and is lighter green in colour. In later growth stages, fronds are larger and fold upward with a definite keel shape. Submerged leaves are finely divided into linear segments that resemble and function as modified roots. Studies by Gordon and Usher, (1997) with the aid of laser scanning confocal microscopy found out that four structures are arising from a node; two floating leaves, one submerged leaf and a lateral bud. The first primordium initiated at a node is the distal floating leaf, followed by lateral bud, then the proximal floating leaf and finally the submerged leaf.

Adaptations in *Salvinia molesta* Mitchell

*Salvinia* exhibits unique adaptive features in morphological, anatomical, physiological and reproductive characters that together help the plant for its wide distribution. These adaptations are summarised below :-

(i) Rapidity of multiplication.

*Salvinia molesta* is a sterile polyploid that reproduces vegetatively through the growth and subsequent fragmentation of its lateral shoots (Harley and Mitchell, 1981). Vegetative propagation is effected by fragmentation. The horizontal fragile rhizome and their lateral branches easily break and the separated parts of the sporophyte develop into new individuals. (Sculthorpe, 1967).

(ii) The root - like submerged leaves with its dense brown hairs act as balancers which help in maintaining buoyancy and probably help in the absorption processes and provide protection for the sporocarps.

(iii) The upper surface of floating leaves are covered with small, stiff, velvetty hairs, which appear like "egg beaters" (Croxdale, 1978) that prevent it from being wetted.

(iv) Rotation of lamina about 90°, during maturation of the plant permits the growth of other beneficial plants such as nitrogen fixing *Azolla* in its vicinity.

(v) Internally the plant is provided with plenty of aerenchyma which adds to buoyancy maintenance.

(vi) Though the dry matter content is comparatively low, the growth rate and efficiency of biomass production is very high. (Penfound, 1956. De Busk, et al., 1981). Under field condition the doubling time with regard to the leaf number is only about 8 days in weed grown areas and about 13 days in open waters where there was no *Salvinia* growth . (Mitchell & Tur, 1975; Westlake, 1969; Toerien, et. al. 1983)

(vii) The plant exhibits relatively high stress tolerance against fluctuations in climatic conditions, salinity, concentration of toxic contaminants etc. (Carry & Wearts, 1980).

(viii) The relatively high proportion of non-digestible lignocellulosic content offers a protective measure against pests and other aquatic feeders.

(ix) In its origin, the plant is a pentaploid interspecific hybrid, hence the plant shows heterosis (hybrid vigour) which help the plant for its invasion in a new area and also for successful establishment there.

Methods of control with special reference to utilization

Inspite of concerted global efforts spanning more than a century for chemical, mechanical and biological control, the aquatic weeds continue to thrive practically unrestrained. Various efforts are fraught with 3 basic disadvantages.

(a) The very high cost for control.

(b) Introduction of chemicals and bioagents on a large scale has the serious risk of environmental pollution.

(c) The destruction of one weed invariably paves the way for another problematic weed, which has greater resistance than the prevailing weed for the given chemical or biological agent. For eg. in Kerala, *Salvinia* infestation in the

1960's took place at a time when efforts were being made to control the then dominant weed water hyacinth through chemical weedicides. (Cook & Gut, 1971), Usher, (1971). *Salvinia* had more tolerance towards the weedicides which were active against water hyacinth. (Joy, 1978).

The present status of various kinds of control measures are:

(A) Chemical

Important weedicides which have been tried against aquatic weeds are:-

(i) Methyl-Chloro-Phenoxy Acetic Acid or MCPA

(ii) 2,4-D (2,4 - Dichloro Phenoxy Acetic Acid) and its Sodium & Amine salts Hexazinone, Diuron etc. Of these weedicides applied so far, 2,4-D is found to be the most effective against water hyacinth world over, while in Kerala Gramoxone (Paraquat) was found to be the most effective amongst the various herbicides tried against *Salvinia*. (William, 1956, George, 1976) Plants were completely destroyed within 5 days of treatment with 5Kg / ha dose, while other weedicides like Agroxone, 2,4-D, Dicotox, Coronox etc required a level of 25 to 40 Kg / ha for similar results. Regeneration tests conducted by putting fresh plants in treated area showed that Gramoxone activity in the soil was lost within 5 to 7 days, while other herbicides were active for 21 to 30 days. (Blackburn, 1974, Kam- Wing, & Furtado, 1977).

There are several factors concerned with the extent of action of the same weedicide, at the same concentration in different water bodies which include

(i) Growth stage of a weed. (ii) Climatic factors. (iii) Water quantity and quality. (iv) The kind of sprayers and nozzles used and the co-solvents and wetting agents present in the herbicide fomulations. (v) The extent of coverage of the weed with herbicides etc. Again chemical control has several harmful environmental consequences, such as (a) Adverse effects on fresh and edible crustaceans and various organisms, which occur in their food chain. (b) Herbicide accumulation in the animal body that eventually affects the consumer (Biomagnification) (c) Persistence in water and soil may adversely affect aquaculture and agriculture (d) Secondary effects created by the ecological imbalances arising in water bodies. (Gupta, 1979, Finlayson, & Farell, 1983).

(B) Biological

Various biological control measures so far tried, have been found to be unsuccessful due to the following reasons. (1) The growth rate of weed has always been faster than the rate at which they are destroyed. (2) It is hazardous to introduce alien fast growing animals in any region as they can become a major pest themselves owing to the absence of natural enemies in the

environment which could control their growth. Examples of biocontrol agents of *Salvinia* include *Pila globosa* (Thomas, 1976), the aquatic snail *Marisia cornuarietis* (Seaman & Porterfield, 1964), *Paulinia acuminata* - a wingless orthopteran (Gaudet, 1976). The particular insect feeders of *Salvinia* include, (i) *Samea multiplicalis* Guenae - a moth, (ii) *Cyrtobagaous singularis* Hustache -a beetle (Room, et. al. 1981 Calder & Sands, 1985), (iii) *Rhopalosiphum nymphae* L. - a cosmopolitan aphid and (iv) *Nymphula responsalis* - pyramid moth (Kam-wing & Furtado, 1977 Bennet, 1966, 1977). Besides the chineese grass carp (*Ctenopharyngodon idella*) (Bailey, 1972), Sea cow or Manatee (*Tricheachus manatus*) (Rady, 1979), the water duck or white Chineese geese (*Kakki campbell*) (Rose, 1971) etc have also been tried but no significant reduction in weed growth were noted. (Abbasi, 1993).

From the history of biological control agents, one finds that there is dramatic success in the beginning followed by either the failure of the biological control agent or replacement of the target weed by some other dominant species resistant towards the biocontrol agent. In many situations a biocontrol agent successful in a given region fails completely in another region. Stress factors such as falling water levels, physical damage by flooding rivers or strong wave action etc had apparently contributed to biological control of *Salvinia molesta*. (Freeman, 1977, Kamath, 1979).

C. Mechanical control (physical)

The aquatic weeds can easily be removed by manual operations or with the help of fluidized machine. However this method is not profitable because missed plants and spores grow again and spread all over the water body within a few days. (De Silva, et al., 1983). The 'Salvinia Week' in Sri Lanka in 1952 was intended to be an all out attempt by the government to clear *Salvinia* from several thousands of acres of water areas (Senarathna, 1943). Although huge amounts of the weeds were removed, complete reinfestation occurred within a few months (William, 1956, Dias, 1967). Economic constrains are also the main reasons for the failure of physical or mechanical control measures. (Velu, 1976). Manual removal can be useful in the early stages of an infestation, but once the weed is established, the very high biomass productivity and the potential for rapid growth make this impractical. (Robson, 1974 Canellose, 1981).

From various studies, it may be deduced that no weed control technique can achieve a permanent freedom from aquatic weeds. Long-term control of the weed requires heavy initial clerance followed by regular periodical removal of the regrown weeds (Pant, 1976). If the cost of periodic harvesting can be offset by proper utilization, the mechanical removal of aquatic plants may provide an answer to the weed infestation problem in an

environmentally safe manner. (Koegel, et. al. 1973, National Academy of Sciences,1976, Nag, 1976).

D. Control through utilization and relevance of Biotechnology

Biotechnology is a fastly developing applied branch of biological science in which information from all basic branches such as physiology, molecular biology, biochemistry, microbiology, environmental science, etc. are utilized in an integrated manner in order to exploit the potentialities of cultured cells, tissues, metabolic products or organisms as a whole for human welfare. Biotechnology embraces diverse aspects such as genetic engineering, tissue culture, enzyme technology, single cell protein (SCP) and mycoprotein production, waste water treatment systems, recycling of organic wastes, biofertilizer production, etc. At present genetic engineering and tissue culture techniques have become important and versatile tools in the hands of agricultural scientists and promise to revolutionize agriculture and industry (Ignacimuthu, 1997). Rest of the fields are relatively in developing stage. In this context the utilization of aquatic weed biomass for various purposes that offers human food and triggers biogeochemical cycles is rather important.

So far aquatic weeds are utilized for the following purposes

I. As Livestock feed :-

Aquatic weeds *Salvinia & Eichhornia* are rich in digestible crude proteins and have been used as a forage crop, which contain about 24% crude protein, 4.5% fat, 6.5% starch and 9.5% fibre. (Boyd, 1968, Goering &

Van Soest, 1970, Kiflewahid, 1975). Major limitations in utilizing these weeds as forage crop are :- (a) high water content (90%) which causes difficulties in transportation. Though pressing reduces as much as 50% of crude protein (Bates & Hentges, 1976), drying of weeds to remove water is uneconomical. If grown over polluted waters, aquatic weeds, accumulate toxicants and utilization of such weeds will be hazardous and it may lead to biomagnification. Again the high lignin content of *Salvinia* reduces its digestibility. (Evans & Evans , 1949, Sullivan, 1959, Allinson, & Osbourn, 1970, Hartley, 1972, Lakshman, 1978).

II. As compost making raw material:-

Aquatic weeds have been used for mulching purposes and as compost, but the major limitations are :-

(a) Weeds harvested from waters that contain toxic pollutants produce compost that is hazardous to humans, animals, crop plants and to the environment in general (Ophel & Fraser, 1970).

(b) In areas where parasites and pathogenic bacteria infect the waters, care must be taken for proper composting, otherwise composting may lead to rapid spread of diseases.

(c) Conversion of weed into slurry ash is also time consumig and uneconomical.

III. As source of paper pulp

The use of pulp from *Salvinia* and *Eichhornia* affects the quality of the paper, because the dried leaves are comparatively brittle and the roots are dark, stiff and gritty. From the cost-benefit analysis it was found that large scale utilization of weeds as raw material in the manufacture of paper is uneconomical . (Bhambie & Bharadwaj, 1979). Again utilization of the weeds for paper manufacture is not an environmentally safe alternative because pulp and paper factories release large quantities of pollulants into water bodies. (Ghole, et. al. 1983).

IV. As Bio-agents for waste water treatment

Due to their hardiness (tolerance and resistance to toxicants, temperature, salinity etc) and fast growth rate, aquatic weeds can survive and grow on waters containing high BOD and toxic chemicals. *Salvinia* and *Eichhornia* have been extensively explored for treating waste water from dairies, piggeries, textile industries, natural rubber factories, metal work industries and also for treating nutrient - rich agricultural drainage effluents. (Wolverton & Mc Donald, 1981, Finlayson, 1983, Erdman, 1985, Abbasi, 1987). The treatment plan as devised by US National Aeronautics and Space Administration (NASA) consists of basically a zig-zag canal in which water hyacinth is grown. (Frank, 1976, Hays, et. al. 1987). During passage through the hyacinth filled canals with a retention time of about 6 weeks most of the pollutants including heavy metals are removed

from the effluents (Md. Asrarul Haque and Sudhirendar 1980). Here again proper disposal of effluent treated weeds becomes unavoidable.

V. As energy source

Of the important technologies available for converting biomass into energy, thermal conversion, thermo-chemical conversion as well as aerobic fermentation are unsuitable for aquatic biomass due to the very high water content and low sugar content. The most appropriate and feasible process for energy production from aquatic biomass is anaerobic digestion. (Lorber, et al., 1984). This process leads to the break down of complex biodegradable organics in multi stage processes (3 Phases : Hydrolysis phase, Acid phase, Methane phase) and the principal end product is methane gas, containing about 35% $CO_2$, traces of ammonia, hydrogen sulphide and hydrogen. (Schwitzguedel & Peringer, 1987, Wang, et. al. 1980). The end product commonly called 'biogas' is a convenient and clean fuel for various uses. (Nair, et al., 1982, Polisetty et al., 1983). In general estimates, fast growing aquatic weeds, *Eichhornia* and *Salvinia* attain annual productivities of 60 tonnes/ha/year on dry weight basis and 800 tonnes/ha/year on wet weight basis. (Gaudet, 1976, Reddy, 1984, Gopal, 1987, Abbasi & Nipaney, 1991). This biomass has the potential of yielding close to 30,000 cubic metre of biogas/ha/year equivalent to 225 million Kcal/ha/year. But there are certain practical problems with regard to designing of digesters, separation of phases etc. (Boyd, 1974,

Hashimoto, 1982, Abbasi & Nipaney, 1984, Reddy & De Busk, 1985, Reddy & Smith, (Eds) 1987).

VI. As a substrate for mushroom cultivation and utilization of the spent substrate as manure are discussed in detail in the following chapters :

Scope of the present study

In general, investigations on the utilitarian aspects of weeds are rare. Major works on aquatic weeds are confined to the control measures, toxicological studies etc. As far as *Salvinia* is concerned, any step to control its profuse growth in an environmentally safe manner and its subsequent utilization for different uses is important.

The present work comprises biochemical and biotechnological investigations, on *Salvinia,* with a view to control this problematic weed, by its efficient utilization.

In the introductory chapter a detailed survey of the reported works in allied area, a thorough description of the experimental material with relevant photographs, various control measures with special emphasis on utilization are given. To describe the specimen in detail, important diagrams are also presented.

In the second chapter , analysis of chemical and major biochemical contents (lignocellulose) is given, which suggests its utility as a substrate for mushroom cultivation.

The third chapter comprises potential application of *Salvinia molesta* for the cultivation of lignocellulolytic oyster mushroom. It was found that the dried weed can be utilized for mycoprotein production even without any supplements.

Chapter four gives an account of the comparative efficiency for lignocellulose conversion by three species of *Pleurotus* viz (*P. sajor-caju, P. florida* & *P. citrinopileatus*). In their biological efficiency and efficiency for lignocellulose conversion, these species showed only slight variations. Due to the wider adaptability of *P. sajor-caju,* it was selected for further experiments.

Results of the biochemical analysis of weed - derived muhroom are discussed in chapter five. Special emphasis is given to the protein, carbohydrate, lipid & mineral composition.

The residue after mushroom harvest, (spent substrate) was found to be rich in nitrogen content due to the mineralization processes. Its utilization as an organic manure for Anthurium plants (*Anthurium andreanum*) and its

multidirectional impacts are discussed in chapter six. Emphasis is given to the effect on plant growth, soil chemical status and impact on microflora (Bacteria, Fungi and Actinomycetes).

In the seventh chapter, utilization of dried *Salvinia* as seed-bed material for the germination of *Anthurium* seeds is described. Since the seeds of this plant do not normally germinate on garden soil, this finding is of much practical importance.

In the last chapter, a brief summary of the whole work is given. The major findings, advantages and limitations in the utilization of this weed as a dried organic substrate are given and suggestions are also given for future works in allied aspects.

**Objectives**

The following studies were taken up as main objectives of this thesis work.

1. Analysis of chemical constituents of sun dried *Salvinia* with special emphasis on lignocellulose and mineral components.
2. Potentiality of dried African weed (*Salvinia molesta* Mitchell) as a substrate for the cultivation of lignocellulolytic oyster mushroom (*Pleurotus sajor-caju*).
3. Evaluation of comparative efficiency for lignocellulose conversion on dried *Salvinia* by three commonly cultivated species of *Pleurotus* viz. *P. sajor-caju, P. florida* and *P. citrinopileatus.*
4. To study the alterations in the nutritive value of mushrooms in response to *Salvinia* as substrate.
5. Investigations on the utility of substrate after mushroom harvest (spent substrate) as an organic garden manure for *Anthurium* plants.
6. To check the suitability of dried *Salvinia* as a seed-bed material for *Anthurium* seed germination.

## CHAPTER II

## Analysis of the chemical constituents in *Salvinia molesta* Mitchell

### Introduction

A detailed analysis of the chemical constituents in any given biomass is a pre-requisite for designing its efficient utilization. Earlier works on the chemical analysis of *Salvinia* collected from different areas have showed considerable variation in the concentration of the constituents. Different factors that may contribute to those alterations include the particular growth stage, environmental factors, presence of contaminants in ambient conditions etc. Notable studies include those of William, (1956), Little and Henson (1967), Bagnall, et al (1973) Moozhiyil and Pallauf (1986) etc. The chemical analysis of fresh plant material by William, (1956) gives the following results. Moisture = 89.3%, organic matter = 6.07%, Ash and Sand = 4.63%, Nitrogen =0.09%, Potash ($K_2O$) = 1.156%, Phosphoric acid ($P_2O_5$) = 0.022% and lime (CaO) = 0.042%. Studies by Thomas et al (1977) based on samples collected from Trissur, Eranakulam and Kottayam Districts in Kerala gave the following results on dry matter basis; dry matter = 10.1±0.21, Crude Protein = 13.2±0.92, Ether extract (Fat, Carotene etc) = 3.7±0.18, Crude fibre (Cellulose) = 23.5±1.1, Nitrogen free extract (soluble carbohydrates) = 46.9±1.3, Total ash = 12.7±0.41, Acid soluble ash (silica) = 2.1±0.31, Calcium (Ca) = 1.35±0.15, Phosphorus (P) = 0.35± 0.03.

Since the samples (*Salvinia*) for the present work were collected from paddy fields subjected to occasional (crop seasonal) application of fertilizers,

insecticides, pesticides etc, its chemical analysis became an unavoidable step before investigating methods for its scientific and systematic utilization.

## Materials and Methods

**(a)** Collection of the sample and pre-treatments :-

Fully mature (Fig 3), mat formed weeds were collected from paddy fields, of Kottayam District (selection were made at random) at the end of the growing season, when the contents attain physiological equilibrium. Weeds were brought to the laboratory on the same day of collection. In order to ensure the removal of dust, soil particles, fertilizer and spray residues, weeds were thoroughly cleaned by washing in water and were spread over a blotting paper so as to drain off excess water. Fresh weight of a sample of about 250g was accurately determined. It was kept in a hot air oven (at a temperature of $70^0$C ), in open shallow trays, until constant weight was attained. Thus care was taken to reduce chemical and biological changes to a minimum. The dry weight was recorded.

(b) Grinding and storage of plant material :-

The dried weeds were ground to a powder using a stainless steel pulverizer. Samples of two different particle size were prepared - viz : 20 mesh fineness for macro methods of analysis and 40 mesh fineness (for micro analytical procedures). After grinding, samples of a particular particle size are mixed

thoroughly and transferred to poly-propylene bottles and stored for analytical purposes.

(c) Quantitative determination of the chemical constituents :-

Important constituents in the weed samples were analyzed by the following methods (i) Organic Carbon-Chromic acid wet digestion method (Walkey & Black, 1934). (ii) Total Nitrogen-Microkjeldahl method (Sadasivam & Manickam, 1992) (iii) Total Phosphorus-Molybdenum yellow colour method (Jackson,1973) (iv) Total Potassium-Flame photometric method (Jackson, 1973) (v) Calcium (Ca) and (vi) Magnesium (Mg) by EDTA method (Piper , 1966), (vii) Lignin and cellulose by AOAC Method. ( Goering & Van Soest, 1975) .

Determination of organic carbon

(Walkey and Black method,1934 )

Soil organic matter is oxidised under standard conditions with excess of potassium dichromate in sulphuric acid solution and the excess dichromate determined by titration against ferrous ammonium sulphate using diphenylamine as indicator.

Nitrogen - (Microkjeldahl method)

Nitrogen present in weed sample in organic form is converted to inorganic ammoniacal form (ammonium sulphate) by digestion with con. sulphuric acid in the presence of mercuric oxide- potassium sulphate mixture. Mercuric oxide is added as a catalyst and potassium sulphate to raise the boiling point of sulphuric acid

from 330$^0$C to 420$^0$C. Ammonia which is fixed as ammonium sulphate is liberated by adding an excess of caustic alkali and determined by distilling off the liberated ammonia into standard boric acid solution and titrated against standard acid( 0.0 2 N HCl ). Along with caustic alkali, sodium thiosulphate is also added to decompose the mercuric ammonium compound formed.( Calculation is based on the fact that 1 ml of 0.1 N acid is equivalent to 1.401mg N).

Sample preparation for the analysis of mineral constituents

1gm oven dried sample was digested in a block digester in 10ml concentrated tri-acid mixture (nitric acid (16 M), sulphuric acid (18 M) and perchloric acid (11.6 M) in the ratio 7:3:1). After complete digestion of the organic matter, digest was cooled and made up to 250ml with distilled water. Aliquots of the diluted digest were used for the determination of mineral constituents.

Determination of Phosphorus

Vanado molybdate method OR Vanado molybo- Phosphoric acid yellow colour method.

The intensity of yellow colour formed by the substitution of oxyvanadium and oxymolybdenum radicals for the oxygen of the phosphate is measured in this method. This method is extremely simple and the colour obtained is more stable.

This method is also free from interferences with a wide range of ionic species in concentrations upto 1000ppm.

Determination of potassium

(Flame photometric method)

The concentration of potassium in the dry ashed extract is determined with a flame photometer. The principle of operation of a flame photometer is based on the fact that quantitative measurement of the characteristic light emitted is possible, when a solution of the element being determined is atomised as a mist into a gas flame.

Determination of calcium and magnesium

(Titrimetric method)

The concentration of calcium and magnesium ions in the sample solution is determined by direct titration with Ethylene Diamine Tetra Acetate (EDTA) in the presence of metal ion indicators. The metal ion indicators added to the test solution forms a stable complex with the metal ion in solution. As the EDTA solution is added, the concentration of the metal ion decreases due to the formation of metal EDTA complex. Near the equivalence point, where no more free metal ions is present, the free indicator will be liberated. This reaction proceeds as the metal ion-indicator complex is less stable than EDTA-metal

complex. The colour of the free indicator is different from that of the indicator-metal complex and so there is a sharp colour change at the end point.

Lignocellulose determination

Refluxing the sample material (dried & powderd *Salvinia*) with acid detergent solution (prepared by dissolving 20g of cetyl trimethyl ammonium bromide in one litre of I N. sulphuric acid), removed the water solubles and materials other than the fibrous component. The residue was filtered, and weighed after drying in a desicator. This gives the weight of Acid Detergent Fibre (ADF). ADF is treated with 72% $H_2SO_4$, filtered and weighed after drying. The loss of weight during acid treatment gives the weight of cellulose. Then the residue is ignited and the ash weighed. The loss of weight on ignition gives the weight of acid detergent lignin. (Van Soest , 1967 ).

Results and Discussion

The results of the analytical work of the weed sample are shown in Table I. Moisture content of aquatic plants in general and *Salvinia* in particular are of very high order , and the dry matter ranges between 5- 15% ( Boyd and Blackburn ,1970 ). In this experiment , the results obtained are 90% and 10% respectively. However, similar to earlier reports ( Boyd , 1974 ) methodological difficulties arose during the determination of fresh weight of *Salvinia* due to the content of adherent water.

The detergent fibre analysis , by Goering and Van Soest method (1975), provided approximate values of acid detergent fibre (ADF), cellulose and lignin . The mean lignin content of mature *Salvinia* obtained here (15.92%) is higher than

that in *Eichhornia crassipes* 11.31% and paddy straw 10.05% (Kiflewahid, 1975 and, Jakson, 1977). The higher lignin content in *Salvinia* is a typical fern character (Swain, 1979).

Present study indicates that *Salvinia* is rich in cell wall materials (cellulose and lignin) thereby the organic matter and organic carbon content are also relatively higher,(4.61% and 2.62% respectively ).

With regard to the mineral composition, the content in *Salvinia* with the exception of phosphorus (0.09%) is relatively high. Table I. These are in agreement with comparable results (William,1956; Gaudet, 1973; and Thomas, et.al. 1977). Studies by Moozhiyil, & Pallauf (1986) with regard to the potential of *Salvinia* as feed source for ruminants, also arrived at similar observations and reported that the higher amount of crude ash (17.3%), lignin(13.7%) and the presence of tanins (0.93%) may reduce the acceptance as well as digestibility and restrict the use of *Salvinia* as a potential feed.

The higher value of macronutrients in the dry matter reflects that, this floating weed is very efficient in nutrient uptake from the growing medium. Similar to the earlier observations, (Moozhiyil & Pallauf,1986) the lignocellulose content in this weed is of very high order. This again points out that, this plant occupies a superior rank in biomass production. Generally lignin and cellulose are the major components in plant cell wall and of these, lignin is a phenolic polymer of coniferyl, synapyl & coumaryl alcohols and its degradation under natural conditions is very slow and it also acts as a barrier for cellulose degradation. (Brown, 1964 .)

In nature, certain Basidiomycetes fungi are equipped with specific wall degrading enzymes such as lignases, cellulases etc. Besides, *Trichoderma viridae* , and certain pathogenic fungi are also capable of degrading lignocellulosic substances (.Loveless, ,1969 , David , et. al 1985). Since, the most efficient and cost-effective means of lignin degradation is by means of fungi, this weed with its high lignocellulosic content, forms a potential substrate for lignocellulolytic fungus such as *Pleurotus*.. More than that, this weed with its relatively high content of macro - nutrients with the exception of phosphorus (0.09%) can be utilized as a mulch and as an organic manure for land plants, especially for those which grow well in soils rich in organic nutrients. The use of *Salvinia* as an organic manure is of great relevance in Kerala soils,which are rich in phosphate contents.

Table I :- Analysis of the constituents of *Salvinia molesta* Mitchell

| Components in sample | Composition gm/100gm dried weed sample |
|---|---|
| * Moisture (in fresh weed) | 90 ± 0.61 |
| Organic carbon (OC) | 2.62 ± 0.37 |
| Lignin | 15.94 ± 0.44 |
| Cellulose | 24.12 ± 0.15 |
| Total Nitrogen (N) | 0.56 ± 0.07 |
| Phosphorus (P) | 0.09 ± 0.02 |
| Potassium (K) | 0.28 ± 0.13 |
| Calcium (Ca) | 1.13 ± 0.17 |
| Magnesium (Mg) | 0.38 ± 0.11 |

Values are the mean of six separate samples ± SEM.

Chemical composition of *Salvinia*

| Components in oven- dried sample | Composition g / 100g dry wt. |
|---|---|
| Crude protein (Nitrogen X 6.25) | 13.2 ± 0.92 |
| Ether extract (Fat, Carotene, etc.) | 3.7 ± 0.18 |
| Crude Fibre | 23.5 ± 1.1 |
| Nitrogen free extract (Soluble carbohydrate) | 46.9 ± 1.3 |
| Total ash | 12.7 ± 0.41 |
| Acid soluble ash (Silica) | 2.1 ± 0.31 |
| Calcium (Ca) | 1.35 ± 0.15 |
| Phosphorus (P) | 0.35 ± 0.03 |

(After Thomas, et al., 1977.)

Components of fresh and dried *Salvinia.*

| Components | Fresh matter (g /100g fresh wt.) | Dry matter (g /100g dry wt.) |
|---|---|---|
| Moisture | 89.30 | --- |
| Organic matter | 6.07 | 56.72 |
| Ash and sand | 4.63 | 43.28 |
| Nitrogen (N) | 0.09 | 0.84 |
| Potash $K_2O$ | 1.156 | 1.46 |
| Phosphoric acid ($P_2O_5$) | 0.022 | 0.207 |
| Lime (CaO) | 0.042 | 0.386 |

(After William, et al 1956)

CHAPTER III

## Potential application of African weed (*Salvinia molesta* Mitchell for the cultivation of oyster mushroom (*Pleurotus sajor-caju* (Fr.) Singer.

### Introduction

Fungal biota play the most important role among natural scavengers in major environmental systems. Because of their heterotrophic mode of nutrition, for deriving nutrients, they degrade complex substrate molecules on which they grow. Presence of cell wall degrading enzymes and their efficient metabolic processes facilitate their absorption processes. In the long run, fungi convert complex organic molecules into simpler forms and finally into elementary state. Thus they play a significant role in biogeochemical cycles, especially in carbon, nitrogen, sulphur and phosphorus cycles.

Mushroom culture (artificial cultivation of edible mushroom) offers a potential tool in the conversion of unusable organic substrate into usable form or in other words fungi by their growth and subsequent fructification convert the organic substrate into edible basidiocarps. Various kinds of lignocellulosic materials have been tried as substrates for oyster mushroom cultivation. These include different types of straw (paddy, wheat, maize etc), banana pseudostem; saw dust, wood waste, sugarcane bagasse etc. (Bano, & Srivastava, 1962, Singh, 1983). Of these substrates tried, the fungus is found to thrive well on the conventionally used paddy straw. This is probably due to its more or less optimal nutrient composition needed for the fungal growth. In other substrates

such as saw dust, sugarcane bagasse etc, contamination takes place frequently after mycelial spreading. Again straws are generally used as cattle feed and its use as substrate for mushroom cultivation will increase the cost of production of mushrooms. Under these circumstances, aquatic weed like *Salvinia* can be utilized as a potential substrate for mushroom cultivation.

Among various floating aquatic weeds, *Salvinia Molesta* (Mitchell) and *Eichhornia crassipes* (Mart) Solms occupy top rank in terms of biomass as well as in their efficiency for propagation, in different parts of the Kerala State. These obnoxious weeds are of no significant use and create many problems in inland waters. Various earlier studies have elucidated their chemical and biochemical nature and established that they can provide adequate nutrients for the growth of saprophytic organisms . (Olah, et al 1987, Room & Thomas, 1986, Sharma & Goel, 1986). Of these two weeds, though E. *crassipes* (EC) has been used in terms of energy recovery in mushroom cultivation, (Gujral, et al 1989), no attempt has ever been made on the feasibility of utilization of *Salvinia molesta* (SM) or its combination with other substrates for mushroom cultivation.

## Materials and Methods

The present investigation was carried out from April to November 1994. Weeds were collected from paddy fields of Kottayam district and were spread over clean dry ground in single layer. They were sun-dried for six to seven days.

Spawn was obtained from Kerala Agricultural University, Kumarakom Division. Oyster mushroom was selected for the present study as it requires less crucial conditions and can tolerate the warm climate of South India. (Ganguli & Chanakya, 1994).

*Substrate preparation* - Since *Salvinia* is small in size and easy to handle there was no need to cut them into pieces before sterilization. Substrate preparation was carried out as follows: Various substrates (1 kg) were immersed in cold water for 12 hr , (Nair, 1990) so that they imbibe water thoroughly and were then washed twice in clean tap water. The excess water was drained off and they were kept in boiling water for 30 min. They were then spread over a clean surface until they retained only about 65 to 70% water.

*Bed preparation* - Beds were prepared in polythene bags (60 × 40 cm) provided with a few holes. Substrates (1 kg/bag) and spawn dose (150 g) were filled in alternate layers (Tewari, 1991). Bed with 100% paddy straw was taken as control.

*Culture conditions* - Beds were kept in a cool and dark shed with sides made of gunny bags which were soaked with water frequently. A temperature

range of $20 \pm 2^0$C and a relative humidity of 92% were maintained in the shed throughout the experiment (Nair, 1990, Thilagavathy, et al 1991).

Analysis of the air-dried substrate used for mushroom cultivation were carried out using standard methods (details are given in chapter II). Cellulose and lignin by (Goering & Van Soest AOAC methods, 1975), Nitrogen (microjeldahl method- Sadasivam & Manickam, 1992), phosphorus (vanado phosphomolybdate yellow colour method, (Jackson, 1973), potassium flame photometer method, (Jackson,1973) and calcium by EDTA method (Piper, 1966) (Table 2).

Composition of commonly used paddy straw and that of *Eichhornia crassipes*, another problematic fresh water weed (already used as substrate for mushroom cultivation) are also given in Table 2 , for a comparative study.

## Results and Discussion

Newly collected weeds were found to be more suitable for mushroom cultivation, since those stored for prolonged periods were found to be prone to the attack of insects and other air-borne fungal spores.

Spawn running was completed within 13- 16 days in all the treatements. Pin heads of fructification appeared on 17th day in SM+PS and SM+EC combinations, followed by EC+PS (18th day ), EC100% (19th day ) and PS 100% amd SM 100% on the 20th day. Biological efficiency which is the percentage conversion of dry substrate to fresh fruit bodies was found to be maximum in SM+PS (77.6%)

followed by SM+EC (74.5%), SM(66.1%) (Fig 3), PS (64.6%), EC+PS (60.4%) and EC (57.3%) (Table I). The results are mean ± SEM of 6 separate experiments. The higher yield observed with mixed substrates was probably due to a more balanced supply of nutrients than that from a single substrate (Janadaik, et al 1976). A comparative analysis of constituents of the substrates shown (Table 2) will make it clear (Van Soest, 1963, William 1956 & Frank, 1976). Occasionally few mushrooms of large size were produced on SM (100%) which may be due to the compact nature of the substrate alone (Fig. 4).

The growth of the fungus on weed substrate was found to be more or less equal to that in paddy straw. The probable reasons for rapid fungal establishment on weed substratum might be the optimal lignocellulosic content, C:N ratio, mineral constituents etc.

While considering the greater need to control the undesirable aquatic weeds and their availability in ample amount, *Salvinia molesta* and other aquatic weeds are reliable substrates for mushroom cultivation in urban areas. It could be generalized that the selection of a particular substrate or combination of substrates is largely determined by their availability (Patil & Jadhav, 1991) and cost - effectiveness.

Table 1- Biological efficiency of oyster mushroom on various substrates

| Sl No. | Substrate | Days taken for spawning | Days for budding | Yield g/kg of substrate | Biological efficiency (%) |
|---|---|---|---|---|---|
| 1 | PS (Control) | 15 | 20 | 646.67 ± 33.58 | 64.6 |
| 2 | SM | 15 | 20 | 661.67 ± 46.14 | 66.1 |
| 3 | EC | 14 | 19 | 573.33 ± 35.84 | 57.3 |
| 4 | (SM+EC) | 13 | 17 | 745 ± 44.55 | 74.5 |
| 5 | (EC+PS) | 16 | 18 | 604.17 ± 32.22 | 60.4 |
| 6 | (SM+PS) | 13 | 17 | 776 ± 42.29 | 77.6 |

PS - Paddy straw, SM - *Salvinia molesta* & EC - *Eichhornia crassipes*

Table 2- Analysis of constituents of the air-dried substrates used for mushroom cultivation ( gm/100gm dried substrate).

| Components | Paddy straw (Control) | *Eichhornia crassipes* | *Salvinia molesta* |
|---|---|---|---|
| Cellulose | 40.08 | 42.23 | 24.12 |
| Lignin | 10.05 | 11.31 | 15.94 |
| Nitrogen (N) | 0.672 | 1.6 | 0.56 |
| Phosphorus (P) | 0.09 | 0.3 | 0.09 |
| Potassium (K) | 1.32 | 3.8 | 0.28 |
| Calcium (Ca) | 0.24 | 1.7 | 1.13 |

# CHAPTER IV

## Comparative efficiency for lignocellulose conversion on aquatic weed substrate (*Salvinia molesta* Mitchell ) by different species of Oyster mushroom (*Pleurotus sajor - caju, P. florida & P. citrinopileatus.*

Introduction

Major constituents in plant biomass are cellulose, hemicellulose, lignin, water soluble constituents (sugars, aminoacids and aliphatic acids), ether & alcohol soluble constituents (fats, oils, waxes, resins & many pigments) and proteins. Of these, cellulose and lignin occupy the major portion. Chief among unutilised lignocellulosic substances include weeds of both terrestrial and aquatic origin. Among fresh water weeds, *Salvinia molesta* Mitchell and *Eichhornia crassipes* Mart Solms are the most widespread and threatening species throughout the Kerala State. Native cellulose is very resistant to enzymatic degradation. The highly crystalline structure and presence of lignin (a complex, high molecular weight polymer of p-hydroxy-cinnamyl alcohols) effectively prevent the attack of cellulases, making the hydrolysis slow and incomplete. Micro organisms in general and fungi in particular can effectively utilise these lignocellulosic substances for their growth. (Siu, 1951). Among fungi, certain Basidiomycetes forms are famous for their efficiency for lignocellulose degradation. (Norman & Fuller , 1942). The basic principle underlying mushroom cultivation is that, by the direct cultivation of cellulolytic organisms on cellulosic substances we can convert the unusable organic matter into usable form. Earlier studies by Zadrazil, (1976), Wood, (1979) reported that growth of mushrooms on substrates ultimately resulted in the utilisation of cellulose, lignin etc to a greater extent even upto

80% loss in substrate colonized by *P. florida.* Water hyacinth with an adequate amount of lignin and cellulose was found to be a potential substrate for mushroom cultivation.(Gujral, et al 1989,Malaya Ghosh&Nandi, 1995).

In the present study, the efficiencies of three different species of oyster mushroom (*Pleurotus sajor-caju, P. florida* & *P. citrinopileatus*) for lignocellulose conversion on dried and processed African weed or water-fern substrate (*Salvinia molesta* Mitchell) have been compared and the potential practical applications of the results discussed here.

## Materials and Methods

### Collection of weeds :-

African weed (*Salvinia molesta* Mitchell) was collected in bulk quantity from paddy fields of Kottayam district. After removing other weed particles and debris, they were spread on clean ground in a single layer. They were sun-dried for six to seven days and stored in plastic bags.

### Pre-treatement :-

Sun-dried weeds (1kg) were kept immersed in tap water for twelve hours, till they imbibed water thoroughly. After that, they were washed twice in clean tap water and steamed for half an hour. These pre-treatements are aimed at loosening the highly crystalline structure of cellulose and extending the amorphous state( Linko , 1977). Later they were spread over a clean surface for draining off excess water. Substrate with about 60-70% water content was used for mushroom cultivation.

Collection of spawn and preparation of beds :-

There are different species *Pleurotus*, which have been brought into commercial cultivation in different parts of the world. These species include, *Pleurotus sajor-caju*, *P. florida*, *P. ostreatus*, *P. flabellatus*, *P. cystidiosus*, *P. eous*, *P. citrinopileatus* ,*P.eryngii*,*P.djamor etc* (Camino Vilaro et al 1995, Mathew, et al 1996,Munoz, et al 1997)

In the present study, three different species of oyster mushroom were used viz; *Pleurotus sajor-caju*, *P. florida* & *P. citrinopileatus*. Spawn was collected from Regional Agricultural Research Station (Kerala Agricultural University) Kumarakom Division. The spawn dose used was 5% in each bed.

Processed substrates and a thin layer of spawn were filled in alternate layers in polythene bags (60×40 cm) provided with a few holes for aeration. They were kept in properly ventillated, cool dark shed with sides made of gunny bags. The gunny bags were soaked frequently so as to retain an optimal temperature of $20 \pm 2^0$ C and a relative humidity of 92%. (Thilagavathy Daniel et. al ., 1991).

Biological efficiency :-

Three harvests were taken in a total period of 45 days. Biological efficiency, which is the percentage conversion of dried substrate into fresh fruit bodies was calculated in individual cases.(Table 1)

Analysis of lignocellulose :-

Six samples (100g each) from different portions of each beds were dried to constant weight at $60^0$ C in an oven to determine the dry weight of substrates.(Prabhu Deai & Shivappa Shetty , 1991).

Determination of acid detergent fibre and lignin :-

Determination of the acid detergent fibre (ADF) provides a rapid method for the assay of the content of lignocellulose in feed stuffs. The residue also includes silica. The ADF is used as a preparatory step for lignin determination.(Van Soest, 1963)

Acid detergent Lignin :-

Refluxing the samples (*Salvinia* before and after the cultivation of mushroom) with acid detergent solution removes the water solubles and materials other than the fibrous components. The residue was filtered and weighed after drying in a desicator. This gives the weight of Acid Detergent Fibre (ADF). ADF was treated with 72% $H_2SO_4$, filtered and weighed after drying. The loss of weight gives the weight of cellulose. Then the residue was ignited and the ash weighed. The loss of weight on ignition gives the weight of acid detergent lignin.. (Goering & Van Soest , 1970) . The Table 2 shows the data of detailed analysis of lignin and cellulose in *Salvinia* before cultivation (SB), and after cultivation using 3 species viz; *Pleurotus sajor-caju* (PSC), *P. florida* (PF) and *P. citrinopileatus* (PC).

Results & Discussion

With regard to the growth characters, in all the three species spawn running was completed in about 15-18 days . Pin - heads appeared withn 18-20 days, which matured within 2-3 days . Three harvests were made in a total period of 45 days

Different species exhibited variation in distribution and size of fruit bodies.Most of *P. citrinopileatus* produced relatively medium size flushes , which are arranged in a clustered manner (Fig 2), *.P florida* produced comparatively large sized flushes with a number of branches for the stipe (Fig 3),where as in *P.sajor-caju* flushes were formed all over the substrate more or less as individual fruit bodies (Fig 1). Probable reasons for these differences include the genotypic differences,the substrate suitability of the species ,the extent of compactness of the substrate, the pattern of spawn running etc.

Of these three species of *Pleurotus* grown on *Salvinia* substrate,though there was no significant difference in yield, *P.florida* recorded the highest yield with 53.5 % bio efficiency followed by *P.citrinopileatus* 51.8% and *P.sajor-caju* the lowest with 51.2% bio efficiency (Table 1). Similar bioefficiency of these species on rubber wood sawdust and paddy straw was reported by earlier studies.(Kothandaraman,et al 1991, Mathew, et al 1996) .

A collection of literature on cellulose utilization by fungi has come forth mainly from extensive investigations including excellent review by Siu, (1951) and Venkata Ram, (1958). Even different strains of same fungal species exhibit varitation in the cellulose degrading abilities (White, et al..1948) and the same fungus may respond differently to the various cellulosic substrates employed (Tracey , 1953) . However, with regard to their cellulose utilization, fungi generally

exhibit a common trend , and utilize cellulose at a slower rate than its hydrolytic product the glucose. This has been attributed to the insolubility of cellulose which limits the activity of the enzyme cellulase to the surface or to insufficient enzyme synthesis (Lilly &Barnett, 1951). Also many variable factors associated with the experimental conditions like availability of some major elements like nitrogen, the pH and temperature ranges, presence of some other carbon sources etc. influence the intensity of cellulolytic activity.

In this experiment, gradation in their biological efficiency is in accordance with their efficiency for lignocellulose utilization,i.e ., *P. florida* shows the highest followed by *P citrinopileatus* and *P. sajor - caju* the lowest. (Table .2). These variations shown in substrate utilisation were probably related to their selective utilization of carbon resources., differences in metabolic activities,especially in the activity of enzymes,adaptations to the growing conditions etc. Again we could arrive at the conclusion that all these three species are equipped with ample amount of various enzymes, mainly cellulase and lignase with which they could degrade even complex organic substances with high lignin content like *Salvinia.*

The soluble components in organic substrates are available relatively readily and are quickly absorbed by the fungi, leaving the insoluble components which must then be degraded. Degradation is brought about by the action of enzymes that are produced within the hyphae and diffuse out of them into the substrate. These breakdown the insoluble compounds into soluble breakdown products, which then diffuse into the hyphae through the cell walls. Since the hyphae are charcteristically immeresed in the substrate, they are consequently bathed in the

soluble nutrients released by the action of their extracellular enzymes like cellulase , lignin peroxidase etc. (Shanmugam & Yadav , 1996) . The effect of these enzymes allows fungi to move through the solid organic matter by eroding channels, that act as routes for hyphal growth , or by rendering soft enough to be penetrated by means of the mechanical pressure exerted by extending hyphal tips.

In India, the total quantity of agricultural wastes and by-products accounts for nearly 25 million tonnes and these are mainly lignocellulosic in composition (Ghose & Gosh, 1978 ) . These materials and aquatic weeds like *Salvinia, Eichhornia, Eleocharis* etc. form potential substrates for the cultivation of *Pleurotus.* The cellulose and lignin content in the substrate form a major factor governing the growth and yield performance of oyster mushrooms ( Zadrazil, 1974; , Sivaprakasam & Kandaswamy,1981 , Joseph et al 1991) Cellulose and lignin are characterstic of plant biomass. The basic unit of each compound is a soluble molecule and these molecules are linked together to form long chain polymers which are probably insoluble. Microbial extracellular enzymes breakdown the atomic linkages between the molecules, and long chains are fragmented in this way, so that their constituent molecules are made available for absorption by the fungus. The ability of an enzyme to break a linkage is related to the physicochemical nature of that linkage. Whether or not a particular enzyme will degrade a particular compound depends on the manner in which that compound's component molecules are joined together , thereby their action is specific. For example , cellulolytic enzymes which can cleave the linkage between glucose molecules in cellulose cannot breakdown various unions between propanoid units in lignin (Cooke, 1977) Whether a particular fungus can attack a particular

natural substrate is thus obviously determined by the chemical composition of that substrate in relation to the enzyme producing properties of the fungus . In other words, the success of the fungus in colonizing on a particular substrate depends on its capability to produce the necessary specific enzymes. In this context, all the three species of *Pleurotus* are found to be efficient for the bioconversion of aquatic weed *Salvinia* into edible fruting bodies.

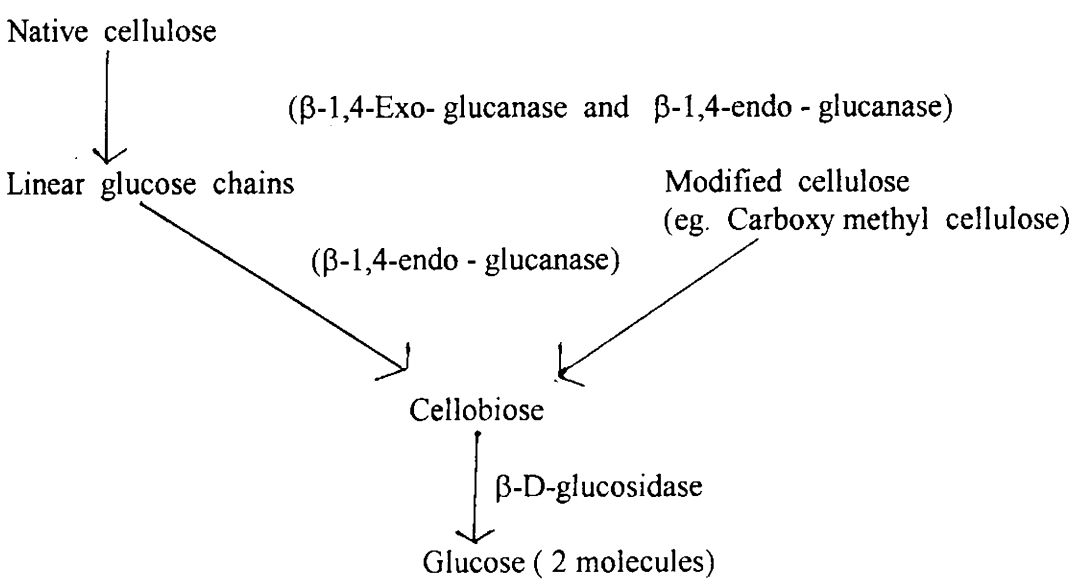
Because of their ability to produce hydrolyzing and oxidising enzymes in greater amount, *Pleurotus* sps. can be cultivated on a variety of lignocellulosic substrates such as rice straw (Bano & Srivastava 1962), wheat straw (Zadrazil 1976), banana pseudostems, saw dust (Block et al, 1958) and waste paper (Hashimoto & Takahashi, 1976). According to Zadrazil (1978), during cultivation of *P. florida* in a total period of 60 Days, about 10% of the original dried substrate was utilised by the fungus and this in turn was converted into fruit bodies. About 50% was liberated as $CO_2$, about 20% as water of decomposition and the remaining 20% is left over as the spent substrate after cropping. Loss of organic matter and lignin, and increase in *in vitro* digestibility of decomposing saw dust, sun flower & rice husks by *Pleurotus cornucopiae* & *P. florida* have been studied by Zadrazil (1980). Higest digestibility was recorded in sun flower substrate degraded by *P. cornucopiae*. Rajarathnam, (1981), has studied the changes in the various constituents of rice straw during the growth of *Pleurotus flabellatus* and noted considerable loss of cellulose and lignin during mycelial growth and fructification.

Since *Pleurotus* is lignocellulolytic in nature, the differences in the biochemical components viz lignin & cellulose in the substrate before and after

mushroom growth indicated the effect of these constituents on mushroom growth and development. Though the percentage reduction of cellulose & lignin in *P florida* grown substrate was significantly higher (Table II) than that of *P. citrinopileatus* and and *P . florida,* the yield performance was found to be more or less similar. From these results we could generalise that the lignocellulose composition of substrate is not the sole factor which determines the sporophore production.

The oyster mushroom produced all three essential enzymes of the cellulolytic complex viz. (i) A combined form of β-1,4-exoglucanase & β-1,4-endoglucanase, (ii) β-1,4 endoglucanase alone and (iii) β-D-glucosidase - essential for the complete saccharification of crystalline cellulose, under aseptic conditions (Rajarathnam, et.al 1979).

A probable mechanism of cellulose degradation by fungi is as follows.




Cowling & Merril, (1966) has enlisted some characteristics of cellulose, which makethem accessible to enzymatic breakdown. These include (1)

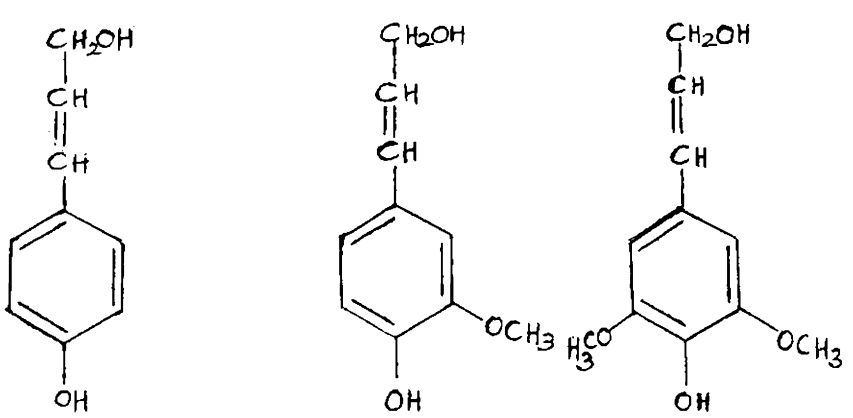
Hydration of fibres (2) The degree of crystallinity of cellulose (3) The polymerization of the cellulose molecules (4) The substances associated with cellulose such as hemicellulose,

pectin, lignin and the type of linkage with them (5) The size and diffusability of the enzyme molecules in respect to the size and surface properties of the capillaries in between the cellulose microfibrils etc.

Oyster mushrooms are efficient in degrading lignin and the percentage reduction of lignin in mushroom grown substrates are higher( Yadav, et al 1988, Singh, et. al. 1989). Dahiya , (1989) pointed out that lignin inhibits the action of cellulases and atleast it partial degradation is necessary for cellulose degradation by microorganisms.

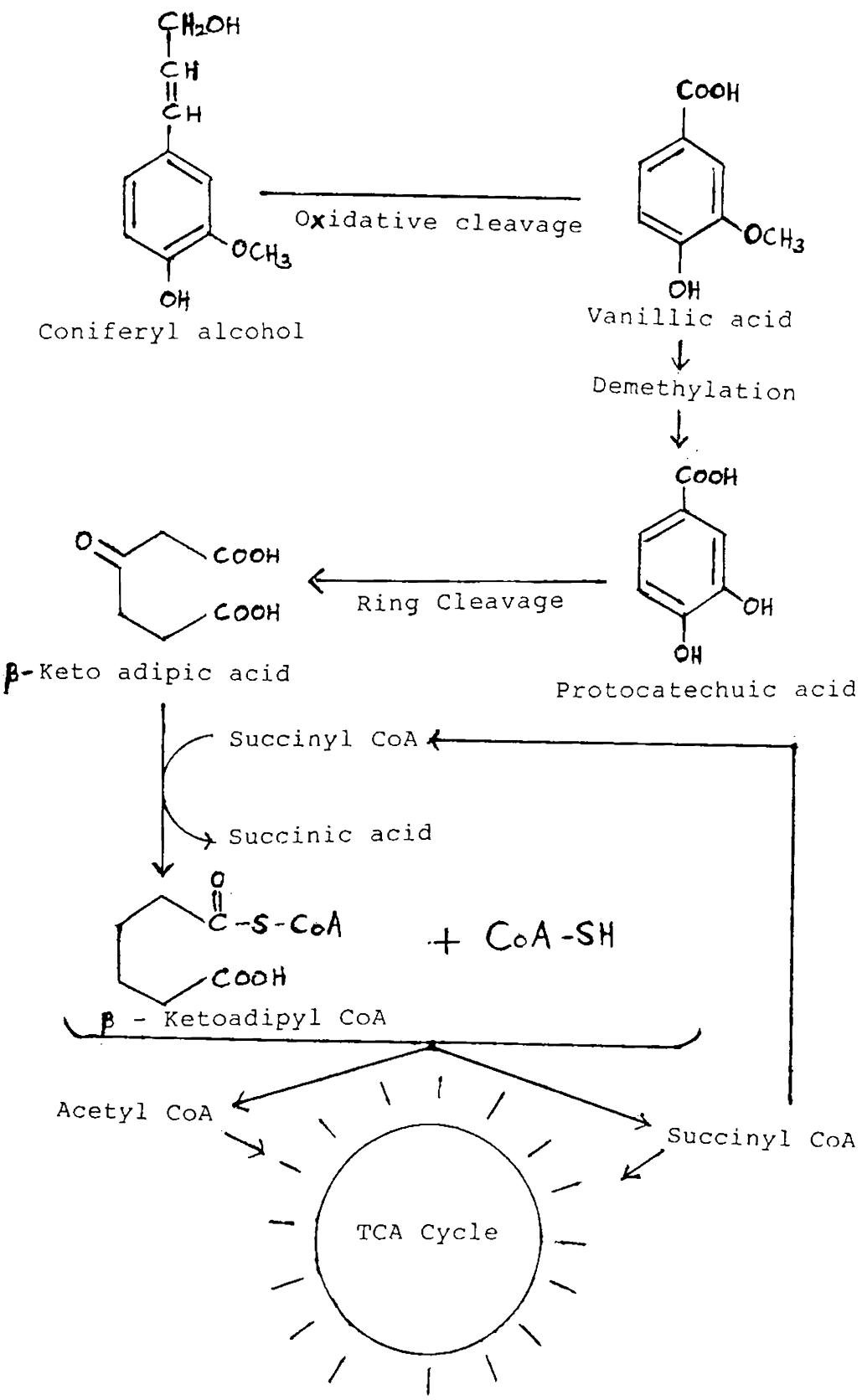
Building units (monomers) of lignin

(I) Coumaryl alcohol (ii) Coniferyl alcohol (iii) Sinapyl alcohol.




In complex lignin materials, these units are polymerised by arylglycerol-β-aryl ether bonds between the monomers in different proportion in different plants.

A hypothetical scheme for lignin degradation by fungi.



Degradation.

Initial step is the cleavage of the arylglycerol-β-aryl ether bonds between the monomers. This is followed by oxidative cleavage of the side chain with the loss of two carbon atoms and the formation of a carboxyl group to give vanillic acid. Vanillic acid is demethylated to proto catechuic acid. Ring cleavage then occurs to β-Keto adipic acid and this in turn is used in the tricarboxylic acid cycle., via β - Keto adipic path way.

β-Keto adipic pathway and final entry in to tricarboxilic acid (TCA) cycle.

The formation of proto-catechuic acid initates this pathway. After the formation of a series of intermediate metabolites the end products formed will be succinate and acetyl CoA. Acetyl CoA of succinate ie. succinyl CoA combined with β-Keto adipic acid to form β-Keto adipyl CoA (6C) which then spilit into acetyl CoA (2C) and succinyl CoA (4C) , and both of them may enter the TCA cycle and their degradation may yield energy as indirect products of β-Keto adipic acid pathway. (Stanier & Ornston ,1973) .

Table 1: Growth characters and comparative biological efficiency of different species of oyster mushroom on *Salvinia* substrates

| Species | Days for spawning | Days for budding | Yield (gm wet wt of mushroom / kg of dried substrate) | % Biological efficiency |
|---|---|---|---|---|
| *Pleurotus sajor-caju* | 16-18 | 21-23 | 512. 65 ± 46.67 | 51.2 |
| *P.citrinopileatus* | 16-18 | 22-24 | 518.49± 35.98 | 51.8 |
| *P. florida* | 15-18 | 22-24 | 535.49±39.28 | 53.5 |

Values are the mean of six separate samples ± SEM.

Table 2. Acid Detergent Fibre (ADF), Cellulose and Lignin content in *Salvinia molesta* before and after mushroom cultivation (spent residue)
(gm/100gm dried sample)

| Constituents | *Salvinia before* cultivation(SB) | SPENT RESIDUE OF | | |
|---|---|---|---|---|
| | | *P. sajor-caju* (Psc) | *P.citrinopileatus* (Pc) | *P.florida* (Pf) |
| Acid Detergent Fibre (ADF) | 39.42 ± 3.43 | 29.27 ± 6.81 | 25.31 ± 3.87 | 16.17 ±10.11 |
| Cellulose | 23.5 ±1.1 | 15.46 ± 0.24 | 14.56 ± 0.54 | 6.87 ± 0.47 |
| Lignin | 15.92 ±1.16 | 12.39 ±0.53 | 10.18 ± 0.52 | 8.61 ±0.44 |

Values are the mean of 6 separate samples ± SEM

# CHAPTER V

## Alterations in the nutritive value of mushrooms in response to *Salvinia* as substrate

Edible mushrooms are considered as delicious as well as a good supplementary item of food. Though fresh mushrooms contain about 90% of moisture (water), from the nutritional point of view, they occupy a position in between meat and vegetables and are arbitrarily known as 'vegetable meat'. They provide high quality proteins and are low in calories. On account of these unique features it is often recommended as an item of special diet to diabetic patients.

Fresh mushrooms are rich in various aminoacids and contain all the essential aminoacids required by an adult individual. Some of the amino acids like tryptophan and lysine which are deficient in vegetable proteins are also present in mushrooms. Free aminoacids constitute about 25-30% of total aminoacids. They are also found to be excellent sources of vitamins such as riboflavin, nicotinic acid, pantothenic acid and contain appreciable amount of thiamine, folic acid and ascorbic acid. The composition may vary for the same species according to the substrate, age, culture conditions etc.

The total carbohydrate content of fresh mushrooms is relatively low; the carbohydrates include different types of sugars such as sucrose, glucose, ribose & different classes of sugar derivatives.

The crude fat in mushrooms constitute less than 1% on fresh weight basis. It can be as high as 15-20% on dry weight basis. It includes all classes of lipid compounds including free fatty acids, mono, di and

triacylglycerols etc. Similar to other edible forms oyster mushrooms are also rich in sterols especially ergosterols.

Mushrooms are also rich in various kinds of minerals and their mineral content is higher than that of many fresh vegetables and fruits. The major constituents are phosphorus and potassium. Copper and iron are present in appreciable amounts. Comparatively low quantities of sodium, calcium, magnesium and some of the trace elements are also present in mushrooms.

The aquatic weed *Salvinia molesta* Mitchell is rich in lignocellulosic contents. Hence it can be utilized as substrate for the cultivation of the lignocellulolytic fungus *Pleurotus* species. The fungus thrives well on dried and processed weed substrate and converts the substrate into edible fruiting bodies. Since the composition of the growth substrate contributes much to the nutrient composition of mushrooms, it is necessary to carry out biochemical analysis of mushrooms grown on *Salvinia molesta* Mitchell as substrate and to compare the results with mushrooms raised on standardised substrate (paddy straw).

## Materials and Methods

Sun dried *Salvinia* (*S. molesta* Mitchell ) and paddy straw (control) were used as substrates for mushroom cultivation. Bed preparation and subsequent steps were carried out following the method of Jandaik, ( 1976). Optimal culture conditions of temperature (25-32°C), and relative humidity (85-90%) were provided throughout the experiment. Experiments were run in hexaplicate and the concentrations of the biochemical components - moisture, ash, protein,

carbohydrates and lipids were determined by standard methods described below. Mineral composition in terms of potassium, phosphorus, calcium and magnesium were also determined using standard procedures.

(I) Moisture and Ash :-

Moisture and ash of the samples were determined by AOAC methods (1975). Moisture content was determined by drying known quantity (100gm) of fresh sample in a hot air oven at 60°C till constant weight was obtained. The loss of weight was used to calculate the percentage of moisture.

Percentage of ash, was found out by igniting known quantity (1gm) of fresh sample at 550°C in a muffle furnace. The residue was weighed. Results were expressed as percentage of ash.

(ii) Protein analysis

For protein analysis, the method developed by Lowry et al (1951) was followed. This is based on the principle that, the blue colour developed by the reduction of the phosphomolybdic and phosphotungstic components in the Folin ciocalteau reagent by the aminoacids tyrosine and tryptophan present in protein, plus the colour developed by the biuret reaction of protein with alkaline cupric tartrate can be measured colourimetrically.

Requirements

(a) 2% Sodium carbonate in 0.1N Sodium hydroxide (Reagent A)

(b) 0.5% Copper sulphate ($CuSO_4$ $5H_2O$) in 1% Potassium Sodium tartrate

(Reagent B)

(c) Alkaline Copper solution: Mix 50ml of A and 1ml of B prior to use (Reagent C)

(d) 50% Folin-Ciocalteau Reagent (Reagent D)

(e) Protein solution - working standard: with a conc. of 0.2mg/ml in 0.1N NaOH.

Procedure :-

Extraction of Proteins

About 500mg of the sample (fruiting body), was weighed accurately and ground well with a pestle and mortar. 5ml of 10% TCA was added to precipitate the proteins. The precipitate was dissolved in 5ml of 2N NaOH. Centrifuged and the supernatant was used for protein estimation.

Estimation of proteins

(I) Pipetted out different known volumes of the working standard into a series of test tubes.

(ii) Pipetted out measured volumes of the sample extract in other test tubes.

(iii) made up the volume to 1ml in all the test tubes using 0.1N NaOH. A tube with 1ml of 0.1N NaOH was used as the blank.

(iv) 5ml of reagent C was added to each tube including the blank. Mixed well and allowed to stand for 10 min.

(v) Then added 0.5ml of reagent D, mixed well and incubated at room temperature.

Blue colour was developed.

(vi) The absorbance of the solution was compared at 660nm.

### (iii) Total carbohydrate

Total carbohydrate content in mushrooms was estimated by anthrone method (Hedge and Hofreiter, 1962). In this method carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural, which forms with anthrone a green coloured product with an absorption maximum at 630nm.

Requirements :-

(a) 2.5N HCl.

(b) Anthrone reagent:- Prepared by dissolving 200mg anthrone in 100ml of ice cold 95% $H_2SO_4$. (Prepared freshly before use.)

(c) Standard glucose solution with a final con. of 0.1 mg/ml.

### Procedure

(I) Weighed accurately about 100mg of the sample, and treated with 5ml of 2.5N HCl in a mortar & pestle and transferred into a boiling tube.

(ii) Hydrolysed by keeping it in a boiling water bath for three hours and cooled to room temperature.

(iii) Neutralised it with solid sodium carbonate until the effervescence ceased.

(iv) Made up the volume to 100ml and centrifuged.

(v) Collected the supernatant and took measured aliquotes for analysis.

(vi) Prepare the standards by taking different known volumes of the working standard.

(vii) Made up the volume to 1ml in all the tubes including the sample tubes by adding distilled water. 1ml of water in another tube was taken as the blank. Cooled the tubes in ice.

(viii) Then added 4ml of ice-cold anthrone reagent to all the tubes.

(ix) Heated all the tubes together for eight minutes in a boiling water bath.

(x) Cooled rapidly and measured the absorbance of the green to dark green colour at 630nm.

### (iv) Analysis of Lipids

Lipids in mushrooms were estimated by phospho sulpho vanillin method (Barnes and Blackstock, 1973).

#### Principle

This method is based on the sulphophosphovanillin reaction as described by Zollner and Kirsch (1962), and by Drevon and Schmidt (1964). It depends on the reaction of lipids with sulphuric acid, phosphoric acid and vanillin to give a red complex.

**Requirements**

**(a)** Chloroform : Methanol mixture (2:1).

**(b)** 0.9% NaCl

**(c)** Phospho sulpho vanillin, prepared by adding 80 ml of orthophosphoric acid to 20 ml of distilled water and then dissolving 2g of vanillin into it.

**(d)** Standard cholesterol solution in chloroform methanol mixture (2:1), with final conc. of 2mg/ml.

**Procedure**

**(I)** About 500mg of the fresh sample was accurately weighed and homogenised with a small quantity of chloroform - Methanol (2:1) mixture.

**(ii)** Made upto 10ml with the solvent and mixed well.

**(iii)** Filtered the homogenate through whatman No.1 filter paper.

**(iv)** Added 2ml of salt solution (0.9%) and shaken well.

**(v)** Transferred the mixture to a small separating funnel and allowed to stand overnight at $4^0$C.

**(vi)** A biphasic layer was formed with the lower phase containing all the lipids. Removed the lower phase and adjusted the volume to 10ml by the addition of chloroform.

**(vii)** Measured 0.5ml of extract into a clean tube. Allowed to dry in a vacuum desicator over silica gel.

**(viii)** Dissolved the residue in 0.5ml of conc. $H_2SO_4$ mixed well, plugged with non-absorbent cotton. Placed in boiling water bath for 10min. Cooled the

tubes to room temperature.

(ix) 0.2ml of acid digest was taken in a separate tube. Added 5ml of vanillin reagent, mixed well and allowed to stand for half an hour and measured the colour at 520nm.

(x) The standards were treated simultaneously with the samples and a calibration curve was constructed to calculate the concentration of lipid in the samples.

(v) Determination of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg)

Organic matter present in oyster mushroom was destroyed by dry combustion and the soluble mineral constituents dissolved in hydrochloric acid.

Accurately weighed about 0.5g of fresh mushroom sample (dried at $60^0$C for 6 hrs) into a silica dish. Ashed this in a muffle furnace at $550^0$C for half an hour. Allowed to cool and carefully moistened the ash with distilled water, keeping a watch glass over the dish and passing a jet of water through the spout. Carefully added about 5ml of 1:1 hydrochloric acid and digested the contents of the dish in a water bath for about half an hour. After cooling, transferred the contents of the dish to a 100ml standard flask and made up to the mark. This solution was used for the determination of calcium and magnesium.

Pipetted out 5ml of this solution into a 50ml standard flask and made upto the mark. This diluted solution was used for the determination of phosphorus and potassium.

Total phosphorus, and potassium content in these processed samples were determined by the methods described by Jackson, (1973) and calcium and magnesium were determined by the methods described by Piper, (1966).

## Results and Discussion

Spawn running (mycelial growth) and production of mushroom flushes were found to be earlier on dried and processed *Salvinia molesta* substrate. This clearly indicates the suitability of the substrate.

Earlier studies reported that, on an average basis, 100 gm of fresh mushroom contain about 90% moisture, 0.5 to 5% carbohydrates, 4.8 % proteins, 0.2 to 1% fat and 0.5 to 1% of minerals. The average calorific value of mushrooms is about 18 kcal /100g and the presence of different minerals adds to its nutritive value . ( Bano & Srivastava, 1962, Crisan and Sands ,1978 ). The results obtained here (Table I & II) are in general agreement with earlier studies, regardless of the substrate.

Moisture content recorded generally depends on the freshness of mushrooms and there is significant loss of moisture after storage (Li & Chang, 1982). Here, the moisture contents obtained for mushrooms raised on paddy straw (control ) and *Salvinia* are 89.11% & 90.03% respectively.

One a dry weight basis ,the protein content of *Pleurotus* was found to be much higher and it ranges about (19.4 -38 .8% ) (Jandaik, 1976). Protein upon fractionation released albumins , globulins, protamins , glutelins etc with the predominance of albumins (Sivaprakasam , 1983). Increase in albumins , globulins and glutelin fractions and decrease in protamins are known to increase

the nutritive value of proteinaceous foods (Nelson,O. E. 1969) . The values obtained for mushroom proteins in this experiment are 2.04 g /100g (in mushroom grown on paddy straw substrate) and 1.68 g /100g (in mushroom grown on *Salvinia* substrate). Studies by Arkoyd, (1966) reported that the proteins content of *Pleurotus sajor - caju* is much higher than in commom vegetables which ranges from 7.6 % in potato to 18.4 % in cabbage but lower than hen's egg (50.6%) and goat meat (83%). Major factors that contribute to the quantity and quality of proteins include species difference (genotype) , nature of the growing substrate, culture conditions , stage of harvesting , stroage conditions etc.

In general, most cultivated mushrooms contain low amount of carbohydrate (Lau, 1982). Studies by Khurana, et al (1983) noted that the reducing sugar content in mushrooms is relatively very low and was found in between 0.64 to 2.2 % of dry weight . Detailed studies by Sivaprakasam, (1983) on carbohydrate contents revealed that sucrose, glucose and ribose were present in the sporophores and their quantity was shown to be decreasing in mature sporophores.. Here, the results obtained for the two groups of mushrooms are 0.048 gm (Control) and 0.05 gm respectively (Table I). The minor differences in carbohydrate levels in mushrooms raised on paddy straw and *Salvinia* substrate indicate that, the nutrient resources obtained from these two substrates for carbohydrate synthesis are equal in amount.

Crude fat content of edible mushrooms has been found to vary from less than 1% to as high as 20% of the dry weight (Crisan and Sands, 1978). The fat content of *Pleurotus sajor -caju* was lower than that of other *Pleurotus* species,

*Termitomyces* sps , and *Volvariella volvacea.* (Zakia, et al 1963; Chang,1972). In three other edible mushrooms viz. *Cantharellus cibarius* the *Coprinus atramentarius* and *Corpinus comatus,* the amount of crude fat is about 8.27%, 7.5% and 3.28% respectively. (Khurana, et.al. 1983). Low quantities of fat and sugars are recommended to counter obesity and diabetes (Jandiaik ,1977). Here the value of lipid content is significantly low in mushrooms raised on *Salvinia* than in mushrooms raised on paddy straw (control).

The high ash content of *Pleurotus sajor- caju* indicated higher mineral incorporation in the sporophores (Starton, 1984). The amount of major mineral contents viz phosphorus , potassium , calcium , magnesium and iron were also higher in *Pleurotus sajor -caju* than in many fruits and vegetables (Arkoyed, 1966). The amount of mineral constituents in both groups of mushrooms (Table 2) has revealed that potassium and phosphorus are the main constituents of ash, of which potassium is particularly abundant. They are low in calcium and magnesium . This is in agreement with the observation made by earlier workers .(Bano & Rajarathnam 1982, Khurana , et al 1983). To a considerable extent, the amount of minerals in mushroom raised on *Salvinia* is higher than in mushroom raised on paddy straw. The source of minerals in mushrooms is probably the growing substrate.

Mushrooms raised on paddy straw (control)

Moisture content was slightly lower in mushroom grown on paddy straw, while the protein and lipid contents were found to be slightly higher than in the mushroom raised on African weed. This variation might be due to the greater

availability of amino acids, lipid fractions etc in decomposing paddy straw. With regard to the mineral composition , potassium (K), phosphorus (P), calcium (Ca) and magnesium (Mg) were found to be lower in mushrooms raised on paddy straw. This can be attributed to the comparatively lower availability of minerals in dried paddy straw.

Mushroom raised on African weed

The relatively higher proportion of moisture content in this group of mushroom is probably due to the higher water holding capacity of the weed substrate. Protein and lipid contents were found to be a little bit lower in these mushrooms, while the percentage of total carbohydrate was found to be slightly higher (Table 1). This can be due to the presence of various kinds of organic acids and humic fractions available in degrading weed.

Again the higher proportion of mineral constituents in terms of potassium, phosphorus, calcium and magnesium in mushrooms raised on African weed can be related with their higher percentage in substrate composition. A comparative analysis of dried *Salvinia* and paddy straw with regard to their composition will reveal it clearly (Table 2 , ChapterIII) The present study clearly revealed that the oyster mushroom *Pleurotus sajor -caju* raised on *Salvinia* forms a good source of protein and minerals and provided with relatively low amount of carbohydrate and lipid contents, thus making the sporophore not only delicious but also a nutritionally rich food. The manufacture of food products with a well balanced content of essential nutritional factors has been recently developed in advanced countries . Thus within the next few years , one of the possible solutions

to the problem of insufficient nutritive resources ought to be the organization of a new branch ,of large scale production of artificial food based on microbial nutrients (Kharatyan , 1978). For this objective , oyster mushroom is proved to be a powerful biological tool, and *Salvinia* as one of the suitable substrates for its cultivation.

Table 1 Biochemical analysis of mushrooms (gm /100gm of fresh mushrooms).

| Proximate composition | Mushrooms raised on (g/100g fresh mushrooms) | |
|---|---|---|
| | Paddy straw(Control) | African weed |
| Moisture | 89.11 ± 0.76 | 90.03 ± 0.62 |
| Protein | 2.04 ± 0.10 | 1.68 ± 0.19 |
| Carbohydrate | 0.048 ± 0.41 | 0.05 ± 0.11 |
| Lipids | 0.34 ± 0.13 | 0.18 ± 0.27 |

The values are the mean of six separate samples ± SEM.

Table 2 . Mineral content of the fruiting bodies (mg /100g fresh weight):

| Minerals analysed | Mineral content in mushrooms raised on | |
|---|---|---|
| | Paddy straw (Control) | African weed |
| Ash | 940.02 ± 0.06 | 981.08 ± 0.53 |
| Potassium (K) | 369.83 ± 0.71 | 382.62 ± 0.32 |
| Phosphorus (P) | 167.31 ± 0.22 | 202.17 ± 0.13 |
| Magnesium (Mg) | 2.42 ± 0.09 | 3.23 ± 0.10 |
| Calcium (Ca) | 2.91 ± 0.12 | 3.07 ± 0.24 |

The values are the mean of six separate samples ± S E M

CHAPTER VI

# Residual substrate after mushroom harvest (spent substrate) A prospective organic manure and its impacts on (i) Soil Chemical Status (ii) Soil Microbial population dynamics (iii) Plant Growth (*Anthurium andreanum*)

## Introduction

The slow rate of degradation of the African weed (*Salvinia molesta* Mitchell) during mulching practices and compost making is primarily due to the predominance of lignocellulosic contents in its composition. (National Academy of Sciences - Series - 1976, Seshadri, et al 1980, Canellos. 1981). Utilization of aquatic weeds for oyster mushroom cultivation reduces their lignocellulosic content considerably (Giavannozzi Sermani et al 1978). The drying and processing of the substrate during bed preparation and subsequent growth of the fungus alter its physicochemical characters, thereby converting the substrate into an excellent medium for the growth of varied kinds of microflora.

The spent substrate contains partially degraded organic materials. Its degradation is the result of enzymatic hydrolysis. The fungi liberate cellulases and lignases in order to break down lignocellulosic matter in the substrate. The utilization of carbon sources and subsequent synthesis of chitin in hyphal wall directly increases the nitrogen content in the spent substrate. Besides, the loss of carbon dioxide during decomposition contributes to increases in nitrogen content, thereby the wide C:N ratio in the dried substrate gets narrowed considerably

(Leong et al 1978, Zadrazil and Brunnert 1981). Later on, as a result of decomposition of plant and animal residues, soil microorganisms release carbon, nitrogen, sulphur, phosphorus and trace elements from organic materials in forms that can be absorbed by plants (mineralization).

The spent (residual) substrate, when dried is somewhat brown in colour with a loose texture. Its water holding capacity is relatively high. Spent substrate, when dried can be powdered easily by hands. Plate 5 (Fig 1). This powdered organic matter can be applied to the garden pots or to fields evenly.

Different uses of spent substrates after mushroom cultivation are : (i) as substrate for other mushrooms (Gerrits, 1969) (ii) as cattle feed (Schanel et al 1966, Herzig et al 1968 and Kaneshiro, 1977) (iii) as garden manure (Zadrazil 1978, Chang 1979) & (iv) as a source of degradative enzymes (Toyama & Ogawa ,1976, Rajarathnam et al 1979, and Tani Guchi et al 1982).

*Pleurotus* species during its growth on suitable substrate releases humic acids and their derivatives, which when added to the soil, add to its fertility (Zadrazil, 1978). Field studies were undertaken by Chang (1979), on suitability of spent cotton waste after the growth of paddy straw mushroom, *Volvariella* as garden manure for radish and tomato and the yield were found to be increased 3 and 7 times respectively. Studies on the utilization of *Pleurotus* spent substrate as garden manure are limited.

Normally, addition of an organic supplement to the soil affects the soil chemical status, soil microbial population dynamics and plant growth. These parameters are studied in this chapter.

## Materials and Methods

### Analysis of the chemical constituents in the spent substrate

In order to estimate the value of spent substrate as an organic manure, its chemical constituents were analyzed. The lignin content of plant residues may serve as an index of the vulnerability of organic residue to microbial attack (Gaur. et. al. 1971). Therefore, the lignocellulosic contents in the spent residue were also measured. The methods employed were similar to those adopted for the analysis of dried *Salvinia* and were as follows.

(i) Organic carbon : Chromic acid wet digestion method (Walkley and Black, 1934) (ii) Total nitrogen: Microkjeldahl method (Sadasivam & Manickam, 1992) (iii) Total phosphorus : Vanadomolybdo Phosphoric acid yellow colour method (Jackson, 1973) (iv) Total potassium: Flame photometric method (Jackson, 1973) (v) Calcium(Ca) and Magnesium (Mg) by EDTA method (Piper, 1966), (vi) Lignin

and cellulose by AOAC method (Goering & Van Soest, 1975). The results of the analysis are given in Table 1.

## Field experiment

### Experimental material and layout

Since Anthurium (*Anthurium andreanum*) prefers moderately humic soil and produces surface roots which come in contact with the partially degraded residue directly, it was selected as the experimental material. Suckers of ornamental variety of red flowered (Avanthi) plants, with flowering frequency of 1/45days with an average height of 10" and with 4 leaves (one year old) were taken.

36 earthenware pots with 8"×10" size were filled with one month cured normal potting mixture (NPM) consisting of unsieved river sand, coconut husk pieces, broken bricks and charcoal pieces in the ratio 3:1:1:1 by weight. Sieved and thoroughly mixed form of this mixture (from 3 to 8cm depth) were taken as sampling material for both pre-testing and subsequent soil analysis.

Locally collected plants were acclimatized in the potting mixture for one month, before the addition of supplements. The potted plants were arranged in a

plot size of 3.5 × 3.5 sq.m. in Randomized Block Design (RBD) (Plate V, Fig. 2), Steel & Torrie, (1981). Six treatments including control were prepared in hexaplicate (Fig 2). The treatments were (i) Normal potting mixture (NPM) taken as control (ii) NPM+5g spent substrate (iii) NPM+10g spent substrate (iv) NPM+15g spent residue (v) NPM+20g spent residue & (vi) NPM+20g Dried *Salvinia* (DS).

Common cultural conditions provided throughout the experiment were:- 75% shade using shade net, so that plants would get 25% sunlight and watering twice in a day. Manures were supplied in the form of sprays including fermented and diluted clear solution of cowdung monthly and mixture of trace elements once in three months. Fungicide applied was Indofil (Dithane M-45) (1g / lit). Diluted neem decoction was given as insecticide in alternate weeks. Care was taken to apply all these materials uniformly in the form of sprays.

## 6.1 SOIL CHEMICAL STATUS

### 6.1 1 Introduction

Soils are complex natural medium on earth's surface, in which plants live, multiply and die and thus provide a perennial source of organic matter which could be recycled for plant nutrition. Besides, soil provides the physical support needed for anchorage of the root system. Generally fertile soil comprises of five different components (i) mineral matters, obtained by decomposition and

disintegration of rocks. (ii) organic matters, formed by the death and decay of plant and animal remains (iii) Soil organisms, both macro organisms like rodents, worms, insects etc and microorganisms like bacteria, fungi, algae, actinomycetes, protozoans etc (iv) soil water, obtained mainly from rain and remaining as a thin film of water surrounding the soil particles. (v) Soil air or gases, obtained from the atmosphere and from the chemical reactions and microbial activities occuring in the soil. Thus soil is actually a three phase system - the solid phase consisting of the mineral and organic matteres, the liquid phase containing water with salts and soluble gases, and the gaseous phase composed of various gases. All these soil factors (edaphic factors) in general, and soil texture, pH, water holding capacity, amount of nutrients etc in particular affect plant growth.

Retention of water in soil is related to pore space and capillary action of soil particles, while pH is dependent on the chemical condition of soil. Since solubility and availability of plant nutrients are related to soil pH, acidic or alkaline soils are not generally as suitable for plant growth as neutral soils. The colloidal clay in soil acts as a large anion and absorbs cations. Having absorbed the cations, the clay particles act as reservoirs of exchangeable ions and release them for plant nutrition as and when needed. Thus the cation exchange or the base exchange properties of soil determine soil fertility and hence plant nutrition. The stability of soil aggregates depends on the organic matter content and the

nature of microbial products which bind the particles together. The interplay of all these factors affects the chemical status of the soil.

Mineral content of soil and their ingredients i.e. macronutrients (elements that are required in relatively large amounts) and micronutrients (elements required in relatively small amounts ) affect all metabolic processes in plants in a number of ways. Inadequacies of these elements may result in characteristic deficiency symptoms for example (i) nitrogen deficiency produces stunted growth and yellowish green leaves(ii) phosphorus- stems become slender and development of anthocyanins in veins (iii) Potassium- leaves become pale green or streaked with yellow colour (iv) magnesium - brittleness of leaves and leaves may wilt or shed, necrosis (localised dead area formation) etc. (v) calcium - leaves become chlorotic (pale yellow in colour),and breakdown of meristematic tissues in stem etc.

Generallly , dead remains of organisms (plants, animals and micro organisms) are subjected to chemical weathering and also to biological degradation by micro organisms. Degradation of organic matter is rapid at first and is accompanied by the release of inorganic ions and $CO_2$. With the continuation of processes, which progress at slower rates, a colloidal carbonaceous residue called humus is formed . Humus consists largely of dark- brown organic molecules rich in phenolic

compounds derived mainly from lignin of plant residues ( Noggle & Fritz, 1986).

In this experiment, the substrate after mushroom harvest (spent substrate), was added in a powdered form as supplement to *Anthurium* pots and its effect on the soil chemical status (nutrient status ) is determined, with a view to find out its suitabilty as an organic manure.

6.1.2 Materials and methods:-

Soil collection and preparation for analysis

Before the addition of spent residue, pre-test samples were collected on the first day of experiment. A portion of fresh samples was kept for microbial culture (Details are given under the heading soil microbial population dynamics). Treated samples (6 treatments, including control) were collected in 30 days interval for 6 months. Samples were collected from all the 36 pots. (6 treatments × hexaplicate). Care was taken to get representative sample from different parts of the pot from 3 to 8cm depth. About 25g soil samples were taken at a time from individual pot in polythene bags and were properly labelled. These were brought to the laboratory on the same day of collection.

Fresh samples of 6 treatments are separated and sieved through a 2mm sieve. Portions were separated into, fresh samples (for pH, Electrical Conductivity

(EC) measurement), and stored samples (i) kept in refrigerator for short period (for organic carbon (OC), available nitrogen (N), and available phosphorus (P) measurements) (ii) air dried sample (<$40^0$C) - for extraction of available potassium (K), exchangeable calcium (Ca) and magnesium (Mg), and also for Cation exchange capacity (CEC).

Nine parameters of the soil - viz: pH, Electrical Conductivity (EC), Available nitrogen (N), Available phosphorus (P), Available potassium (K), Exchangeable calcium (Ca) and magnesium (Mg) and cation exchange capacity (CEC) of the soil samples were analyzed using the following standardized procedures (Karthikakuttyamma, 1989)

## pH and Electrical Conductivity (EC) of the soil

pH is a good measure of the intensity of acidity or alkalinity of a soil - water suspension and this provides a good identification of the soil chemical nature. Electrical Conductivity (EC) or the measure of current carrying capacity, gives a clear idea of the total soluble salts present in the soil.

### Procedure

To 5gm of fresh soil sample, 25ml of water was added and stirred occasionally until equilibrium was attained. (i.e. 1:5 soil suspension was prepared.)

This unfiltered solution was used to determine pH and electrical conductivity of the soil.

1. pH of the soil

pH of the fresh soil samples was noted by taking readings using pH - meter after proper calibration of the instrument, using standard buffers of different ranges. The values are tabulated and compared.

2. Electrical Conductivity (EC) of the soil

The electrode of the conductivity meter was put in an aqueous soil sample (1:5 suspension) and the readings were noted after proper calibration of the instrument. Care was taken to note the conductivity reading within one hour of preparation of soil suspension. The EC of each soil sample was noted in millimhos/ cm in 1:5 soil suspension.

3. Organic Carbon (OC)

The organic carbon content in soil samples was analysed by Walkley and Black (1934) method. This is based on the principle that the soil organic matter is oxidised under standard condition with excess of potassium dichromate in sulphuric acid solution and the excess dichromate determined by tritration aganist ferrous ammonium sulphate using diphenylamine as indicator.

Weighed 0.5gm of finely ground (0.2mm sieved) soil into a 500ml conical flask. To this. 10ml of 1N potassium dichromate solution was added and swirled the contents. Then added 20ml of con. $H_2SO_4$ (having a pinch of silver sulphate dissolved in it), and mixed it by gentle swirling and the flask was allowed to stand on a sheet of asbestoes for 30 minutes. After the completion of reactions, diluted the contents by adding 200ml of distilled water. Then added 10ml of phosphoric acid (85%) and 1ml diphenylamine indicator. The colour changed to bluish purple. Titrated the contents with 0.5N ferrous ammonium sulphate, very carefully, until the blue colour changed to brilliant green. The end point was very sharp in this titration. From the titre values the percentage of organic carbon was calculated.

4. Estimation of available nitrogen

Alkaline permanganate method (Piper, 1966) was employed for the estimation of available nitrogen in the soil sample. This is based on the following principle. A known weight of soil is mixed with excess of alkaline potassium permanganate solution, distilled, and the ammonium formed is absorbed in known volume (10ml) of 4% boric acid solution. The quantity of excess ammonia formed is determined by back titration against standard acid.

2gm of the soil sample was transferred to a distillation flask. Then 20 ml distilled water was added into it (including those portions used for washing). To this solution, 100ml of freshly prepared 0.32% $KMNO_4$ solution was added. Then

added 100ml of 2.5% freshly prepared NaOH and immediately the flask was attached to the distillation apparatus and distilled the contents to get 30ml of the distillate in 10ml of 4% boric acid solution (Total 40ml). The excess quantity of ammonia formed was determined by titration aganist standard HCl acid. Finally the percentage of available nitrogen in the soil was calculated.

5. Determination of available phosphorus

Bray and Kurtz method (Jackson, 1973) was employed for the determination of available phosphorus. In this method, available phosphorus was extracted using Bray II reagent (0.03 N Ammonium fluoride in 0.1N HCl) and the phosphorus extracted determined colourimetrically by Molybdenum blue method. As far as possible, fresh samples were used to minimise the error, otherwise air dried ($<40^0$C) samples were used. Sample treatments were uniform for a particular time interval samples.

2gm of soil samples were transferred to a dried conical flask. Then 20ml of Bray II reagent was added into it. The contents were shaken in a reciprocating shaker for 5 minutes and rapidly filtered into a dried conical flask using double folded Whatman No.1 filter paper, 1ml of filtrate was transferred to a 50ml conical flask and 2ml of 0.6% boric acid was added and the contents were shaken well. To this, 1ml of 1.5% chloromolybdic acid reagent and a few drops of chlorostannous acid working solution (reductant) was added. The contents were

shaken well. After 5 minutes, the colour was read in a spectrophotometer at 660nm and the values were noted. The blank solution was prepared in a similar manner with 1ml Bray II reagent instead of soil extract. Potassium hydrogen phosphate solution (2.5 ppm) was used as working standard.

Sample preparation for available Potassium (K), Exchangeable Calcium (Ca) & Magnesium (Mg)

Cations present in the exchange complex of soils are removed by leaching the soil with 1N neutral (pH 7) ammonium acetate solution. Exchangeable cations are then estimated in this ammonium acetate extract.

5gm of soil sample was taken in a conical flask and 25ml of neutral ammonium acetate was added into it. Then the contents were shaken in a reciprocating shaker for 5 minutes and filtered into a dried conical flask using double folded whatman No.1 filter paper. The extract thus obtained was used for the estimation of available potassium, calcium and magnesium.

6. Determination of available potassium

Flame photometer method

The concentration of potassium in the ammonium acetate filtrate was determined with a flame photometer. The principle of operation of a flame

photometer is that, quantitative measurement of the characteristic light emitted is possible when a solution of the element being determined is atomised as a mist into a non-luminous gas flame.

Flame photometer was set up according to the instructions and a series of standard solutions and soil extract were aspirated into it. After preparing a standard graph by relating galvanometer reading to concentration of potassium in standard solutions, concentration of potassium in the soil samples was found out.

7. & 8. Estimation of available calcium (Ca) & magnesium (Mg)

In order to remove ammonium acetate and dispersed organic matter, (that would otherwise interfere with the titration with EDTA) a known volume (5ml) of ammonium acetate extract was evaporated to dryness in a China dish and the residue was dissolved in a very small portion (2ml) of aqua-regia. This was again evaporated to dryness and the residue was dissolved and made up to the original volume of the extract using distilled water. Then the concentration of calcium (Ca) and magnesium (Mg) was found out by titration with EDTA.

Principle

The concentration of calcium (Ca) and magnesium (Mg) ions in the sample solution is determined by direct titration with Ethylene Diamine Tetra Acetic acid (EDTA) in the presence of metal ion indicators. The metal ion indicators added to the test solution form a stable complex with the metal ion in solution. As the EDTA solution is added, the concentration of the metal ion decreases due to the formation of metal EDTA complex. Near the equivalence point, where no more free metal ions are present, the free indicator will be liberated. This reaction proceeds as the metal ion-indicator complex is less stable than EDTA-metal complex. The colour of the free indicator is different from that of the indicator-metal complex and so there is a sharp colour change at the end point.

Interfering ions like zinc, copper etc are masked by adding potassium cyanide. Hydroxylamine hydrochloride was added to reduce ferric ion to ferrous ion so that iron can also be masked by cyanide.

(a) Determination of calcium (Ca)

Pipetted out 5ml processed extract of soil into a China dish. This was followed by the addition of 3ml of 5% Hydroxylamine-hydrochloride, 5ml of 3.5% potassium cyanide & 4ml of 50% potassium hydroxide solution. Then the contents were diluted to 30ml with distilled water. To this diluted sample 0.1g of Patton

and Reeders indicator was added and stirred well with a glass rod. Titrated the contents against standard EDTA solution (0.01N). A sharp colour change from wine red to purple blue is obtained at pH values between 12 and 14. 5ml of the blank solution was also titrated as above.

Standardisation of EDTA (For Ca)

Pipetted out 5ml of calcium standard solution into a china dish. Then added 4ml of 50% potassium hydroxide solution and 0.1g of Patton and Reeder's indicator and titrated against EDTA till a pure blue end point was obtained. Carried out a blank using reagents alone.

Determination of Calcium (Ca) and Magnesium (Mg)

Pipetted out 5ml of solution into a china dish. Then added 5ml of potassium cyanide solution and 10ml of Ammonium chloride - Ammonium hydroxide buffer (pH 10). This was followed by the addition of Solochrome Black T. indicator; and then titrated against standard EDTA. At the end point the wine red colour of the solution changed to pure blue. 5ml of the blank solution was also titrated as above.

Standardisation of EDTA (For Ca & Mg)

Pipetted out 5ml of calcium standard solution into a china dish. Then added 10ml of buffer solution and two drops of Solochrome Black T indicator and titrated against EDTA. Carried out a blank using reagents above.

The first titre value was a direct measure of calcium present in 5ml sample & the amount of magnesium present is obtained from the difference between the first and second titre values. Then the concentration of calcium and magnesium in mg/100gm of soil was calculated.

9. Determination of cation exchange capacity of soils

Principle

In a known quantity of soil sample, the cations are displaced by ammonium ions by leaching the solution with 1N neutral ammonium acetate solution, the excess of ammonium acetate is removed with alcohol. Absorbed ammonium ions are then determined by steam distillation.

10gm of soil sample was accurately weighed and transferred to a 500ml conical flask. Then 250ml of neutral ammonium acetate solution was added into it. The contents were shaken occasionally for half an hour and kept overnight. Then, filtered the contents through whatman No.44 filter paper and the filtrate was collected in a 1 lit measuring flask. The soil was then transferred completely on to the filter paper and continued to leach the soil with the neutral ammonium

acetate solution. (Using 25ml at a time). The leachate was allowed to drain off completely before the addition of fresh aliquotes. The residue on the filter paper was used for the determination of cation exchange capacity of the soil.

This residue was washed with 60% alcohol to eliminate excess of ammonium acetate. Inorder to eliminate excess ammonium acetate completely, a pinch of solid ammonium chloride was added to the soil on the filter paper and washed with 60% alcohol till the filtrate was free of chloride (tested using dilute $HNO_3$ and silver nitrate on a drop). After that, the soil with the filter paper was transferred into a distillation flask and added 200ml of water followed by 2gm of magnesium oxide. Then steam distillation was carried out and collected the distillate in a known excess of 0.1N Sulphuric acid to which 2 drops of methyl red were added. Back titrated the excess of the acid with 0.1N sodium hydroxide. Then the cation exchange capacity of the soil samples was calculated in milli - equivalant / 100gm soil.

### 6.1.3. Results and Discussion

Spent substrate analysis

In comparison with the contents in dried *Salvinia* (Chapter II, Table 1), the spent substrate is having high content of total nitrogen and low content of other

nutrients (Table 1). These differences might be due to the high mineralization process of total nitrogen and higher utilization of other nutrients by the fungus.

Soil chemical status

Decomposing organic remains consist mainly of nitrogenous and non nitrogenous compounds. The nitrogenous compounds are proteins, nitrogenous bases like alkaloides, purines etc together with amides, amines, amino acids and nitric compounds. The non--nitrogenous compounds are mainly carbohydrates, lignins, tannins, organic acids , fats and oils, waxes, resins, pigments etc. In addition, all plant remains contain varying amounts of minerals.

All these organic compounds incorporated into the soil do not remain as such for a longer period. They are immediately attacked by a variety of micro organisms, worms and insects in the moist soil. The decomposition by these organisims yield simpler and new substances and ultimately alters the chemical composition of the soil .

**pH** :- Throughout the experiment the pH of the soil samples, tend to remain near slightly acidic or neutral range (Table II, 1 ) . Generally pH is dependent on the chemical condition of the soil (Russel, 1962). However, the non- significant fall in pH levels can be related to the release of organic acid fractions during decomposition processes. Application of humic acid fractions is found to increase the

yield in *Glycine max* (Varshney and Gaur, 1974). Neutral soils generally favour the growth of such micro organisms which are responsible for the conversion of organic forms of nitrogen, phosphorus, and sulphur into inorganic forms which can be absorbed by plants.

**Electrical Conductivity (EC)**

Electrical conductivity (EC), provides a measure of total soluble salts (expressed in millimhos /cm ). The composition of the soil solution and the electrical conductivity of the soil are resulting from an interplay of several factors such as nutrient uptake by plants, surface adsorption, evaporation and drainage, addition of fertilizers and microbial activities on organic matter (Samuel, et al., 1995). In this experiment the values of EC also tend to remain in a normal range, without any significant difference. (Table II, 2 ). From the results, we can infer that , the formation of soluble salt forms is also meagre during the degradation of the spent substrate.

**Organic Carbon (OC)**

When micro organisms grow and multiply on organic debris, carbon is utilized for building the cellular material of microbial cells, with the release of carbon dioxide, methane and other volatile substances. In this process, micro organisms also assimilate nitrogen, phosphorus, potassium and sulphur which get bound in the cell protoplasm. Therfore the C/N, C/P, C/K or C/S ratios in the soil are governed by the extent of organic matter utilized by soil micro orgnisms .

Thus under natural conditions chemical status of a particular soil is mainly determined by three parallel processes going on during decomposition. (i) degradation of plant and animal residues into inorganic forms by cellulases and other microbial enzymes (ii) the increase in the biomass of micro organisms by the building up of polysaccharides and proteins (immobilization) (iii) the accumulation or liberation of end products. The term mineralization is used to designate the conversion of organic complexes of an element to its inorganic state. The second process i.e. microbial immobilization is opposite in magnitude to mineralization and that may reduce the availability of nutrients to plant growth (Gaur, et al 1971, Allison, 1973). The release of metabolic end products by the micro organisms and their death will increase soil organic matter and as a result organic carbon content of the soil also found to be increased during spent substrate decomposition (Table II, 3 ).

**Available Nitrogen**

The gradual increase in available nitrogen in accordance with the increasing level of treatments followed by slow decline clearly indicates the decomposition of organic matter and subsequent utilization of nitrogen by the plants (Table III, 1). In a similar study by Mondal & Chattopadhyay, (1993) with regard to the effect of chemical fertilizers on soil nitrogen, a decreasing trend was observed which is attributed to the utilization by plants and to possible leaching effects. Nitrogen is a constituent of many important biomolecules like proteins DNA, RNA , Chlorophyll, $NAD^+$,

$NADP^+$, FAD etc. Organic matter from diverse plant - tissues vary widely in their C/N ratio. Optimum levels of C/N ratio in the range of 20 - 25 with (1.4-1.7 % N), seem to be ideal for maximum microbial decomposition since there will be no immediate release of mineral nitrogen residues over and above the amount required for microbial synthesis (Gray, and William, 1971).

**Available Phosphorus**

Phosphorus is an important constituent of nucleic acids and phospholipids. The level of phosphorus content in the normal potting mixture is relatively higher. However, the decreasing trend of available phosphorus level might be due to the higher rate of microbial metabolic activities, root respiratory activities and subsequent synthesis of metabolites (ADP, ATP etc.) and also due to microbial immobilization (Table III, 2 ). Stark, (1972) showed that fungal hyphae had 104 - 273% greater phosphorus content than the needle litter in a temperate *Pinus* forest which indicated immobilization of this element.

**Available Potassium**

The immediate source of potassium for plants is that in the soil solution. Plants require potassium in higher amounts for normal growth as it provides necessary ionic environment for the synthesis of proteins inside the plant cells. The potassium uptake by a particular plant mainly depends on the following factors (1) the potassium ion concentration close to the root surface, (2) on the

rate at which potassium ions are transported through the soil solution to the root surface, (3) its replenishment from soil colloids or from degrading organic matter and (4) on the extent to which roots ramify through the soil. The rate of uptake may vary depending on the plant species, the growth stage and the potential growth rate under the prevailing environmental conditions. Here the significant increase in the available potassium content (Table III, 3) can be related to the triggered liberation of potassium ions from degrading spent substrate and from soil colloids. Studies by Mondal & Chattopadhyay (1993) reported that application of higher levels of organic nutrients influenced positively on the available potassium content in the soil.

**Calcium & Magnesium**

The uptake of calcium and magnesium by plants are generally dependent on the salinity status of ambient condition. Crops grown in saline soils seem to accumulate 2mM of $Ca^{++}$ and $Mg^{++}$ per gram dry matter (Krishnamurthy & Bhagwat, 1991). Calcium and magnesium occur as constituents of silicates in the mineral fraction of the soil. They are present as exchangeable cations and in the soil solution, they may also be present as chlorides, sulphates, carbonates or other salts.

In nearly all soils which are neutral or only slightly acidic, calcium ions occupy most of the exchange sites on clays and organic matter. Calcium functions both as a structural component and as a cofactor for many enzymes.

As an exchangeable ion in neutral and slightly acidic soils, magnesium is usually second in abundance to calcium . Magnesium ion resembles calcium in its behaviour and ion exchange reactions. Magnesium serves as a structural component and is involved as a cofactor in many enzymatic transfer reactions. It forms a component in chlorophyll and it is also required to maintain the structural integrity of ribosomes and chromatin fibres. With the addition of spent substrate to *Anthurium* pots both of these exchangeable ions showed significant increase in their availability (Table IV, 1 & 2). This reflects the rapidity of release of these ions from the potting mixture, which includes considerable amount of sand.

**Cation exchange capacity(CEC)**

The term cation exchange capacity (CEC) refers to the capacity of the soil to exchange cations, rather than its capacity for exchangeable cations. The humic substances in the potting mixture have a very large influence on the cation exchange capacity of the soil. Direct relationship exists between soil pH and CEC. Even with a unit rise in soil pH, the CEC may increase several times. In this experiment the significant changes in CEC (Table IV, 3 ) is directly related to the formation of humic fractions from the added supplements, to the microbial metabolic reactions and also to the rate of ion uptake by the plants. Any changes in CEC directly influence the ion exchange mechanism for mineral absorption by the plants. Addition of spent substrate ultimately brings about an ionic gradient necessary for mineral uptake.

**TABLE. I. ANALYSIS OF SPENT SUBSTRATE.**

| Components | gm / 100gm |
|---|---|
| Organic carbon (OC) | 2.21 ± 0.18 |
| Nitrogen (N) | 1.12 ± 0.08 |
| Phosphorus (P) | 0.05 ± 0.14 |
| Potassium (K) | 0.12 ± 0.02 |
| Calcium (Ca) | 0.56 ± 0.27 |
| Magnesium (Mg) | 0.19 ± 0.22 |

Values are the mean of six separate samples ± SEM.

**TABLE : II SOIL CHEMICAL STATUS**

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE | (1) pH | | | | | |
|---|---|---|---|---|---|---|
| 0 DAY | 5.45 ± 0.28 | | | | | |
| TREATMENTS IN WEIGHT ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 5.61 ± 0.11 | 5.33 ± 0.25 | 5.15 ± 0.11 | 5.71 ± 0.22 | 5.37 ± 0.21 | 5.53 ± 0.29 |
| 60 | 6.01 ± 0.17 | 5.67 ± 0.22 | 5.70 ± 0.13 | 5.80 ± 0.40 | 5.46 ± 0.46 | 5.54 ± 0.37 |
| 90 | 5.69 ± 0.29 | 5.55 ± 0.36 | 5.48 ± 0.41 | 5.39 ± 0.17 | 5.35 ± 0.25 | 5.18 ± 0.13 |
| 120 | 6.40 ± 0.11 | 5.55 ± 0.14 | 5.31 ± 0.33 | 5.64 ± 0.13 | 5.33 ± 0.28 | 5.68 ± 0.18 |
| 150 | 5.81 ± 0.19 | 5.85 ± 0.17 | 6.02 ± 0.39 | 5.75 ± 0.19 | 5.21 ± 0.22 | 5.39 ± 0.34 |
| 180 | 6.51 ± 0.31 | 5.43 ± 0.44 | 5.52 ± 0.15 | 5.71 ± 0.21 | 5.31 ± 0.26 | 5.67 ± 0.29 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE | (2) ELECTRICAL CONDUCTIVITY ( EC ) millimhos / cm | | | | | |
|---|---|---|---|---|---|---|
| 0 DAY | 0.301±0.04 | | | | | |
| TREATMENTS IN WEIGHT ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 0.306 ± 0.11 | 0.313 ± 0.03 | 0.308 ± 0.01 | 0.318 ± .03 | 0.314 ± 0.04 | 0.319 ± 0.01 |
| 60 | 0.304 ± 0.01 | 0.312 ± 0.03 | 0.315 ± 0.01 | 0.323 ± .01 | 0.319 ± 0.04 | 0.318 ± 0.04 |
| 90 | 0.307 ± 0.04 | 0.318 ± 0.02 | 0.303 ± 0.02 | 0.304 ± .03 | 0.309 ± 0.03 | 0.304 ± 0.05 |
| 120 | 0.318 ± 0.05 | 0.315 ± 0.01 | 0.301 ± 0.04 | 0.313 ± .02 | 0.314 ± 0.02 | 0.315 ± 0.05 |
| 150 | 0.310 ± 0.03 | 0.313 ± 0.01 | 0.319 ± 0.02 | 0.314 ± .04 | 0.305 ± 0.03 | 0.313 ± 0.05 |
| 180 | 0.318 ± 0.03 | 0.307 ± 0.02 | 0.315 ± 0.02 | 0.330 ± .03 | 0.321 ± 0.02 | 0.317 ± 0.03 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE | (3) ORGANIC CARBON ( OC ) g / 100 g | | | | | |
|---|---|---|---|---|---|---|
| 0 DAY | 2.40 ± 0.19 | | | | | |
| TREATMENTS IN WEIGHT ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 2.36 ± 0.14 | 2.44 ± 0.22 | 2.41 ± 0.20 | 2.50 ± 0.17 | 2.58 ± 0.08 | 2.48 ± 0.19 |
| 60 | 2.39 ± 0.17 | 2.48 ± 0.31 | 2.45 ± 0.35 | 2.54 ± 0.17 | 2.59 ± 0.18 | 2.47 ± 0.29 |
| 90 | 2.33 ± 0.16 | 2.57 ± 0.41 | 2.55 ± 0.29 | 2.50 ± 0.33 | 2.61 ± 0.14 | 2.67 ± 0.21 |
| 120 | 2.36 ± 0.41 | 2.42 ± 0.20 | 2.61 ± 0.24 | 2.74 ± 0.19 | 2.67 ± 0.31 | 2.51 ± 0.18 |
| 150 | 2.40 ± 0.30 | 2.48 ± 0.22 | 2.77 ± 0.13 | 2.65 ± 0.33 | 2.81 ± 0.11 | 2.58 ± 0.50 |
| 180 | 2.31 ± 0.38 | 2.53 ± 0.12 | 2.71 ± 0.22 | 2.67 ± 0.09 | 2.78 ± 0.27 | 2.64 ± 0.15 |

**DS** - Dried *Salvinia*, CNT - Control

**Values** are the mean of six separate samples ± S E M.

**Graph** showing general trend are given at the end of the tables.

## Analysis of variance Tables

Table II. Soil Chemical status (1.) pH

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 0.4419 | 0.08838 | 1.119 |
| Treatment sum of squares | 5 | 1.5271 | 0.30422 | 0.325 |
| Error sum of squares | 25 | 6.7962 | 0.271848 | |
| Total | 35 | 8.7592 | | |

Table II. Soil Chemical status (2.) Electrical conductivity (EC)

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 0.0002 | 0.00004 | 1.136 |
| Treatment sum of squares | 5 | 0.00037 | 0.000074 | 2.102 |
| Error sum of squares | 25 | 0.0088 | 0.0000352 | |
| Total | 35 | 0.00145 | | |

Table II. Soil Chemical status (3.) Organic carbon (OC)

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 0.363 | 0.0726 | 13.248* |
| Treatment sum of squares | 5 | 0.122 | 0.0244 | 4.452* |
| Error sum of squares | 25 | 0.137 | 0.00548 | |
| Total | 35 | 0.622 | | |

* F values are significant at 5% level, ie. $P < 0.05$.

**TABLE : III SOIL CHEMICAL STATUS**

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE | (1) AVAILABLE NITROGEN (N) mg/100g | | | | | |
|---|---|---|---|---|---|---|
| 0 DAY | 26 ± 0.51 | | | | | |
| TREATMENTS IN WEIGHT ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 22 ± 0.20 | 30 ± 0.22 | 27 ± 1.31 | 28 ± 1.04 | 36 ± 0.80 | 25 ± 0.40 |
| 60 | 25 ± 0.35 | 28 ± 1.10 | 38 ± 0.50 | 35 ± 0.70 | 43 ± 1.01 | 28 ± 0.44 |
| 90 | 37 ± 0.48 | 40 ± 0.40 | 34 ± 0.55 | 36 ± 0.35 | 44 ± 0.50 | 32 ± 0.39 |
| 120 | 27 ± 0.30 | 33 ± 0.34 | 35 ± 0.57 | 38 ± 0.08 | 49 ± 0.60 | 30 ± 0.40 |
| 150 | 28 ± 0.20 | 31 ± 0.28 | 40 ± 0.42 | 35 ± 0.38 | 45 ± 0.50 | 37 ± 0.20 |
| 180 | 24 ± 1.02 | 28 ± 0.32 | 37 ± 0.53 | 31 ± 0.50 | 38 ± 0.50 | 34 ± 1.01 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE | (2) AVAILABLE PHOSPHORUS (P) mg/100g | | | | | |
|---|---|---|---|---|---|---|
| 0 DAY | 5.5 ± 0.32 | | | | | |
| TREATMENTS IN WEIGHT ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 5.0 ± 0.60 | 5.2 ± 1.01 | 4.7 ± 0.43 | 5.3 ± 0.38 | 5.9 ± 0.44 | 5.1 ± 0.32 |
| 60 | 5.7 ± 1.30 | 5.2 ± 0.29 | 5.0 ± 1.05 | 5.3 ± 0.40 | 5.5 ± 0.32 | 4.8 ± 0.37 |
| 90 | 5.4 ± 0.30 | 4.5 ± 0.39 | 4.6 ± 0.42 | 5.1 ± 0.80 | 5.0 ± 0.52 | 5.0 ± 0.52 |
| 120 | 6.2 ± 1.31 | 5.0 ± 0.38 | 4.8 ± 0.53 | 4.9 ± 0.40 | 4.6 ± 0.50 | 5.1 ± 0.46 |
| 150 | 5.9 ± 0.53 | 4.6 ± 0.32 | 4.8 ± 0.20 | 4.5 ± 0.50 | 4.3 ± 1.08 | 4.7 ± 0.31 |
| 180 | 5.5 ± 1.04 | 4.7 ± 0.30 | 4.2 ± 0.35 | 4.5 ± 0.55 | 4.3 ± 1.01 | 4.6 ± 0.09 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE | (3) AVAILABLE POTASSIUM (K) mg / 100 g | | | | | |
|---|---|---|---|---|---|---|
| 0 DAY | 7.5 ± 0.44 | | | | | |
| TREATMENTS IN WEIGHT ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 7.0 ± 0.48 | 7.0 ± 0.37 | 7.5 ± 0.39 | 7.3 ± 0.37 | 7.9 ± 0.74 | 6.9 ± 0.36 |
| 60 | 7.1 ± 0.40 | 6.9 ± 0.53 | 7.7 ± 0.55 | 7.6 ± 0.53 | 8.0 ± 0.42 | 7.4 ± 0.25 |
| 90 | 7.5 ± 0.57 | 7.5 ± 0.53 | 7.5 ± 0.55 | 8.1 ± 0.57 | 8.2 ± 0.30 | 6.7 ± 0.39 |
| 120 | 6.9 ± 0.51 | 7.3 ± 0.28 | 7.7 ± 0.55 | 8.2 ± 0.39 | 7.6 ± 0.40 | 7.1 ± 0.31 |
| 150 | 7.3 ± 0.30 | 7.6 ± 0.40 | 7.8 ± 0.31 | 7.8 ± 0.39 | 7.6 ± 0.32 | 6.8 ± 0.33 |
| 180 | 6.8 ± 0.32 | 7.5 ± 0.39 | 8.0 ± 0.40 | 8.1 ± 0.40 | 7.8 ± 1.20 | 6.9 ± 0.31 |

DS - Dried *Salvinia*, CNT - Control

Values are the mean of six separate samples. ± S E M.

Table III. Soil Chemical status (1.) Available nitrogen

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 801.55 | 160.31 | 14.341* |
| Treatment sum of squares | 5 | 335.89 | 67.178 | 6.009* |
| Error sum of squares | 25 | 279.45 | 11.178 | |
| Total | 35 | 1416.89 | | |

Table III. Soil Chemical status (2.) Available phosphorus

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 3.119 | 0.6238 | 5.125* |
| Treatment sum of squares | 5 | 1.7423 | 0.3484 | 2.862* |
| Error sum of squares | 25 | 3.0427 | 0.121708 | |
| Total | 35 | 7.904 | | |

Table III. Soil Chemical status (3.) Available potassium

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 4.566 | 0.9132 | 12.683* |
| Treatment sum of squares | 5 | 0.3433 | 0.0686 | 0.9527 |
| Error sum of squares | 25 | 1.8008 | 0.072 | |
| Total | 35 | 6.7101 | | |

* F values are significant at 5% level, ie. $P < 0.05$.

**TABLE : IV SOIL CHEMICAL STATUS**

DAYS AFTER THE ADDITION OF SPENT SUBSTRATE 0 DAY

(1)

EXCHANGEABLE CALCIUM
(mg / 100 gm)
5.5 ± 0.35

| TREATMENTS IN WT. ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
|---|---|---|---|---|---|---|
| 30 | 5.3 ± 0.37 | 5.5 ± 0.40 | 5.8 ± 0.23 | 6.1 ± 0.30 | 6.0 ± 0.28 | 5.6 ± 0.30 |
| 60 | 5.0 ± 0.33 | 5.7 ± 0.35 | 5.6 ± 0.30 | 5.8 ± 0.32 | 6.2 ± 0.37 | 5.9 ± 0.38 |
| 90 | 5.2 ± 0.20 | 5.5 ± 0.35 | 6.3 ± 0.24 | 5.9 ± 0.30 | 6.2 ± 0.50 | 6.0 ± 0.50 |
| 120 | 5.0 ± 0.30 | 6.0 ± 0.47 | 5.9 ± 0.25 | 6.0 ± 0.55 | 6.7 ± 0.50 | 5.8 ± 0.45 |
| 150 | 5.7 ± 0.44 | 5.6 ± 0.22 | 6.0 ± 0.35 | 5.8 ± 0.53 | 6.4 ± 0.62 | 5.3 ± 0.45 |
| 180 | 5.7 ± 0.32 | 5.5 ± 0.30 | 5.9 ± 0.50 | 6.2 ± 0.55 | 6.3 ± 0.55 | 6.0 ± 0.53 |

DAYS AFTER THE ADDITION OF SPENT SUBSTRATE 0 DAY

(2)

EXCHANGEABLE MAGNESIUM
(mg / 100 gm)
1.8 ± 0.30

| TREATMENTS IN WT. ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
|---|---|---|---|---|---|---|
| 30 | 1.5 ± 0.30 | 1.5 ± 0.35 | 1.7 ± 0.44 | 1.9 ± 0.40 | 1.8 ± 0.25 | 1.6 ± 0.55 |
| 60 | 1.6 ± 0.28 | 1.6 ± 0.32 | 1.7 ± 0.41 | 1.7 ± 0.31 | 2.1 ± 0.50 | 1.6 ± 0.41 |
| 90 | 1.5 ± 0.17 | 1.6 ± 0.42 | 1.8 ± 0.10 | 1.9 ± 0.32 | 2.0 ± 0.35 | 1.9 ± 0.50 |
| 120 | 1.5 ± 0.18 | 1.8 ± 0.15 | 1.7 ± 0.12 | 1.8 ± 0.50 | 1.8 ± 0.17 | 1.9 ± 0.32 |
| 150 | 1.8 ± 0.20 | 1.9 ± 0.37 | 2.4 ± 0.16 | 2.1 ± 0.25 | 2.0 ± 0.50 | 2.1 ± 0.13 |
| 180 | 1.6 ± 0.20 | 1.7 ± 0.17 | 1.9 ± 0.19 | 2.0 ± 0.20 | 1.9 ± 0.53 | 2.2 ± 0.22 |

DAYS AFTER THE ADDITION OF SPENT SUBSTRATE 0 DAY

(3)

CATION EXCHANGE CAPACITY
m cq. / 100 g
6.8 ± 0.42

| TREATMENTS IN WT. ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
|---|---|---|---|---|---|---|
| 30 | 6.2 ± 0.20 | 6.0 ± 0.38 | 6.6 ± 0.30 | 6.8 ± 1.08 | 6.3 ± 0.43 | 6.7 ± 0.50 |
| 60 | 6.5 ± 0.55 | 6.2 ± 0.35 | 7.0 ± 0.43 | 6.9 ± 0.37 | 6.7 ± 0.37 | 6.5 ± 0.51 |
| 90 | 6.3 ± 0.45 | 6.5 ± 0.40 | 6.8 ± 0.39 | 7.2 ± 0.20 | 7.2 ± 0.53 | 6.8 ± 0.42 |
| 120 | 6.5 ± 0.50 | 7.2 ± 0.53 | 7.0 ± 0.28 | 7.2 ± 0.48 | 7.2 ± 0.56 | 6.9 ± 0.57 |
| 150 | 7.0 ± 0.52 | 6.9 ± 0.50 | 7.8 ± 0.22 | 7.4 ± 0.44 | 7.5 ± 0.75 | 7.2 ± 0.55 |
| 180 | 6.6 ± 0.52 | 6.9 ± 0.57 | 7.4 ± 0.51 | 7.7 ± 0.64 | 8.1 ± 1.09 | 7.7 ± 0.55 |

DS - Dried *Salvinia*, CNT - Control

Values are the mean of six separate samples ± S E M.

Table IV. Soil Chemical status (1.) Exchangeable calcium

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 3.313 | 0.662 | 11.129* |
| Treatment sum of squares | 5 | 0.2733 | 0.0546 | 0.9179 |
| Error sum of squares | 25 | 1.4837 | 0.05948 | |
| Total | 35 | 5.07 | | |

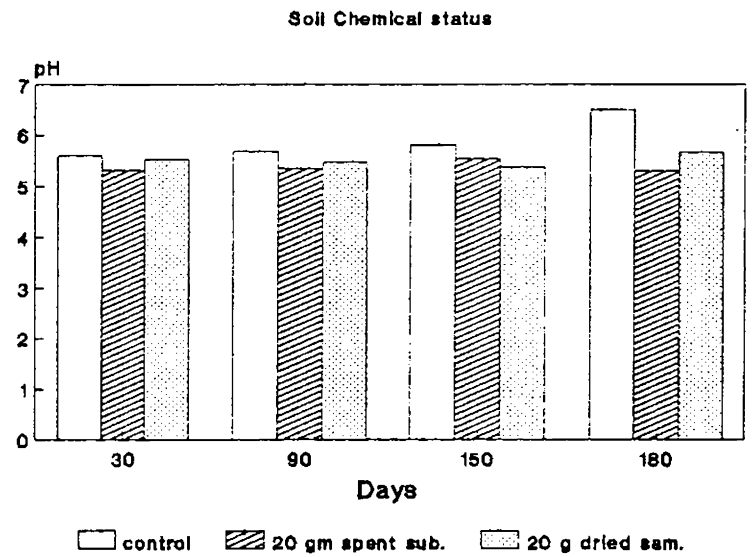
Table IV. Soil Chemical status (2.) Exchangeable magnesium

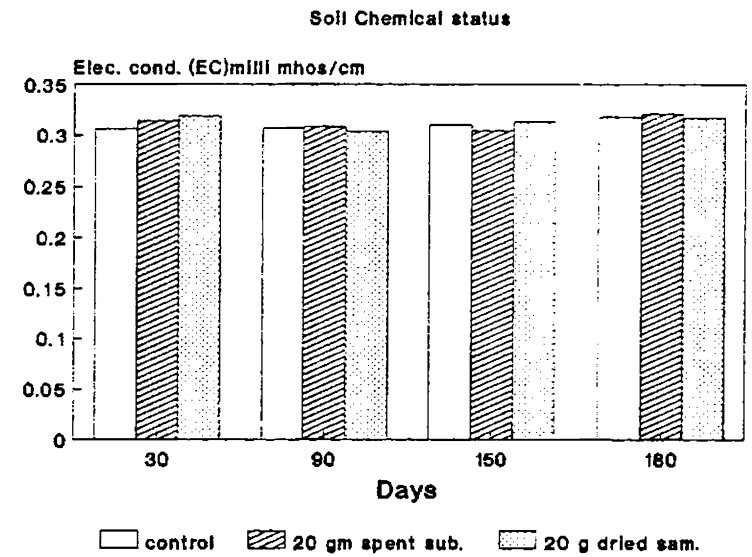
| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 0.5983 | 0.11966 | 6.683* |
| Treatment sum of squares | 5 | 0.5816 | 0.11632 | 6.496* |
| Error sum of squares | 25 | 0.4476 | 0.017904 | |
| Total | 35 | 1.6275 | | |

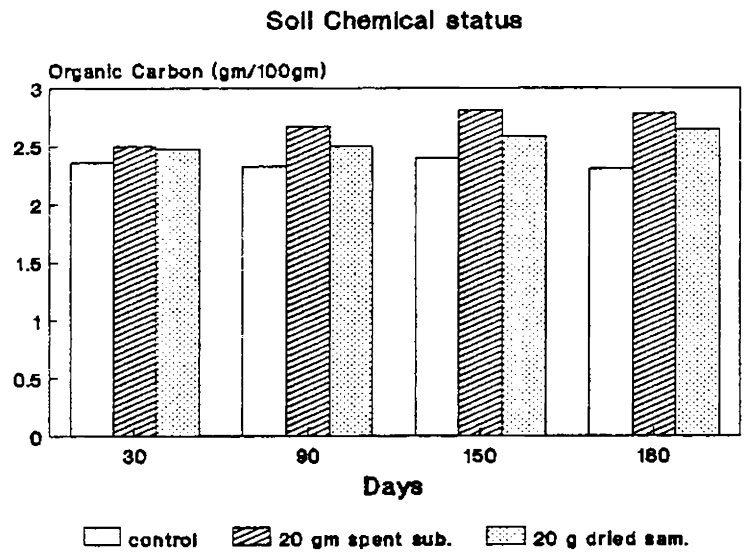
Table IV. Soil Chemical status (3.) Cation exchange capacity (CEC)

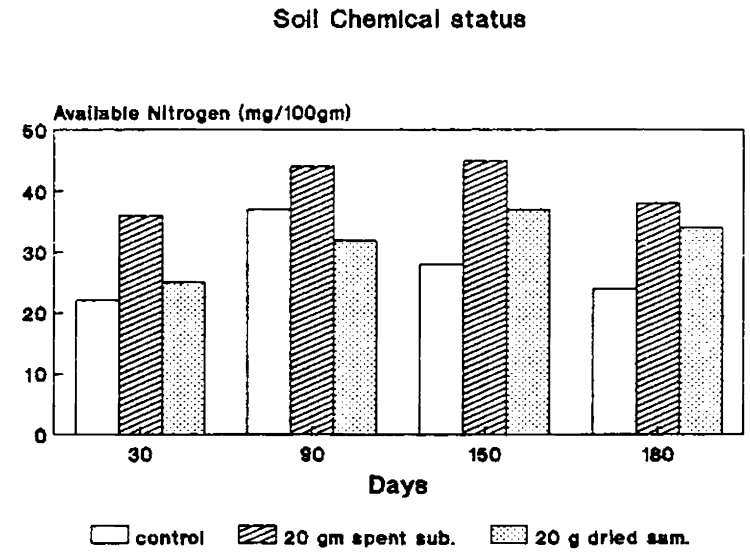
| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 3.1126 | 0.6225 | 6.97* |
| Treatment sum of squares | 5 | 3.769 | 0.7538 | 8.441* |
| Error sum of squares | 25 | 2.2344 | 0.0893 | |
| Total | 35 | 9.116 | | |

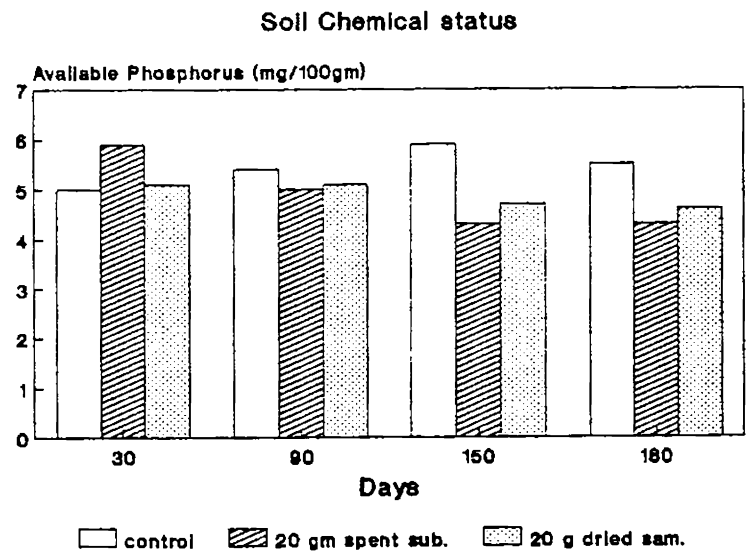
* F values are significant at 5% level, ie. $P < 0.05$.

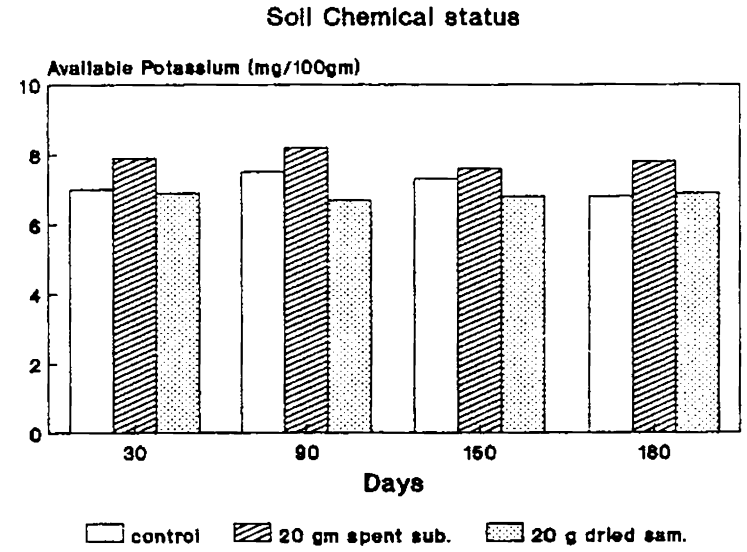

Soil Chemical status
pH
7
6
5
4
3
2
1
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.

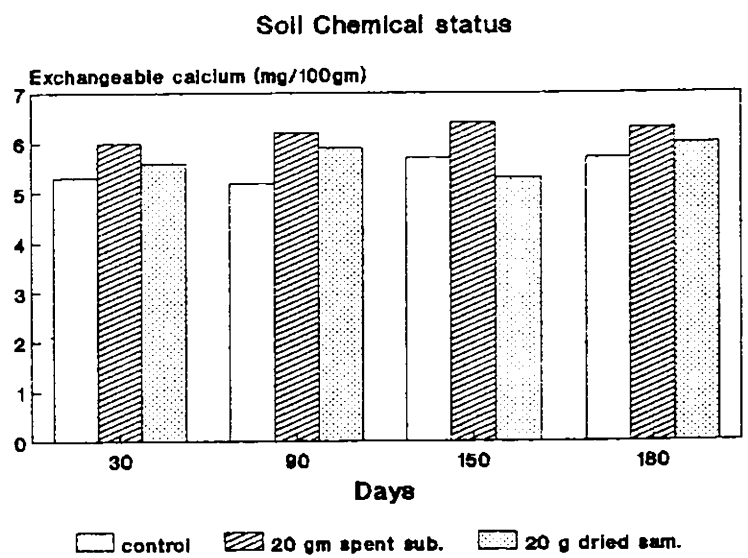

Soil Chemical status
Elec. cond. (EC)milli mhos/cm
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.

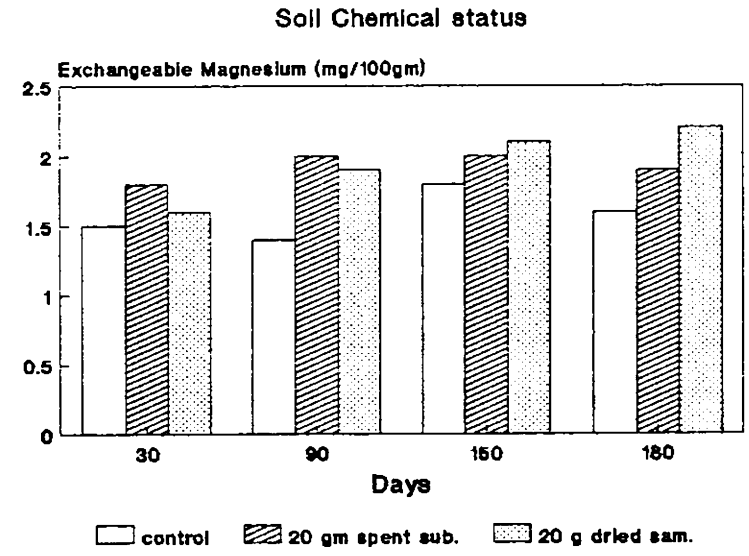

Soil Chemical status
Organic Carbon (gm/100gm)
3
2.5
2
1.5
1
0.5
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.

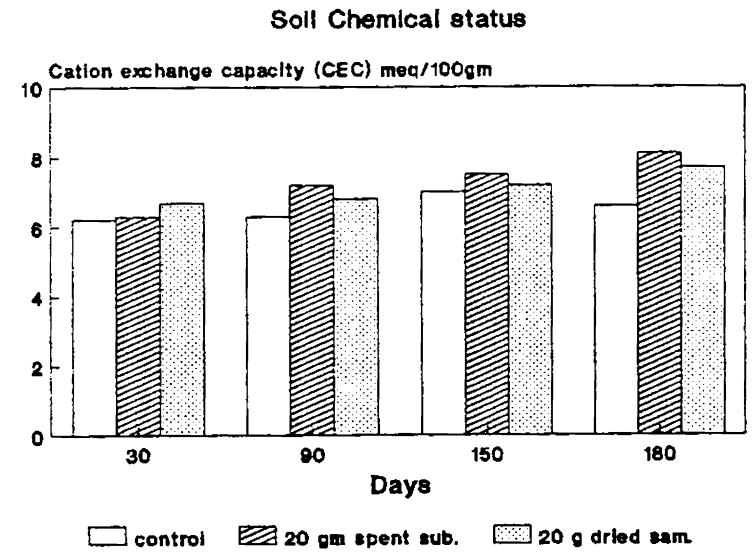

Soil Chemical status
Available Nitrogen (mg/100gm)
50
40
30
20
10
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Soil Chemical status
Available Phosphorus (mg/100gm)
7
6
5
4
3
2
1
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Soil Chemical status
Available Potassium (mg/100gm)
10
8
6
4
2
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Soil Chemical status
Exchangeable calcium (mg/100gm)
7
6
5
4
3
2
1
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Soil Chemical status
Exchangeable Magnesium (mg/100gm)
2.5
2
1.5
1
0.5
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Soil Chemical status
Cation exchange capacity (CEC) meq/100gm
10
8
6
4
2
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.

## 6-2.Soil Microbial population Dynamics

### 6-2. 1 Introduction

The qualitative and quantitative nature of micro organisms inhabiting the soil influences the soil fertility to a greater extent. These micro organisms can be classified into bacteria, actinomycetes, fungi, algae and protozoa. Rhizosphere, (the zone where roots and soils meet) is a special environment and the micro organisms in this area affect plant growth in a number of ways. Certain soil - inhabiting microorganisms produce diseases of great significance. Others are beneficial, they partly inhibit the growth of disease organisms or kill them. In some cases invasion of roots by micro organisms is desirable. This is true for the root - nodule bacteria of the genus *Rhizobium* that fix nitrogen, as well as for mycorrhizal fungi which assist roots in accumulating phosphate and other essential minerals. The processes of nutrient cycling, growth stimulation or inhibition and diseases are of great significance but these are, very complex population effects, rather than the result of simple interactions between roots and known micro organisms.

The sum of various inter -relationships of the rhizosphere micro organisms and roots can benefit plant growth by influencing the availability of essential nutrients, by producing plant growth regulators and also by suppressing root pathogens.

Important activities of soil micro organisms include (i) mineralization i.e. by the decomposition of plant and animal residues, soil micro organisms release carbon, nitrogen, sulphur, phophorus and trace elements from organic materials, in forms that can be absorbed by plants. (ii) Solvent action of micro organisms i.e. certain soil micro organisms promote phosphorus solubilization by the production of chelators. These chelators form complexes with metal ions and increase their solubility. (iii) Competence with roots-- micro organisms require many of the same nutrient elements that are essential to plants for their growth. When these minerals are in short supply, the rhizosphere population will compete with roots for nourishment. (iv) Micro organisms also produce vitamins, aminoacids, hormones and other growth regulating substances. Rhizosphere micro organisms are variously credited for promoting rate of seed germination, root elongation, root hair development, nutrient uptake and plant growth.

In the present study, quantitative determination of the three major groups of soil micro organisms viz. bacteria, actinomycetes and fungi with special importance to their population dynamics in response to the addition of spent substrate (an organic supplement) are carried out. Special emphasis is given to the quick response ( 5 days interval for 20 days) and to the delayed response (30 days interval for 180 days).

6.2.2 Materials and Methods:

Freshly collected samples (random selection was made from both rhizosphere and non-rhizosphere area) were used for pre-testing and subsequent count of microbial population (from 3 to 8cm depth). Counts were taken for total bacteria, actinomycetes and fungi. Microbial counts were taken (i) at 5 days interval for the first 20 days, in order to evaluate the quick response of microbes towards this partially degraded spent substrate added as the organic supplement (ii) and also in 30 days interval for 180 days to evaluate delayed response.

Total number of bacteria, actinomycetes and fungi was cultured by pour-plate technique after serial dilution. The specific media used for culture were, nutrient agar for total bacteria (Aneja, 1993), Kenknight and Munaier's medium - for total actinomycetes (Subba Rao, 1981) and Rose-Bengal agar for fungi (Martin, 1950). Cultures were prepared in duplicate for all treatments and the mean values were taken as microbial count and were expressed in (Bacteria $\times$ $10^7$, Actinomycetes $\times$ $10^6$ & Fungi $\times$ $10^4$).

Composition of media used for microbial culture

(I) Bacteria - nutrient agar medium.

| Component | Weight in gm |
|---|---|
| Beef extract | 3 |
| Peptone | 5 |
| Agar | 15 |

Beef extract and peptone were dissolved in 500ml of distilled water. Boiled 15gm of agar in another 500ml water and these two were mixed together. Then heated until the peptone was dissolved. Adjusted the pH to 6.7 using bromothymol blue as indicator.

(ii) Kenknight and Munaier's medium - for Actinomycetes

| Components | Weight in gm |
|---|---|
| Dextrose | 1 |
| $KH_2PO_4$ | 0.10g |
| $NaNO_3$ | 0.10g |
| KCl | 0.10g |
| $MgSO_4$ $7H_2O$ | 0.10g |
| Agar | 15g |
| Distilled water | 1000ml |

All these components were dissolved in distilled water by heating. Then sterilized the medium in an autoclave at $120^0$ C (15 lbs pressure for 30 minutes).

(iii) Fungal medium - Rose Bengal agar

| Components | Weight in gm |
|---|---|
| Glucose | 10 |
| Peptone | 5 |
| $KH_2PO_4$ | 1 |
| $MgSO_4$ $7H_2O$ | 0.50 |
| Streptomycin | 0.03 |
| Agar | 15 |
| Rose-Bengal | 0.035 |
| Distilled water | 1000ml |

All components were dissolved in distilled water by boiling and sterilized the medium in an autoclave. The antibiotic was sterilized separately and added aseptically to the sterilized medium.

### 6.2.3 Results and Discussion

Major constituents in spent substrate include cellulose, hemicellulose ,lignin, remnants of chitinous fungal hyphae, humic acids fractions etc. Examples of micro organisms capable of degrading these constituents are :, (i) Cellulose & hemicellulose :- (fungi) - *Alternaria, Aspergillus, Rhizoctonia, Rhizopus Trichoderma*

(bacteria ) - *Achromobacter, Angiococcus, Bacillus, Cellulomonas, Clostridium, Pseudomonas species* etc (actinomycetes) - *Micromonospora, Nocardia, Streptomyces.* (ii) Lignin :- (fungi) - *Clavaria, Clitocybe, Collybia, Hypholoma, Lepiota, Mycena* (bacteria) - *Pseudomonas, Flavobacterium* etc. (iii) Chitinous substances :- (fungi) *Fusarium, Mucor, Mortierella, Trichoderma, Gliocladium, Thamnidium, Absidia* etc (bacteria) *Cytophaga, Achromobacter, Bacillus, Beneckea, Flavobacterium, Micrococcus, Pseudomonas* (actinomycetes) *Streptomyces, Nocardia, Micromonospora* etc.(Gray & William, 1971).

Patrik, et. al (1964) reported that ,the size of particles in the organic matter, the nature and abundance of micro organisms involved, the extent of availability of C,N,P and K, the moisture content of the soil, its temperature, pH and aeration, presence of inhibiting substances (such as tannins) are some of the major factors which influence the rate of organic matter decomposition.

Bacteria constitute the most abundant group of micro organisms and in normal fertile soils 10-100 million bacteria may be present per gram of soil. Their number may increase depending on the organic matter content of the soil. The bulk of soil bacteria are heterotrophic and they utilize readily available source of energy like sugars, starch cellulose etc. Other important activities include decomposition of protein with the liberation of ammonia (ammonification), formation of nitrates and nitrites (nitrification), transformation of nitrogenus

compounds into elementary nitrogen (denitrification), fixation of atmospheric nitrogen (nitrogen fixation) etc. Autotrophic bacteria, which occupy a smaller portion of the biomass in soil and utilise inorganic sources such as iron *(Ferrobacillus)* and sulphur *(Thiobacillus)* by oxidation and reduction reactions are not directly involved in organic matter decomposition. (Hughes & Rose, 1971).

There are numerous accounts of fungal colonization of leaf litter.(Visser and Parkinson,1975, Monarch et. al, 1986, Sinha, 1992, Pathak & Asha Sinha, 1995). Soil fungi generally belong to four main groups, viz Phycomycetes, Ascomycetes, Basidomycetes and Fungi imperfectii. Their chief activity is the decomposition of organic compounds, both nitrogenous and non-nitrogenous, that are added to the soil in the form of manures and plant residues (Peterson, 1958). On the contrary, the metabolic processes of certain pathogenic forms also produce toxins (Pringle & Scheffer, 1964).

Actinomycetes are a group of organisms that are intermediate between the bacteria and fungi. Actinomycetes also take an active part in the decomposition of all sorts of organic substances and humus formation. After the decomposition of fresh organic matter by bacteria and fungi, the actinomycetes take the field and attack the more resistant substances left decomposed by other organisms (Lakshmikumari, 1961).

Though the mode of activities and products of metabolic processes vary depending on the type of microorganisms, their overall increase in number will provide a direct indication of their successful establishment on a substratum. In this experiment, the quantitative changes of soil microbes (bacteria, actinomycetes and fungi) in response to the addition of spent substrate were studied on suitable media. Results showed significant increase in number of microbes in samples (delayed response Table VI, 1, 2, 3) collected from spent substrate-added pots. Though the results were not significant in the earlier stages (Table V, 1, 2, 3) at a later stage, the spent substrate was found to be suitable for the growth of microorganisms inhabiting in and around the rhizosphere of *Anthurium* plants. This is probably due to the better availability of liberated nutrients for microbes at a later stage. The probable reason for the reduction in microbial population of earlier response samples is the replacement of non-thermophilic forms by the thremophilic forms.

Bacteria are the most dominant group of microorganisms in soil and their population decreases as the depth of the soil increases. According to Winogradsky, (1925) soil microorganisms include two broad categories viz. autochthonous and zymogenous organisms. The Autochthonous or indigenous population is always uniform and constant in soil, since their nutrition is derived from native soil organic matter. On the other hand, zymogenous or fermentative organisms require an external source of energy and their normal population in soil is low. When specific substrates are added to the soil the number of zymogenous

bacteria increases and gradually declines when the added substrate is exhausted. With the addition of spent substrate a similar event might have occured and the significant increase of number of microorganisms (in delayed response samples) can be attributed to this.

In the early stages of decomposition, bacteria and protozoa are very active. With the increase in temperature they die out and then thermophilic microorganisms especially some bacteria, actinomycetes and fungi become active. The significant increase of number of microorganisms in samples (delayed response) is attributed to this type of microbial population dynamics.

Since the nitrogen content of spent residue is relatively higher, its decomposition also occurs at a rapid rate. And, being in the powdered form, it is easily attacked by the soil bacteria. The hemicelluloses encrusting the cellulose in the decomposing plant material are first attacked by fungi, resulting in the evolution of heat and $CO_2$. Then the cellulose is attacked and there is rapid reduction in the cellulose content. The growth of *Pleurotus* reduces the lignocellulosic contents considerably, this in turn triggers the decomposition of spent residue.

In the early stages of decomposition, heterotrophic bacteria are highly active. In the course of breakdown, various intermediary substances are formed. Other bacteria, actinomycetes and fungi which are capable of attacking the intermediate products now become active and they utilise $CO_2$ to synthesise various carbon compounds. They oxidise sulphur, ammonia etc to obtain energy for the anabolic processes. Thus the original organic matter is reduced in part to simpler substances which are utilised by various microorganisms for their body build up. Some of the breakdown products dissolve in soil water and are available for plant growth. The insoluble plant residues constitute the soil organic complex or humus (Rangaswami and Bagyaraj, 1996).

**TABLE V - MICROBIAL POPULATION DYNAMICS - QUICK RESPONSE**

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE 0 DAY | (1) TOTAL BACTERIA (x $10^7$) 7.2 ± 0.57 | | | | | |
|---|---|---|---|---|---|---|
| TREATMENTS IN WEIGHT ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 5 | 9.7 ± 0.18 | 7.4 ± 0.53 | 8.9 ± 0.24 | 8.0 ± 0.09 | 8.2 ± 1.20 | 7.0 ± 0.18 |
| 10 | 8.0 ± 0.20 | 7.2 ± 0.27 | 7.8 ± 0.77 | 22.4 ± 0.41 | 8.8 ± 1.37 | 8.7 ± 0.61 |
| 15 | 5.5 ± 0.28 | 8.9 ± 1.11 | 6.2 ± 0.23 | 7.5 ± 0.40 | 7.8 ± 0.71 | 8.6 ± 0.53 |
| 20 | 6.3 ± 0.47 | 7.5 ± 1.38 | 7.7 ± 0.45 | 9.3 ± 0.19 | 7.2 ± 0.53 | 6.7 ± 0.50 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE 0 DAY | (2) TOTAL ACTINOMYCETES (x $10^6$) 1.8 ± 0.19 | | | | | |
|---|---|---|---|---|---|---|
| TREATMENTS IN WEIGHT ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 5 | 2.4 ± 0.14 | 2.1 ± 0.27 | 2.9 ± 0.16 | 3.9 ± 0.28 | 3.1 ± 0.35 | 2.7 ± 0.32 |
| 10 | 3.0 ± 0.10 | 3.5 ± 0.13 | 4.1 ± 1.31 | 5.3 ± 0.42 | 4.8 ± 0.28 | 2.6 ± 0.19 |
| 15 | 2.8 ± 0.17 | 3.3 ± 0.52 | 4.5 ± 0.39 | 6.9 ± 0.31 | 6.1 ± 0.16 | 3.1 ± 0.24 |
| 20 | 2.5 ± 1.12 | 13.8 ± 0.54 | 4.3 ± 0.22 | 4.7 ± 0.18 | 8.4 ± 0.27 | 4.3 ± 0.15 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE 0 DAY | (3) TOTAL FUNGI (x $10^4$) 1.32 ± 0.21 | | | | | |
|---|---|---|---|---|---|---|
| TREATMENTS IN WEIGHT ⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 5 | 3.1 ± 1.28 | 3.9 ± 0.71 | 4.7 ± 0.15 | 5.3 ± 1.12 | 4.9 ± 0.81 | 4.3 ± 0.19 |
| 10 | 2.7 ± 0.09 | 4.1 ± 0.12 | 4.4 ± 0.78 | 7.1 ± 1.31 | 9.0 ± 0.47 | 5.6 ± 0.18 |
| 15 | 1.7 ± 0.32 | 5.0 ± 1.13 | 6.8 ± 0.53 | 9.9 ± 0.25 | 11.1 ± 1.18 | 4.0 ± 0.09 |
| 20 | 4.3 ± 0.24 | 7.0 ± 0.40 | 8.1 ± 0.22 | 5.7 ± 0.19 | 20 ±0.17 | 5.7 ± 0.17 |

DS- dried *Salvinia* CNT- control.

Values are the mean of six separate samples ± S E M

Table V. Microbial population dynamics - Quick response (1.) Total bacteria

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 3 | 56.7088 | 18.902 | 2.107 |
| Treatment sum of squares | 5 | 37.4946 | 7.498 | 0.835 |
| Error sum of squares | 15 | 134.6629 | 8.97 | |
| Total | 23 | 228.8663 | | |

Table V. Microbial population dynamics - Quick response (2.) Total actinomycetes

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 3 | 38.4396 | 12.8132 | 2.594 |
| Treatment sum of squares | 5 | 33.5266 | 6.7053 | 1.357 |
| Error sum of squares | 15 | 74.1534 | 4.94 | |
| Total | 23 | 146.1196 | | |

Table V. Microbial population dynamics - Quick response (3.) Total fungi

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 3 | 40.64 | 13.55 | 1.08 |
| Treatment sum of squares | 5 | 101.92 | 20.38 | 1.638 |
| Error sum of squares | 15 | 186.62 | 12.44 | |
| Total | 23 | 329.18 | | |

**TABLE VI - MICROBIAL POPULATION DYNAMICS - DELAYED RESPONSE**

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE 0 DAY | (1) TOTAL BACTERIA (x $10^7$) 7.2 ± 0.42 | | | | | |
|---|---|---|---|---|---|---|
| TREATMENTS IN WEIGHT⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 7.6 ± 0.21 | 15.7 ± 0.37 | 24.0 ± 0.33 | 18.0 ± 0.48 | 29.8 ± 0.10 | 10.1 ± 1.02 |
| 60 | 10.0 ± 0.36 | 10.8 ± 0.55 | 26.2 ± 0.71 | 33.0 ± 0.53 | 31.3 ± 1.08 | 8.3 ± 0.12 |
| 90 | 7.2 ± 0.43 | 15.0 ± 0.10 | 30.1 ± 1.22 | 26.0 ± 0.47 | 36.5 ± 0.59 | 10.7 ± 0.42 |
| 120 | 10.2 ± 0.50 | 12.3 ± 0.38 | 25.8 ± 1.27 | 24.4 ± 1.42 | 26.0 ± 0.56 | 13.2 ± 0.70 |
| 150 | 10.0 ± 1.32 | 23.0 ± 0.59 | 18.7 ± 0.61 | 27.3 ± 0.74 | 30.0 ± 0.21 | 15.7 ± 1.09 |
| 180 | 10.4 ± 1.03 | 15.5 ± 0.42 | 20.0 ± 1.40 | 19.0 ± 1.02 | 28.8 ± 0.41 | 18.3 ± 0.63 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE 0 DAY | (2) TOTAL ACTINOMYCETES (x $10^6$) 1.8 ± 0.30 | | | | | |
|---|---|---|---|---|---|---|
| TREATMENTS IN WEIGHT⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 4.0 ± 0.59 | 6.0 ± 0.40 | 8.1 ± 0.20 | 6.7 ± 1.28 | 7.9 ± 0.79 | 4.5 ± 0.35 |
| 60 | 4.6 ± 0.19 | 13.0 ± 0.18 | 12.3 ± 0.35 | 20.0 ± 0.52 | 27.6 ± 1.45 | 8.3 ± 0.40 |
| 90 | 3.3 ± 0.43 | 10.1 ± 0.50 | 9.5 ± 0.80 | 16.3 ± 0.57 | 15.1 ± 0.36 | 4.0 ± 0.43 |
| 120 | 1.9 ± 0.79 | 7.6 ± 1.11 | 15.4 ± 0.85 | 17.1 ± 0.56 | 18.7 ± 1.16 | 5.6 ± 0.35 |
| 150 | 4.2 ± 0.11 | 5.8 ± 0.32 | 17.6 ± 0.40 | 25.6 ± 0.47 | 10.2 ± 0.50 | 7.0 ± 0.05 |
| 180 | 7.0 ± 0.53 | 7.8 ± 0.64 | 12.0 ± 0.27 | 18.1 ± 0.18 | 19.7 ± 0.13 | 9.1 ± 0.45 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE 0 DAY | (3) TOTAL FUNGI (x $10^4$) 1.32 ± 0.53 | | | | | |
|---|---|---|---|---|---|---|
| TREATMENTS IN WEIGHT⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 2.9 ± 0.21 | 13.3 ± 0.41 | 10.0 ± 0.50 | 11.1 ± 0.50 | 4.5 ± 0.20 | 5.6 ± 0.64 |
| 60 | 3.6 ± 0.39 | 10.1 ± 0.39 | 19.6 ± 1.20 | 11.7 ± 0.39 | 13.7 ± 0.57 | 6.9 ± 0.22 |
| 90 | 4.7 ± 0.52 | 15.0 ± 1.09 | 14.0 ± 0.74 | 7.8 ± 0.34 | 17.1 ± 0.45 | 7.3 ± 0.21 |
| 120 | 2.0 ± 0.50 | 12.9 ± 0.32 | 24.1 ± 0.64 | 17.7 ± 0.80 | 24.3 ± 1.39 | 10.0 ± 1.01 |
| 150 | 3.3 ± 0.45 | 14.5 ± 0.58 | 17.2 ± 0.52 | 8.9 ± 0.28 | 18.1 ± 0.72 | 10.7 ± 0.74 |
| 180 | 1.9 ± 0.33 | 7.4 ± 0.34 | 20.0 ± 0.64 | 13.0 ± 0.75 | 16.8 ± 0.08 | 9.1 ± 0.32 |

DS - Dried *Salvinia*, CNT - Control
Values are the mean of six separate samples ± S E M

Table VI. Microbial population dynamics - Delayed response (1.) Total bacteria

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 55.048 | 11.0096 | 0.6565 |
| Treatment sum of squares | 5 | 2013.075 | 402.615 | 24.016* |
| Error sum of squares | 25 | 419.117 | 16.7646 | |
| Total | 35 | 2487.24 | | |

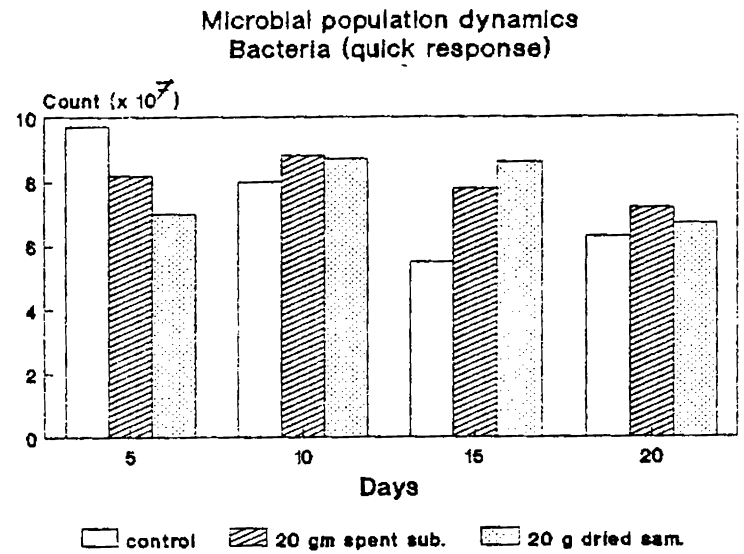
Table VI. Microbial population dynamics - Delayed response (2.) Total actinomycetes

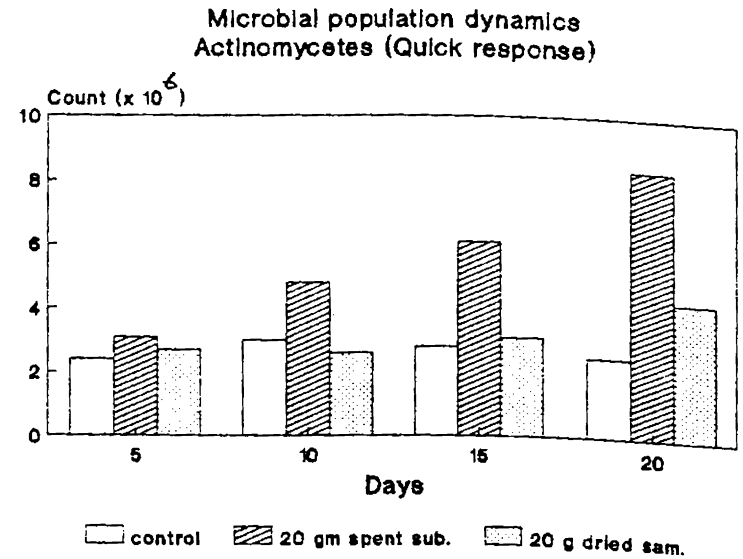
| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 226.07 | 45.214 | 3.193* |
| Treatment sum of squares | 5 | 881.82 | 176.36 | 12.456* |
| Error sum of squares | 25 | 354.086 | 14.16 | |
| Total | 35 | 1461.976 | | |

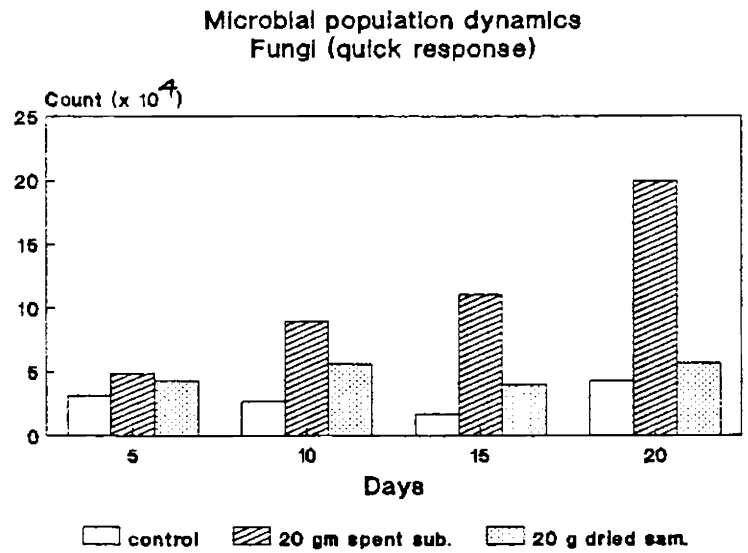
Table VI. Microbial population dynamics - Delayed response (3.) Total fungi

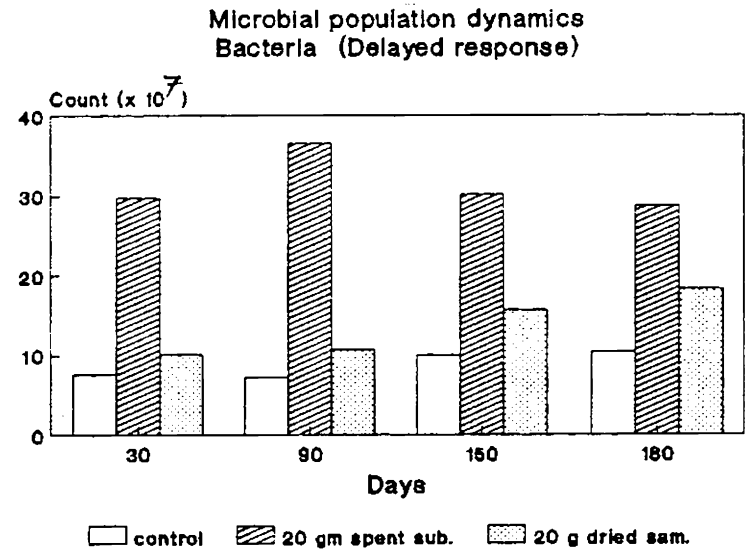
| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 164.06 | 32.812 | 2.738* |
| Treatment sum of squares | 5 | 815.53 | 163.106 | 13.613* |
| Error sum of squares | 25 | 299.53 | 11.9812 | |
| Total | 35 | 1279.12 | | |

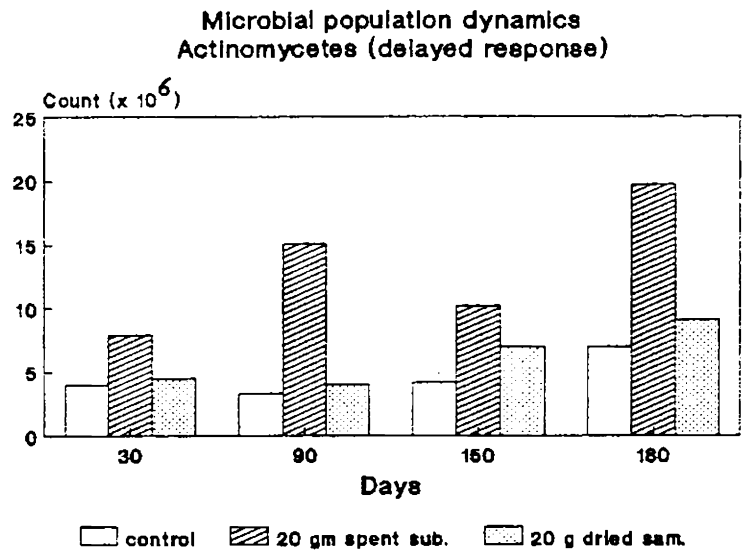
* F values are significant at 5% level, ie. $P < 0.05$.

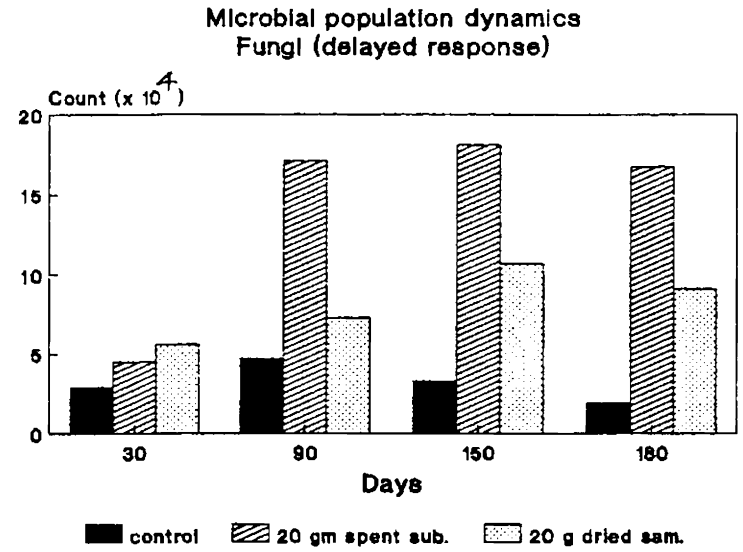

Microbial population dynamics
Bacteria (quick response)
Count (x 10⁷)
10
8
6
4
2
0
5
10
15
20
Days
control
20 gm spent sub.
20 g dried sam.


Microbial population dynamics
Actinomycetes (Quick response)
Count (x 10⁶)
10
8
6
4
2
0
5
10
15
20
Days
control
20 gm spent sub.
20 g dried sam.


Microbial population dynamics
Fungi (quick response)
Count (x 10^4)
25
20
15
10
5
0
5
10
15
20
Days
control
20 gm spent sub.
20 g dried sam.


Microbial population dynamics
Bacteria (Delayed response)
Count (x 10^7)
40
30
20
10
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Microbial population dynamics
Actinomycetes (delayed response)
Count (x 10^6)
25
20
15
10
5
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Microbial population dynamics
Fungi (delayed response)
Count (x 10^4)
20
15
10
5
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.

## 6.3. Plant Growth Analysis

6.3. 1 Introduction:-

Growth is the most obvious manifestation of life which is the net result of the metabolic processes occurring in the plant body, thereby, an irreversible increase in size, area or volume of cells, tissues and organs takes place. In higher plants growth is confined to meristems (localized type) and these meristematic regions are composed of (i) the region of cell formation (ii) the region of cell enlargement (iii) the region of cell maturation.

The pattern of development during vegetative phase is influenced by various internal and external factors viz. genetic, nutritional, environmental and hormonal. Among these factors, three major environmental factors affecting growth include (i) light intensity and photoperiodism (the periodic effect of lightness and darkness on physiological activities in plants). (ii) Temperature and thermoperiodicity and (iii) Nutritional balance. The third one deserves particular importance with regard to the addition of organic supplement (spent substrate). An adequate supply of nutritional organic substances is necessary for plant growth. They provide energy which is necessary for growth and also supply materials for the building up of new protoplasm and cell walls.

Growth analysis is a powerful tool in studying complex interactions between plant growth and the environment. Growth can be measured in many different ways. For example, an increase in plant height, individual leaf size (length, width & area), plant fresh weight, plant dry weight partitioned among organs such as roots, stem leaves, fruits, cell numbers in tissues and organs and concentration of specific chemical constituents (total chlorophyll, nucleic acids, soluble nitrogen, protein nitrogen, lipids, carbohydrates) in tissues and organs are examples of growth data that have been reported (Noggle and Fritz, 1986).

6.3.2. Materials and Methods.

Due to their semi - epiphytic mode of life, *Anthurium* plants generally show slower but steady pattern of growth. Growth kinetics in terms of the following 6 parameters were studied in this investigation.

(i) Frequency of formation of new leaves (Fig 3)

(ii) Leaf Area Ratio (LAR)- expressed in $cm^2/gm$.

(iii) Total chlorophyll content (a & b) - mg /g of fresh leaves.

(iv) Frequency of formation of new and healthy surface roots (Fig 4)

(v) Frequency of flowering (Fig 5)

(vi) Frequency of sucker formation (Fig 6)

Among these parameters (i), (iv), (v) & (vi) were noted by visual observation and were noted down at 30 days interval. Leaf area ratio was computed using graph paper & digital balance and amount of total chlorophyll (a & b) were measured by Arnon's method (Arnon, 1949).

Estimation of chlorophyll

Principle

Chlorophyll can be extracted in 80% acetone and the absorption at 663 nm and 645 nm are read in a spectrophotometer. Using the absorption coefficients, the amount of total chlorophyll can be calculated.

Procedure

1. Weighed out 1g of finely cut and well mixed representative sample of leaf into a clean mortar.
2. Ground the tissue to a fine pulp with the addition of 20ml of 80% acetone.
3. The extract was centrifuged (5,000 rpm for 5min) and transferred the supernatant to a 100ml volumetric flask.
4. Again ground the residue with 20ml of 80% acetone, centrifuged and transferred the supernatant into the same volumetric flask.
5. Repeated this procedure until the residue became colourless. Washed the mortar and pestle thoroughly with 80% acetone and collected the clear washings in the volumetric flask.
6. Made up the volume to 100ml with 80% acetone.
7. Read the absorbance of the solution at 645 and 663 nm, against the solvent (80% acetone) blank.

Then the amount of chlorophyll present in the extract i.e.mg chlorophyll /g fresh leaf samples was calculated using the following equation.

$$\text{mg total chlorophyll / g fresh leaf tissue} = \frac{20.2\,(A\,645) + 8.02\,(A\,663) \times V}{1000 \times W}$$

Where A - absorbance at specific wavelength

V - final volume of chlorophyll extract in 80% acetone

W - Fresh weight of the tissue extracted.

### 6.3.3 Results and Discussion

The relationship between organic matter and plant growth may be direct or indirect. Organic matter is a natural substrate for saprophytic micro organisms and provides nutrition to plants indirectly through the activity of soil micro organisms. It is essential for the formation of soil aggregates and hence affects the soil structure, and ultimately determines the extent of soil aeration and rooting habit of plants. Generally organic matter helps in the conservation of soil nutrients by preventing erosion and surface run-off of nutrients.

Though certain decomposition products of plant remains such as syringaldehyde, ferulic acid, p-hydroxybenzoic acid etc have been reported to be phytotoxic ( Rovira, 1965), these products seem to accumulate under water

logged anaerobic conditions. In well aerated soil, the presence of these toxic compounds are rather negligible.

Major synthetic functions in higher plants include photosynthesis, protein synthesis, lipid synthesis etc. together with the synthesis of small molecules and energy rich molecules. In fact, all the events of growth and development are the ultimate results of physiological functions. In order to meet the requirements of these functions, plants need frame- work elements (carbon, hydrogen and oxygen), non-metals like nitrogen, sulphur and phosphorus and metals like potassium, calcium, magnesium, iron etc and also trace elements. In the long run, these elements form proteins and other organic constituents of the plant and later on, as a result of cell division, cell enlargement and cell differentiation, various tissues and organs are formed.

In this experiment, out of the six parameters under investigation, four of them viz. (i) frequency of formation of new leaves (ii) leaf area ratio (iii) total chlorophyll and (iv) frequency of formation of new and healthy surface roots showed significant difference in spent substrate added pots (Table VII 1,2,3 & Table VIII,1 ). Frequency of flowering and frequency of sucker formation were also found to be earlier in spent substrate added pots. (Table VIII, 2,3 ). Of these, formation of new leaves, leaf area ratio and total chlorophyll content are directly related to the nutritional status of the soil, especially to the nitrogen and magnesium levels.

While other characters such as formation of new and healthy surface roots, frequency of flowering and frequency of sucker formation etc can be related to the overall soil nutrient status especially to the increased potassium and calcium content.

Studies by Roy Chowdhary, (1995) in *Colocasia*, a member of Aroidae family (*Anthurium* also belong to Aroidae) reported that the leaf area ratio is a reliable index in plant growth analysis and greater leaf area is associated with higher dry matter production and yield. Similar observations with regard to the importance of leaf area ratio on plant growth in other crops were noted by earlier workers (Ramanujam and Indira, 1983, Roy Chowdhary and Ravi, 1994). Ramanujam, (1985) reported that maintenance of optimum leaf area index was important for better yield in Cassava. In this experiment the leaf area ratio of plants which are growing in pots with added spent substrate was found to be increasing in accordance with the treatment dose. From this observation, we could generalize that spent substrate triggers overall plant growth.

The leaf analysis data on the total chlorophyll content were also found to vary significantly in accordance with the treatment dose. The maximum chlorophyll concentration was obtained in plants with 20 gm spent substrate added condition. This is in agreement with the observations made by Mondal and Chattopadhyay, (1993) in custard apple under field conditions.

From the multidimensional impacts of added organic spent substrate, we could arrive at the following conclusions with respect to the plant growth.

(a) The added substrate promoted successive formation of new leaves especially in pots with 15g and 20g added substrate.

(b) The leaf area ratio ($cm^2/g$) were found to be increased with the increase of added substrate in a significant manner.

(c) Significant increase of total chlorophyll in a similar manner also is a direct measure of enhanced plant growth.

(d) Formation and ramification of new and healthy surface roots were higher in pots supplemented with spent substrate .

(e) Flowering starts in pots supplemented with 20g spent substrate and the overall frequencies were higher in pots supplemented with 15g and 20g spent substrate

(f) Frequency of sucker formation was found to be triggered in pots containing 15g and 20g spent substrate . To a certain extent sucker formation was also observed in pots with dried *Salvinia.*

**TABLE VII - PLANT GROWTH PATTERN AFTER THE ADDITION OF SPENT SUBSTRATE**

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE | (1) TOTAL NUMBER OF NEW LEAVES AMONG SIX PLANTS | | | | | |
|---|---|---|---|---|---|---|
| 0 DAY | LEAVES ALREADY FORMED WERE NOT TAKEN INTO ACCOUNT | | | | | |
| TREATMENTS IN WEIGHT⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 2 | 1 | 2 | 2 | 3 | 1 |
| 60 | 2 | 3 | 2 | 4 | 3 | 2 |
| 90 | 4 | 3 | 3 | 2 | 6 | 3 |
| 120 | 2 | 4 | 5 | 5 | 4 | 3 |
| 150 | 4 | 2 | 6 | 3 | 5 | 4 |
| 180 | 2 | 4 | 2 | 3 | 3 | 2 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE | (2) LEAF AREA RATIO (cm2 / gm) | | | | | |
|---|---|---|---|---|---|---|
| 0 DAY | 36.2 ± 0.41 | | | | | |
| TREATMENTS IN WEIGHT⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 36.4 ± 1.09 | 37.3 ± 0.51 | 37.6 ± 0.38 | 36.9 ± 0.44 | 37.3 ± 0.09 | 37.6 ± 0.22 |
| 60 | 37.7 ± 0.51 | 37.8 ± 0.55 | 40.4 ± 0.57 | 39.0 ± 0.32 | 38.8 ± 0.40 | 37.0 ± 0.32 |
| 90 | 38.3 ± 0.60 | 38.0 ± 0.32 | 39.9 ± 0.40 | 40.3 ± 1.42 | 41.9 ± 1.15 | 37.2 ± 0.14 |
| 120 | 39.1 ± 1.12 | 39.4 ± 0.42 | 42.3 ± 0.51 | 40.2 ± 0.35 | 41.5 ± 0.18 | 38.4 ± 0.33 |
| 150 | 39.0 ± 0.21 | 39.6 ± 0.38 | 41.3 ± 0.30 | 41.9 ± 0.50 | 41.5 ± 0.42 | 38.1 ± 0.50 |
| 180 | 40.9 ± 0.50 | 39.9 ± 0.19 | 410 ± 0.73 | 42.0 ± 1.14 | 42.7 ± 0.31 | 39.5 ± 0.48 |

| DAYS AFTER THE ADDITION OF SPENT SUBSTRATE | (3) TOTAL CHLOROPHYLL (a & b) (mg / 100 gm) | | | | | |
|---|---|---|---|---|---|---|
| 0 DAY | 67.4 ± 0.52 | | | | | |
| TREATMENTS IN WEIGHT⇒ | CNT | 5 g | 10 g | 15 g | 20 g | 20 g (DS) |
| 30 | 68.1 ± 0.32 | 68.7 ± 0.50 | 68.0 ± 0.55 | 69.1 ± 0.41 | 68.5 ± 0.19 | 68.6 ± 1.02 |
| 60 | 68.9 ± 0.34 | 69.4 ± 0.44 | 71.8 ± 0.53 | 72.7 ± 1.10 | 70.1 ± 1.22 | 70.0 ± 0.55 |
| 90 | 69.3 ± 1.08 | 72.0 ± 0.50 | 75.3 ± 0.59 | 79.2 ± 0.40 | 78.0 ± 1.30 | 71.1 ± 0.32 |
| 120 | 70.1 ± 1.10 | 73.0 ± 0.38 | 78.6 ± 0.40 | 79.7 ± 0.55 | 79.6 ± 0.55 | 73.4 ± 0.35 |
| 150 | 72.0 ± 0.30 | 75.4 ± 0.42 | 81.2 ± 1.37 | 80.3 ± 0.42 | 81.3 ± 0.59 | 76.1 ± 0.18 |
| 180 | 74.5 ± 0.43 | 79.9 ± 0.50 | 81.0 ± 0.50 | 80.9 ± 1.20 | 82.6 ± 0.37 | 78.7 ± 0.38 |

(1) Values in horizontal rows (time interval replication) represent successive formation of new leaves.
(2) & (3) Values are the mean of six separate samples ± SEM
CNT- control DS- dried *Salvinia*

Table VII. Plant growth pattern after the addition of spent substrate (1.) Frequency of new leaves

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 20.92 | 4.184 | 3.887* |
| Treatment sum of squares | 5 | 8.92 | 1.784 | 1.657 |
| Error sum of squares | 25 | 26.91 | 1.0764 | |
| Total | 35 | 56.75 | | |

Table VII. Plant growth pattern after the addition of spent substrate (2.) Leaf area ratio (LAR)

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 57.891 | 11.5782 | 17.23* |
| Treatment sum of squares | 5 | 37.324 | 7.465 | 11.11* |
| Error sum of squares | 25 | 16.801 | 0.672 | |
| Total | 35 | 112.016 | | |

Table VII. Plant growth pattern after the addition of spent substrate (3.) Total chlorophyll

| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 201.85 | 40.37 | 11.83* |
| Treatment sum of squares | 5 | 539.87 | 107.974 | 31.634* |
| Error sum of squares | 25 | 85.33 | 3.413 | |
| Total | 35 | 827.05 | | |

* F values are significant at 5% level, ie. $P < 0.05$.

## TABLE VIII PLANT GROWTH PATTERN AFTER THE ADDITION OF SPENT SUBSTRATE

### (1) ROOT FORMATION

| Days after the addition of spent substrate ↓ | Number of newly formed surface roots in 6 plants after treatment with | | | | | |
|---|---|---|---|---|---|---|
| | No spent substrate (Control) | 5g spent substrate | 10g spent substrate | 15g spent substrate | 20g spent substrate | 20g dried *Salvinia* |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 2 | 2 | 1 | 3 | 4 | 2 |
| 60 | 3 | 4 | 3 | 3 | 3 | 4 |
| 90 | 3 | 2 | 2 | 4 | 7 | 3 |
| 120 | 1 | 3 | 2 | 5 | 3 | 3 |
| 150 | 3 | 4 | 3 | 6 | 5 | 3 |
| 180 | 2 | 3 | 5 | 4 | 6 | 5 |

Values in horizontal rows represent successive formation of new roots in a group of six plants.

## TABLE VIII : PLANT PATTERN AFTER THE ADDITION OF SPENT SUBSTRATE

## (2) FREQUENCY OF FLOWERING

| Days after the addition of spent substrate ↓ | Frequency of flowering among six plants after tretment with | | | | | |
|---|---|---|---|---|---|---|
| | No spent substrate (Control) | 5g spent substrate | 10g spent substrate | 15g spent substrate | 20g spent substrate | 20g dried *Salvinia* |
| 0 | - | - | - | - | - | - |
| 30 | - | - | - | - | - | - |
| 60 | - | - | - | - | - | - |
| 90 | - | - | - | 1 | - | - |
| 120 | - | - | - | - | 1 | - |
| 150 | 1 | - | 1 | 1 | 2 | - |
| 180 | - | 2 | 1 | 1 | 2 | 1 |

Values in horizontal rows represent formation of flowers successively in a group of six plants.

## TABLE VIII : PLANT GROWTH PATTERN AFTER THE ADDITION OF SPENT SUBSTRATE

## (3) FREQUENCY OF SUCKER FORMATION

| Days after the addition of spent substrate ↓ | Frequency of sucker formation among 6 plants after treatment with | | | | | |
|---|---|---|---|---|---|---|
| | No spent substrate (Control) | 5g spent substrate | 10g spent substrate | 15g spent substrate | 20g spent substrate | 20g dried *Salvinia* |
| 0 | - | - | - | - | - | - |
| 30 | - | - | - | - | - | - |
| 60 | - | - | - | - | - | - |
| 90 | - | - | - | - | - | - |
| 120 | - | - | - | - | 1 | 1 |
| 150 | 1 | 2 | 1 | 2 | 2 | 1 |
| 180 | - | 1 | 2 | - | 1 | 1 |

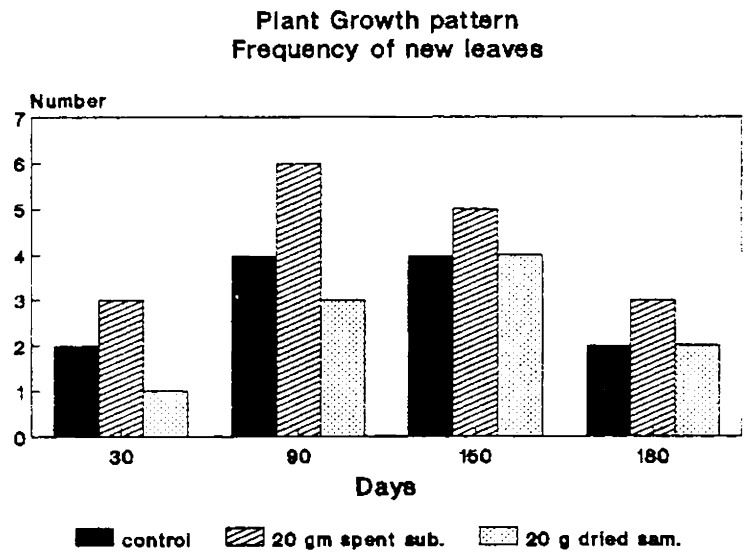
Values in horizontal rows represent successive formation of suckers in a group of six plants.

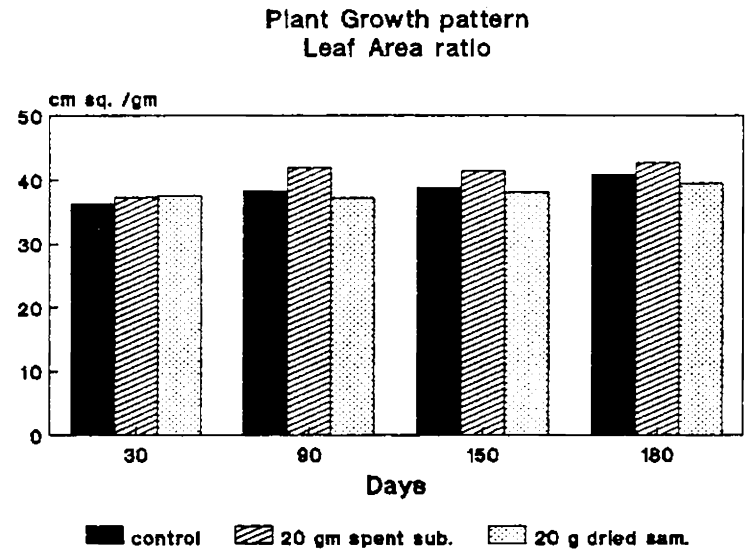
Table VIII. Plant growth pattern after the addition of spent substrate (1.) Frequency of new surface roots

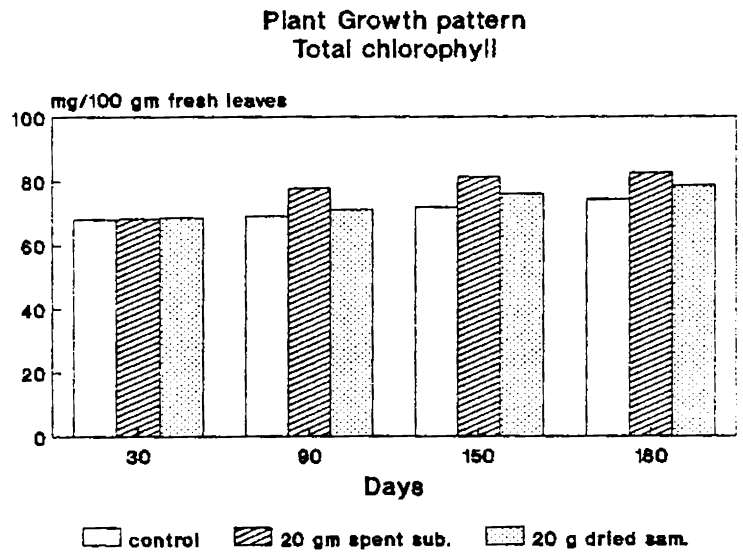
| Source of Variation | degrees of freedom | sum of squares | mean of squares | F - value |
|---|---|---|---|---|
| Replication sum of squares | 5 | 24.14 | 4.828 | 4.357* |
| Treatment sum of squares | 5 | 14.47 | 2.894 | 2.612* |
| Error sum of squares | 25 | 27.70 | 1.108 | |
| Total | 35 | 66.31 | | |

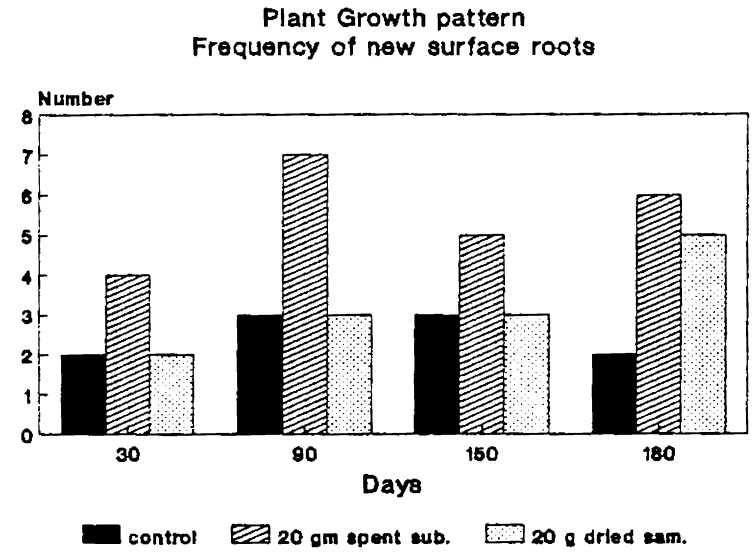
* F values are significant at 5% level, ie. $P < 0.05$.

(2.) and (3.) Frequency of flowering and sucker formation - Since flowering and sucker formation are seen towards the end of the experiment, the values are not statistically analyzed.


Plant Growth pattern
Frequency of new leaves
Number
7
6
5
4
3
2
1
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Plant Growth pattern
Leaf Area ratio
cm sq. /gm
50
40
30
20
10
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Plant Growth pattern
Total chlorophyll
mg/100 gm fresh leaves
100
80
60
40
20
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.


Plant Growth pattern
Frequency of new surface roots
Number
8
7
6
5
4
3
2
1
0
30
90
150
180
Days
control
20 gm spent sub.
20 g dried sam.

## CHAPTER VII

### Seed bed prepared from African weed (*Salvinia molesta* Mitchell) a reliable material for Anthurium (A. *andreanum*) seed germination.

Introduction :

Seeds germinate and develop only under favourable environmental conditions including adequate moisture, optimal temperature and enough supply of oxygen. Apart from these factors, the availability of nutrients, the presence of toxic substances and the activity of microbes in the immediate surroundings influence the germination processes.

In *Anthurium,* seeds are produced naturally or seed setting can be induced by hand pollination . Selfing or crossing can be made, but the percentage of natural seed germination is very low. Plants are commonly propagated by vegetative means i.e. from suckers, root-stock cuttings etc. They are also produced in large numbers by tissue culture (*in vitro*) techniques.(Pierik, 1975).

In these plants, raising seedlings to their flowering stage requires about one and a half to two years. Though it is a lengthy process, breeding techniques and subsequent raising of seedlings are necessary to evolve new and improved hybrid varieties. In *Anthurium* inflorescence, normally the male phase starts with the emergence of four stamens which are clustered around the stigma of each flower.(Croat, 1980 ). In a well- fertilized inflorescence (candle or spadix) about 100-200 berries may develop. Seeds become mature within a period of 6-8 months after pollination. Seeds should be harvested at proper stage, when they are pushed out of the candle. These mature seeds will have a pulpy coating, that must be removed before sowing.

Seedlings of *Anthurium* are commonly raised on specially devised seed- beds of cotton, finely sieved river sand etc. Good seed-bed preparation is essential for successful field and garden sowing and it should have a loose physical texture that produces close contact between seed and substrate base or soil, so that moisture can be supplied continuously to the seed. Such a medium should provide good aeration. Excessive aeration causes rapid drying and so it is avoided. Adequate moisture should be available to carry the seeds through the germinating and early seedling stage. At the same time there should be good drainage so that water does not accumulate or impede oxygen supply to the seed. Organic matter in the soil will improve texture, and the soil can be conditioned by incorporating a green manure, peat moss or other organic materials.

The floating aquatic weed *Salvinia* (*Salvinia molesta* Mitchell), when dried and cured (partially decomposed) provides adequate amount of nutrients, aeration and moisture for the growth of various organisms. Its common use as a mulch with slow rate of degradation and relatively higher resistance to phytopathogens suggests its role as a potential seeding medium.

Materials and Methods :

Collection of parental material (A. *andreanum*):

Red flowered (Avanthi variety) Fig I (1) *Anthurium* plants with flowering frequency of 1/45 days were taken as parental material. Fully matured spadix of about 8 months age (Fig.I (2)) were collected and their seeds were dissected out. The surrounding jelly was removed without injuring the soft seeds. Then the seeds were thoroughly washed in tap water. They were spread on blotting paper and were shade dried for one day.

Seed-bed preparation :

A shallow pot of 9" diameter and 4" height was taken. It was provided with a basal hole for drainage. A layer of brick pieces were spread as a basal layer. Then a layer of dried *Salvinia* of about 1.5" thickness was filled and the whole material was sprinkled with water and covered using a polythene sheet with holes for aeration and maintaining moisture (Fig. 2) and preserved for one week (for curing).

Seeding :

After curing for about one week, the polythene cover was removed, seeding was done after making shallow holes in the substrate and they were then covered with small pieces of *Salvinia.* Over the polythene cover a slight pressure was applied using small pebbles. The whole structure was kept under thoroughly moistened condition.

In order to compare the relative suitability of different media, similar experiments were carried out using cotton and finely sieved river sand as media (control) and the extent of germination was determined in each case (Table 2). For statistical analysis, experiments were carried out in hexaplicate and S.E.M were calculated. The sequential stages in germination were observed based on the development of plumule, radicle and differentiation of the first leaf. (Fig.4)

Results and Discussion :

Within the span of one week all the seeds showed early signs of germination. First of all the radicle (dark green coloured) emerged within about 96 hours together with a tuft of root hairs that penetrated into the moistened substrate. The rapid germination and establishment of seedlings on weed substrate (Fig 3) were attributed to the optimal conditions provided by the substratum. This condition was created by the interaction of various kinds of microflora on decomposing plant remains that released different types of

growth promoting substances. The degradation process also released many types of nutrients and micromolecules needed for plant growth. The chemical analysis of weed substratum (Table 1) elucidated the suitability of the medium. The water holding capacity of dried *Salvinia* was found to be optimal, which was enhanced by the presence of epidermal hairs, while that of cellulosic cotton fibre was found to be a little higher which resulted in the decay of a few seeds. At the same time the low water holding capacity of sand with high silicate content led to the desiccation of some seeds. Since *Anthurium* seedlings (a humic loving plant) germinated on seed bed prepared from *Salvinia* were well adapted to natural conditions, acclimatization of sprouted seeds on small pieces of normal potting medium (broken bricks, charcoal pieces, coconut husk and unsieved sand in the ratio 1:1:1:3 by weight) with a supplementation of one part dried *Salvinia* was found to be less crucial.

The basic processes involved in seed germination are water imbibition, cell expansion, hydrolysis of food reserves in endosperm and cotyledon, transport of soluble metabolites to the embryo and synthesis of cellular constituents in the embryo accompanied by cell division. All these processes are outlined in graph 1, where fresh weight of seed and seedling is plotted against the time of beginning of water imbibition (Phase 1).

Water imbibition results in the hydration of embryonic axis whereby several hydrolytic enzymes such as proteinases, nucleases, lipases and phytases become activated and alpha amylase synthesized *de novo*. The reserve foods in seeds such as starch, fats, proteins, nucleic acids, phytin etc are mobilized and metabolised to provide substrate for continued growth of the embryonic axis. No changes in dry weight take place during the first 24 to 36 hours following the onset of water imbibition. Hydration, however enables

the cells in the embyonic axis and cotyledon to attain full turgor followed by reorganisation of subcellular organelles and cellular membranes.

In comparison with other ornamental plants, and crop plants, radicle emergence is slow in *Anthurium* (It takes about 96 hours). The hydration of all the cells in the embryo, cotyledon, endosperm etc occurs early in phase II resulting in cell expansion and the size increase. Then as a result of triggering of respiratory activities some loss of dry weight is observed. (Graph 1).

Generally in monocots, cells within the hydrated embryo secrete gibberellic acid which moves to the aleuron layer, where it initiates the synthesis of a number of enzymes. Epistein , et . al (1980) have shown in maize that the released Indole-3- acetic acid ( IAA ) and inositol from the endosperm are translocated to the embryonic axis, to supply both IAA and inositol for further growth of seedling. Towards the end of phase II, the dry weight of shoot system and root system increases exponentially and the plant becomes autotrophic.

During phase III, the plant growth is supported by leaf photosynthesis and uptake of water and inorganic solutes by the roots. During the early stages of phase III, some substances probably auxins and cytokinins are necessary to maintain the growth of the emerging seedlings. Eventually, the developing seedling attains complete independence of seed reserves.

Earlier studies reported that fern extracts have got inhibitory effect on seed born mycoflora and other pathogenic fungi. (Gupta, .& Singh, 1984, Pathak, 1991 Yasmeen & Saxeena, 1992). Robert, (1985) found out that major constituents released during the decomposition of fern remains include terpenoids, flavonoids etc. Experiments by Hanne and Dennis, (1998) proved beyond doubt the remarkable role of decomposing

wood debris in Orchid seed germination, probably owing to the presence of mycorrhizal components. Similarly, released chemical components and the presence of beneficial fungi in decomposing *Salvinia* might have prevented the growth of pathogenic microorganisms in and around the germinating seedlings due to the chemical effects or by competitive growth. All these factors ultimately resulted in significant increase of *Anthurium* seed germination on *Salvinia* seed-bed in comparison with the normally used sand bed and cotton pad which are deficient in these components.

Microbial effects on germination include beneficial actions such as the decay of hard pericarps, microbial weakening of the seed coat, production of extracellular products with plant hormone activity etc (Campbell, 1985). The classical example is the production of gibberellin by *Gibberella fujikuroi* which causes a disease characterized by growth distortion in rice. Inoculation of the seed with bacteria such as *Azotobacter, Clostridium, Bacillus, Pseudomonas* and *Arthrobacter* can produce increase in crop yield (Brown, 1974). Some of the methods of seed-treatment with bacteria increase germination rate and affect later root and shoot growth in ways similar to the effect of indole-3-acetic acid and gibberellins. In this experiment also the presence of microbes in the nearby areas of the seed affect the germination process positively and probably by the production of growth promoting substances.

It has been found that mature seeds of a number of water plants (*Alisma plantago – aquatica, Hippuris vulgaris, Sagittaria sagittifolia* and various species of *Potamogeton, Sparaganium* and *Scirpus*) germinate more readily in impure water containing bacteria than in pure water. It is believed that extracellular secretion from the bacteria may stimulate the germination of these seeds (Subhash, 1994). This again supports the fact that microbes on cured *Salvinia* might have a role in promoting the germination process of *Anthurium* Seeds.

There is considerable scope for the cultivation of *Anthurium* in Kerala. The existing climatic conditions viz. atmospheric humidity of 80% and above, temperature range from $20 - 35^{0}C$ and abundant sunlight etc. are ideal for growing *Anthurium.* The increasing popularity of *Anthurium* as a cut flower and as an ornamental plant warrants increased requirement of planting materials. In this situation, a reliable seeding substrate from a widespread weed *Salvinia,* ideal for *Anthurium* seed germination is really worthwhile.

Table 1. Biochemical analysis of air dried *Salvinia*

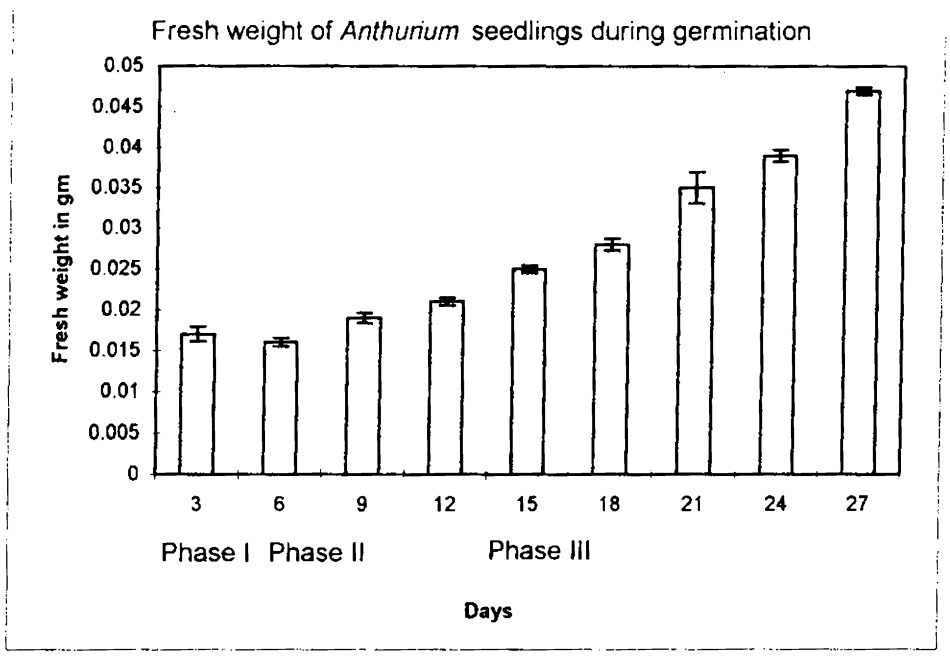
| Components | g/100g dry wt. |
|---|---|
| cellulose | 24.12 |
| lignin | 15.94 |
| Nitrogen (N) | 0.56 |
| Phosphorus (P) | 0.09 |
| Potassium (K) | 0.28 |
| Calcium (Ca) | 1.13 |
| Magnesium (Mg) | 0.38 |

Table II. Germination percentage

| Medium | Germination % |
|---|---|
| Sand | 66.66 |
| Cotton | 83.33 |
| *Salvinia* | 91.66 |

F value significant at 5% level i.e. ($P<0.05$)

**Graph I**


Fresh weight of *Anthurium* seedlings during germination
Fresh weight in gm
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
3
6
9
12
15
18
21
24
27
Phase I
Phase II
Phase III
Days

## Chapter VIII

## Summary and conclusions

Among various floating weeds, *Salvinia molesta* Mitchell forms one of the most problematic weeds in Kerala. Though various types of control methods are adopted for eradicating this weed, control through utilization in an environmentally safe manner is found to be the best one.

The present work is focussed mainly on the utilization of this weed-biomass on a biochemical and biotechnological basis.

Before designing scientific and systematic utilization of any given biomass, the detailed analysis of its chemical componets is essential. Hence, as the preliminary part of the experimental works, samples of *Salvinia* were analysed for its chemical constituents.

Chemical analysis revealed that major constituents in *Salvinia* are cellulose, lignin and minerals. In nature, certain Basidiomycetes fungi like *Pleurotus* are equipped with lignocellulolytic enzymes. Oyster mushroom is a powerful tool in the bioconversion of lignocellulosic wastes which are abundantly present in aquatic weed *Salvinia molesta* Mitchell. So the suitability of this weed as substrate for mushroom cultivation was investigated and the results were published in Indian Journal of Experimental Biology.*

There are different cultivated species of *Pleurotus* like *Pleurotus sajor-caju*, *P.florida, P.citrinopileatus* etc. In order to check the suitability of the specific species to the weed substrate, the comparative bioefficiency and efficiency for

lignocellulose conversion of these three species were carried out. Though these species showed slight variation in terms of biological efficiency and in their efficiency for lignocellulose conversion to a certain limit, due to the wider adaptability, *Pleurotus, sajor-caju* was selected for further experiments (Proceedings -in Press)**.

The composition of the substrate contributes much to the nutritive value of mushrooms. Hence, alterations in the nutritive value of mushrooms (in terms of total carbohydrates, proteins, lipids and minerals) in response to *Salvinia* as substrate were analyzed.

Substrate after mushroom harvest (spent substrate) can be utilized for various purposes such as cattle feed, as a source of degradative enzymes, as a substrate for other mushrooms and as garden manure. But studies are limited with regard to the utilization of *Pleurotus* spent substrate as garden manure. So the value of spent substrate as an organic supplement and its multidimensional impacts on soil chemical status, soil microbial population dynamics and plant growth (*Anthurium andreanum*) were carried out.

The physical texture and the nature of the chemical constituents of dried *Salvinia* suggest its utility as a prospective seed-bed material. In *Anthurium* (a humic loving plant), the percentage of natural seed germination is very low and its seeds are commonly raised on sieved river sand or on cotton pad with great care. So, as the final aspect of this work, the utility of dried *Salvinia* as a seed - bed material for *Anthurium* seed germination was investigated. It was found that

the percentage of seed germination in this seeding medium is significantly higher than that in control.

Major findings of this work have got much relevance in designing measures to utilize different types of plant biomass, especially aquatic weeds, with the aid of a powerful biological tool, the lignocellulolytic fungus, *Pleurotus*.

The following conclusions were made from the present investigations

1. Biochemical analysis of *Salvinia* revealed that, the weed biomass is predominantly lignocellulosic in composition.
2. One possible way of utilizing this lignocellulosic weed in an environmentally safe manner is its use as a substrate for the cultivation of lignocellulolytic fungus, the oyster mushroom (*Pleurotus).*
3. Comparative bioefficiency and efficiency for lignocellulose degradation by three species of *Pleurotus* viz (*P. sajor-caju, P. florida* and *P. citrinopileatus)* revealed that all these three species are equally suited with regard to their bioefficiency, whereas their efficiency for lignocellulose degradation was observed in the order *P. florida* > *P. citrinopileatus* > *P. sajor-caju.* From these results, it became evident that the substrate degradation is not at all directly related to the bioefficiency, but the fruitbody formation and biodegradation are influenced by an interplay of several factors like prevailing environmental conditions, the species differences, culture practices etc. Again *Pleurotus sajor-caju* shows wider adaptability on *Salvinia* substrate.

4. From the results of experiment to evaluate the effect of growth substrates (*Salvinia* and Paddy straw as control) on the nutritive value of mushrooms,we could infer that these two substrates provided probably similar precursors for protein and carbohydrate synthesis in mushrooms and in particular, *Salvinia* provided lesser amount of metabolites for lipid synthesis and higher amount of minerals in mushrooms.
5. Field study to find out the suitability of spent substrate (substrate after mushroom harvest) as an organic manure for *Anthurium* plants revealed that about 15-20 gram of sun-dried and powdered spent substrate is relatively better for increasing several parameters of soil chemical status, soil microbial populations (in terms of total bacteria, actinomycetes and fungi) and plant growth to a significant manner.
6. From the studies with regard to the utilisation of dried *Salvinia* as a seed-bed material for the germination of *Anthurium* seeds, it becomes clear that this dried weed substratum is highly suited for *Anthurium* seed germination.
7. From various steps of the entire work certain additional information are also brought about, these include (a) special adaptations of *Salvinia* for its wide dispersal (enlisted in the introductory chapter). (b) major advantages and limitations with respect to the utilisation of *Salvinia* etc. These are summarised below.

**Advantages of utilization of *Salvinia* :-**

Any Plant luxuriantly growing out of place is a weed. In this sense *Salvinia* forms one of the troublesome aquatic weeds in Kerala. The advantages of its utilization can be summarized as follows.

(i) Utilization of weed biomass for various purposes such as (a) as substrate for mushroom cultivation (b) as a seed - bed material (c) as an organic manure (spent substrate after mushroom harvest) are effective methods of weed control i.e control through utilization.

(ii) The utilization of dried *Salvinia* for mushroom culture and of spent substrate (substrate after mushroom culture) as organic manure in a sequential manner is an environmentally safe approach.

(iii) Since the lignocellulolytic *Pleurotus* degrades dried and processed weed to a considerable extent, its final degradation into simpler molecules in a particular terrestrial system will be triggered.

(iv) Due to the availability of weed in ample amount, its use as a substrate for mushroom cultivation is cost effective, in comparison with the commonly used paddy straw.

(v) The high lignocellulose content in *Salvinia* offers a protective measure against the attack of air borne fungi and insect pests. Hence, dried *Salvinia* can be stored for a long period without degradation.

(vi) The use of spent weed substrate as an organic supplement to the normal potting mixture, in the long run enriches the soil chemical status, increases soil microbial population and facilitates plant growth.

(vii) The use of dried *Salvinia* as a seed- bed material is a reliable and cost effective method for *Anthurium* seed germination.

(viii) Indirectly, weed control through utilization ensures the accessibility of water resources and paddy fields for increased crop yield, fish production, easier navigation, better irrigation and improved aesthetic value.

**Limitations with regard to the utilization of *Salvinia***

Being an aquatic weed, the major limitation in the utilization of *Salvinia* is its high water content ( about 90% ). This creates difficulties in the collection of weeds, its transportation and drying processes and thus increases the cost of labour. Secondly, due to its high lignocellulosic content, this weed biomass can be biologically degraded and utilized only with the help of lignocellulolytic microorganisms like *Pleurotus*.

With regard to the mushroom cultivation procedures, occasionally mushroom beds are prone to the attack of certian weed moulds, diseases and pests. But these problems are common for all types of substrates, including paddy straw.

A few examples of weed mushrooms, diseases and pests and their control measures are enlisted below.

| I. Weed moulds :- | Control Measures |
|---|---|
| 1. *Coprinus lagopus* | Use fresh spawn and substrate as far as possible and pasteurize the substrate properly |
| 2. *Coprinus comatus* | |

| II. Diseases :- | Causative organism | Control measures |
|---|---|---|
| A. Fungal :- | | |
| 1. Soft mildew('cob web') | *Dactylium dendroides* | Treatment with Dithane Z -78 (0.2%) on affected parts |
| 2. Olive green mould | *Chaetomium olevacearun* | |
| 3. Bubble disease. | *Mycogone perniciosa.* | |
| B. Bacterial :- | | |

| | | |
|---|---|---|
| Brown & sunken blotches on pileus | *Pseudomonas tabaci* | Proper sanitation in and around mushroom shed |

C. Viral diseases.

| | Control measures. |
|---|---|
| 1. Die back disease | 1. Heat treatment of the floor of shed at $70^0$ C for 12 hrs after the harvest of the crop |
| 2. Watery stipe | 2. Trays & equipments must be treated with sodium pentachlorophenate (0.5 to 1% solution) |
| 3. X- disease. | 3. Disinfect the mushroom shed with 4% formaldehyde solution. |

| III | Pests | Contol measures |
|---|---|---|
| | A. Small larvae of flies | |
| | 1. *Lycorella fenestralis* | |
| | 2. *Neoscaria pauciseta* | 1.Addition of lindane dust at the rate of 0.8 g / kg of the substrate |
| | B. Spring tails | |
| | 1. *Lepidocyrtus cyaneus* | 2. Spraying of malathion (50% EC |

2. *Isotoma simplex* in 10 lit of water) two days after spawning (EC - Effective concentration)

C. Mites

1. *Rhizoglyphus sps*
2. *Tyrophagus lintnerii*

D. Nematodes

1. *Ditylenchus mycelophagus*
2. *Aphelenchoides sps*

**Future studies in allied apsects**

All the experimental works described here open up certain new avenues for exploration. Important ones among them are the following :-

(i) Optimization of mushroom cultural conditions and addtion of supplements to dried *Salvinia* substrate for increased biological efficiency.

(ii) Identification of more suitable strains of *Pleurotus* and other lignocellulolytic organisms for the degration of *Salvinia.*

(iii) Characterization of specific enzymes and micro organisms responsible for the degradation of *Salvinia.*

(iv) Detailed studies of rhizosphere microflora and their metabolic reactions in response to the addition of spent substrate.

(v) Extensive field experiments with regard to the suitability of organic spent substrate to other crop plants including leguminous plants.

(vi) Seed- bed experiments using dried *Salvinia* after the inoculation of mycorrhizal fungi like *Rhizoctonia* for the germination of Orchid seeds and other seeds with very long dormancy periods.

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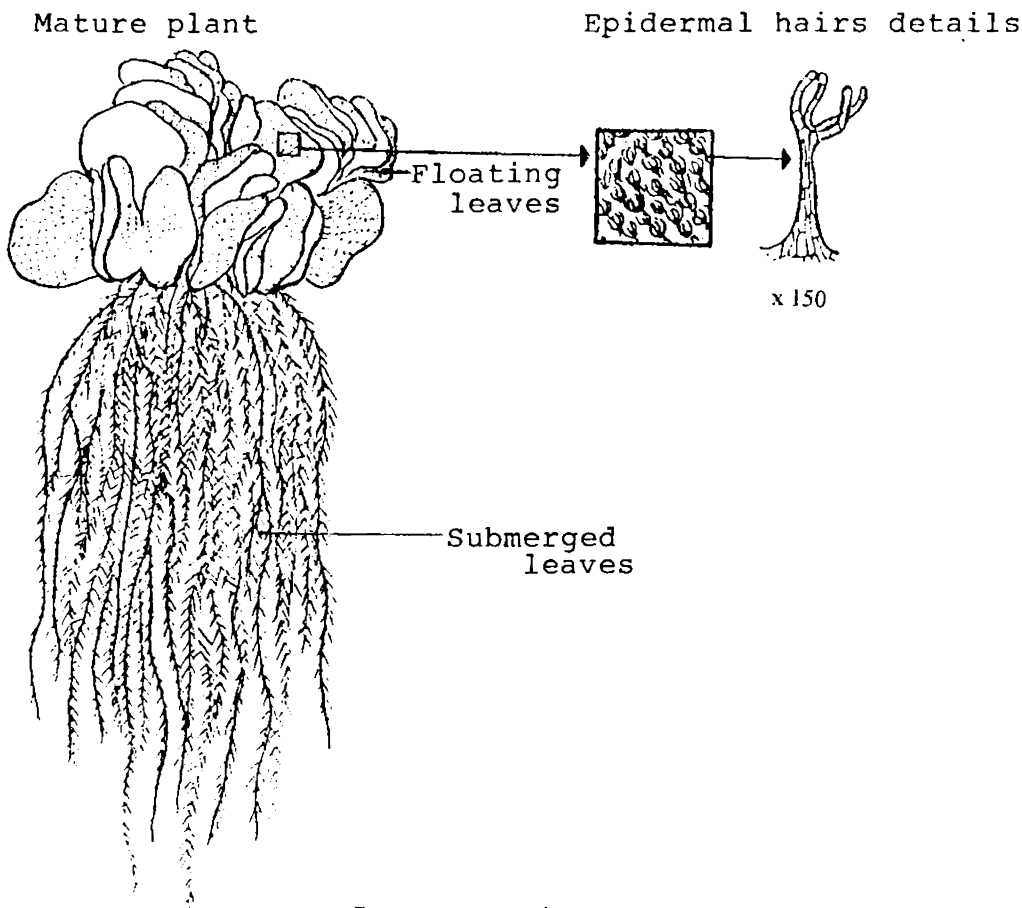
## PUBLICATIONS

* Potential application of African weed (*Salvinia molesta* Mitchell) for the cultivation of oyster mushroom (*Pleurotus sajor-caju* (Fr.) Singer) - published in: **Indian Journal of Experimental Biology,** vol. 33, 1995, 806-808.

** Comparative efficiency for lignocellulose conversion on aquatic weed substrate (*Salvinia molesta* Mitchell) by different species of oyster mushroom (*Pleurotus sajor-caju, P. citrinopileatus* and *P. florida*) - presented in: **National symposium on 'Frontiers in Applied Environmental Microbiology'**, Dec.11-13, 1995, organized by School of Enironmental studies, Cochin University of Science and Technology. - Proceedings in press.

SKETCH 1 SALVINIA - MORPHOLOGICAL FEATURES




Sporocarp stage

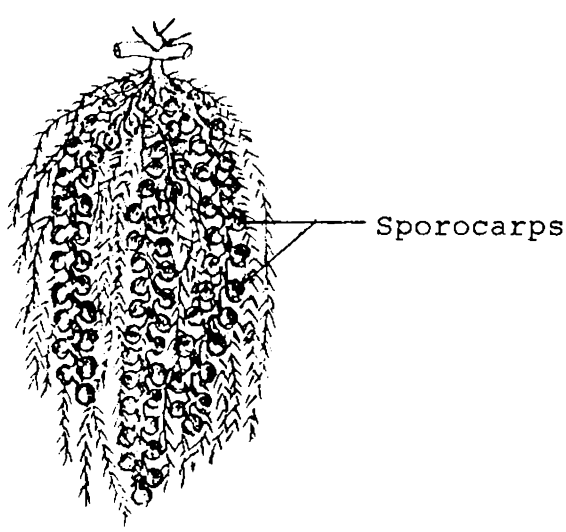


PLATE I.

A paddy field in Kottayam District infested by *Salvinia*

PLATE II.

Growth Stages of *Salvinia molesta* Mitchell

Fig 1. Juvenile Stage

Fig 2. Intermediary Stage

(Contd..... )

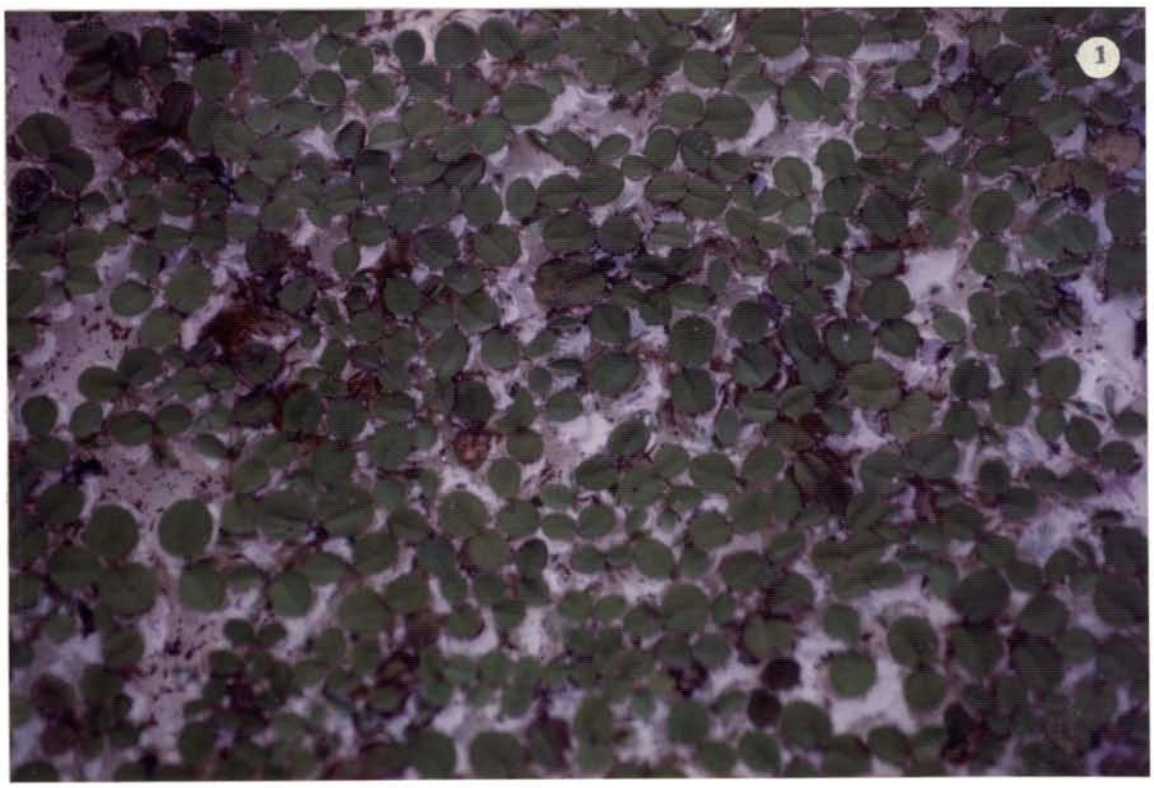

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Fig 3. Mature Stage

Fig 4. Mature stage showing thick mat formation


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PLATE III.

Mushroom beds prepared on :-

1. Paddy straw (Control)
2. *Salvinia molesta* Mitchell
3. Oyster mushroom flushes on *Salvinia molesta* (100
   before first harvest.
4. Rarely formed extra large fruit body on
   *Salvinia molesta* (100%)


1
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4

PLATE IV.

*Pleurotus* Species used for mushroom cultivation.

Fig 1. *Pleurotus sajor-caju*

Fig 2. *Pleurotus citirinopileatus*

Fig 3. *Pleurotus florida*


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PLATE V.

Fig 1. Spent substrate powdered form.

Fig 2. Experimental plants initial stage.

(Contd.....)


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Fig 3. New leaf formation

Fig 4. New surface root formation

(Contd.....)


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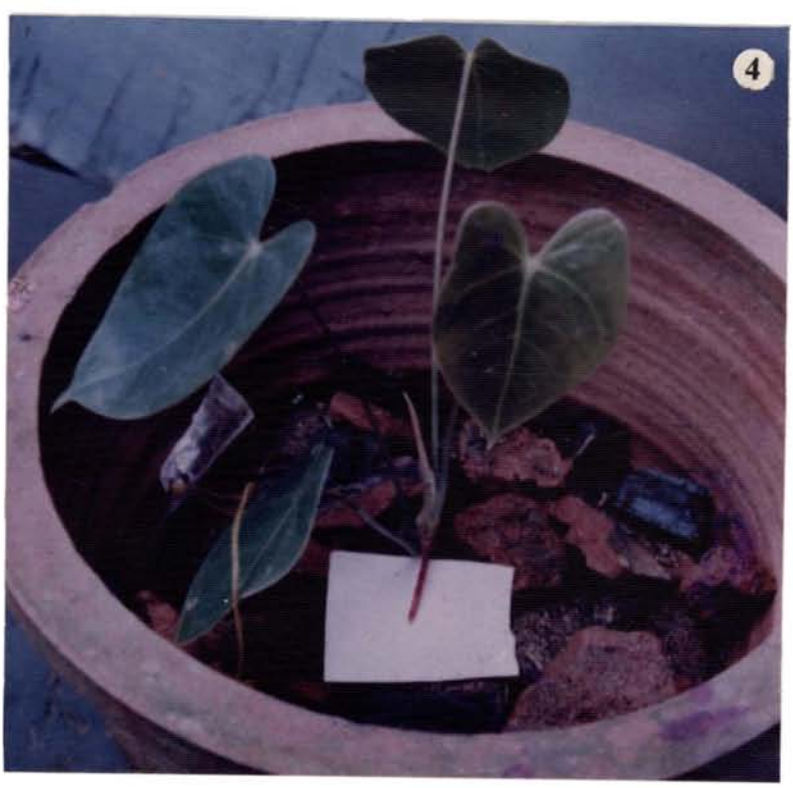
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Fig 5. Sucker formation

Fig 6. Flowered stage

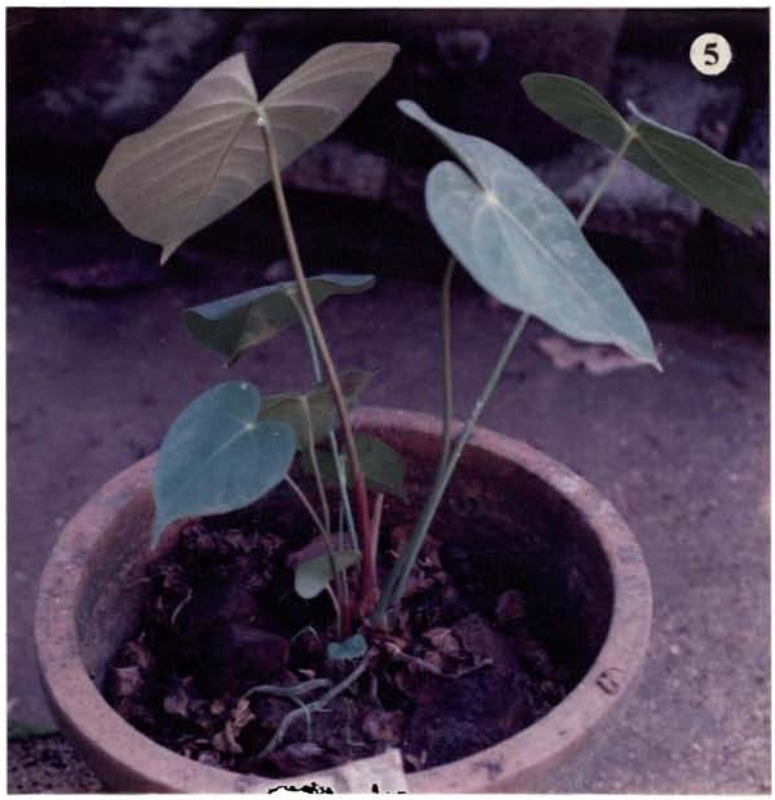
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PLATE VI.

Anthurium seed germination - on seed-bed prepared from *Salvinia molesta*

Fig 1. (1) Mature inflorescence (spadix)
(2) Mature spadix with seeds.

Fig 2. Experimental set up.

Fig 3. Germinating seeds.

Fig 4. Sequential Stages of *Anthurium* Seed germination.


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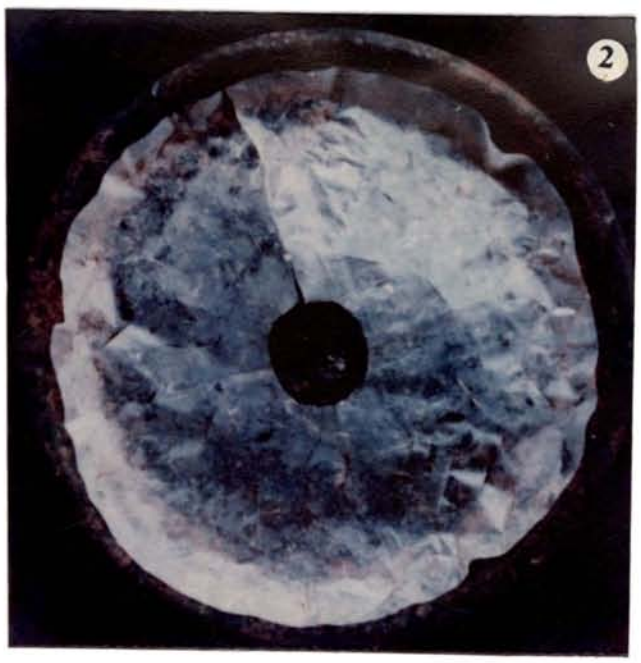
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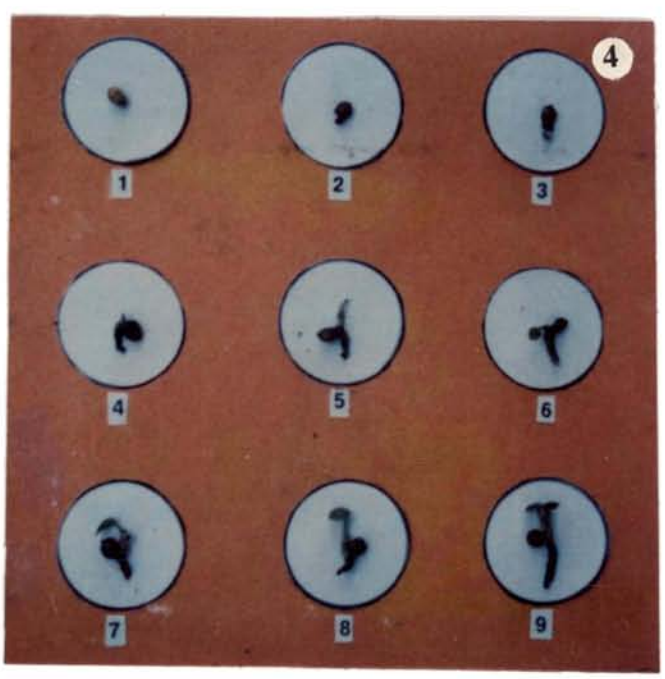
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