A STUDY ON THE ANTIMICROBIAL PROPERTIES OF *EXCOECARIA AGALLOCHA* L. THE TEMPLE TREE OF CHIDAMBARAM

By

M. DEEPA

Reg. No. 97 PLS 02

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN - DEEMED UNIVERSITY, COIMBATORE - 641 043

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CERTIFIED AS BONAFIDE RESEARCH WORK.

Sarojini Sukumaran. 12.5.99.

Signature of
Head of Department

12/5/99

Signature of
Guide

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CONTENTS

CONTENTS

LIST OF PLATES

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

CHAPTER I

INTRODUCTION

Ever since independence, India has been trying to improve her agricultural field. The inability to control the agricultural pests was a major problem that the agriculturists were facing. With the advent of Integrated Pest Management and the various control measures, the agriculturists were able to tackle many of the problems faced by them.

Chemical control paved way for biological control and more and more methods are being devised and designed with the aim to promote agriculture and to protect the environment. Safer, lesser expensive and effective alternatives to chemical control are desirable and an integrated, inter disciplinary approach to pest management is in utmost need. A second green revolution based on environmentally safer practices and technology is very essential. It may be noted that for any farm practice, minimising risks is often more important than maximising profits.

It is at this juncture that the control of pests through biological means gained wide acceptance. Most of the diseases in plants are caused by insects, bacteria, fungi and viruses. Exclusive dependence on the chemical means did not yield sustained solutions to the existing problems in controlling pests. The biopesticides substituted chemical pesticides and is being successfully utilised by the farmers and agriculture extension workers as an effective remedy to the pest problems all over India.

1.1. PLANTS AND PLANT PARTS AS PESTICIDES

Plants have enough potential to control many of the harmful pests that cause high damage to the agricultural products. Pests and diseases can be effectively managed by good crop management practices without using chemical pesticides.

Garlic and turmeric extracts proved to be effective as pesticides in day-to-day life. Spraying with leaf extracts of *Andrographis paniculata* is a normal practice to reduce white fly incidence in plants. Growing Thulsi (*Ocimum sanctum*) in the courtyard will keep away the common pests to an extent. A decoction made from tobacco leaves is also used as an effective pesticide.

1.2. BIOFUNGICIDES

Man has been fighting against the plant pathogens to protect his plants from complete destruction. He developed advanced techniques to achieve his goal - the latest among them being the use of plant parts as antimicrobial agents. Many of the plant diseases have been traced to be due to the attack of micro-organisms namely fungi, bacteria and viruses.

Control of the diseases caused by fungi is a serious concern for farmers since the fungal pathogens produce a variety of spores or propagules such as Chlamydospores, Oospores, Sclerotia and other resting spores which are otherwise difficult to be controlled with the available fungicides. Plant parts such as exudates, extracts of leaf, stem, root, etc. serve as effective bio fungicides. Thus, biofungicides are fast catching up the attention of more and more people, especially, since it

offers much better control and less risk for health.

Research works done on the effect of plant extracts show that they have high potential to inhibit the growth of these fungi by preventing sporulation or germination. Studies done by Chowdhury and Sinha (1989) on the differential effects of root extracts and exudates from Chickpea shows that some fungitoxic compounds were present in the resistant host plant that contributes towards host defence through inhibitory action on the pathogen. Plant extracts were also used for the control of fungal disease of Mulberry (Sarvamangala *et al* ., 1993).

Many of the agricultural commodities like cotton and vegetables viz. tomato, potato, carrot, etc. are affected by the plant pathogens like *Fusarium oxysporum, Helminthosporium oryzae* and *Alternaria tennuis*. Food products are also affected by these pathogens causing huge losses which can be effectively kept under control. As now-a-days, scientific agriculture move towards sustainable organic farming, botanical fungicides and insecticides are being widely recommended.

The plant *Excoecaria agallocha Linn. (F . Euphorbiaceae)* has been found to possess biocidal properties. The present study focuses on the effectiveness of biological means in the control of these pathogens. In addition to these fungi, *Saccharomyces cerevesiae,* commonly known as Baker's Yeast, is also selected. The criterion for study in all the three test fungi is the spore germination whereas that of *Saccharomyces cerevesiae,* it is only the bud formation.

It is to be reconciled that not much work is available on the anti microbial properties of the plant extract towards plant pathogenic fungi and

bacteria. It is hoped that the present investigation carried out with the following objectives would add additional information on the antimicrobial spectrum of this interesting plant.

-to understand the antimicrobial activity of the plant *Excoecaria agallocha Linn.* against yeast and certain plant pathogenic fungi.

-to determine the probable inhibitory principles of the plant extracts.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The available literature pertaining to the antimicrobial properties of *Excoecaria agallocha Linn.* as relevant to the present investigation is reviewed and presented in this chapter. In addition to this, the antifungal properties of the same plant are particularly reviewed.

2.1. BIOLOGICAL CONTROL OF PLANT PATHOGENIC FUNGI

The increasing occurrence of opportunistic and systemic mycoses associated with the use of immunosuppressive drugs has led to new efforts in the search of novel antifungal compounds. At the same time, there is a continuing interest in the discovery of antifungal agents that are effective against plant pathogenic fungi.

The massive overuse and frequent misuse of organic pesticides has led to problems like resurgence of secondary pests, development of resistance in pests, elimination of natural enemies of pests, toxicity hazards to man, plants, domestic animals and wild life, contamination of soil, water and food chain and wholesale pollution of the environment.

Integrated Pest Management (IPM) utilising the best available bio control techniques while using the minimum chemical pesticides, is an effective remedy to the pest problems in India. The natural resources are tapped in a very commendable manner in sustainable agriculture. Biological control of plant pathogens is found to be antagonistic against certain harmful bacteria and fungi. It is currently accepted as a key practise

in sustainable agriculture (Azcon and Barea,1996).

2.2. PLANT EXTRACTS AS ANTIFUNGAL AGENTS

Plant extracts as antifungal agents provide a new avenue for the control of phytopathogens and thereby the diseases caused by them. The inhibitory effect of *Azadirachta indica* on *Curvularia lunata* was proved by Bhowmick and Varadhan (1981). The conidial germination of *Curvularia pallescens* was found to be affected by the extracts of *Eucalyptus spp.* (Rajiv and Sachan,1979).

Results of the studies done by Gupta and Singh (1983) on the effect of the extracts of *Datura stramonium, Calotropis procera* and *Cannabis sativa* on teliospore germination revealed that these extracts were highly inhibitory, the maximum inhibition of 97.6 per cent was observed in 500 ppm *D. stramonium* extract.

Leaf extract and powder of *Vernonia amygdalina* were evaluated for antifungal activity against *Cochliobolus lunatus and Fusarium semitectum.* They were found to have 66.2 per cent inhibition of spore germination and 9.8 per cent inhibition of sporulation (Ekpo, 1991).

Leaf extracts of *Azadirachta indica, Calotropis gigantia, Catharanthus roseus, Eucalyptus spp., Parthenium hysterophorus* and *Pongamia pinnata* showed promising results in inhibiting spore germination of the pathogens causing leaf rust and leaf spot diseases in mulberry (Sarvamangala *et al.*, 1993). Leaf extracts of *Eucalyptus spp.* and *C. gigantia* reduced leaf spot disease incidence by 63.6 per cent and 56 per cent respectively.

The germination of ascospores of the stem rot

pathogen of chickpea, *Sclerotinia sclerotiorum* was found to be inhibited by the alkaloids isolated from *Fumaria indica* (Singh *et al.*, 1994).

Petroleum- ether fraction of the extract of *Clerodendron siphonenthus* inhibited the mycelial growth and sclerotial and conidial germination of several fungi (Chatterjee and Chowdhury, 1995). The isolates of certain pathogenic fungi were found to be inhibited by turmeric oil extracted from *Curcuma longa* at dilutions of 1:40-1:80. After applying turmeric oil, the lesions in the experimental animals were found to disappear within 6 - 7 days (Apisariyakul *et al.*, 1995).

Laboratory studies conducted by Quasem and Abu (1995) showed that the aqueous extracts of different weed species like *Crepis aspera, Chenopodium murale, Ranunculus asiaticus, Erodium cruciatum, and Euphorbia helioscopia* reduced the colony growth of some plant pathogens like *Penicillium digitatum.* However, the inhibitory effect varied between extracts. *C. murale, C. aspera* and *R.asiaticus* extracts completely inhibited the growth of *P. digitatum.*

The in vitro antifungal activity of tea tree oil *(Melaleuca alternifolia)* was evaluated against *Candida albicans* (Nenoff *et al.,* 1996). It was found effective in the treatment of fungal infections of the skin when applied in the form of ointment. The antifungal activity of lemon grass oil and lemon grass oil cream extracted from *Cymbopogon citratus* leaves were evaluated for their antifungal activities and were found effective (Wannissorn *et al.,* 1996).

Perez and Suarez (1997) screened some Argentinian medicinal plants like *Lithrea ternifolia, Cassia occidentalis, Psidium guineense, Punica*

granatum and *Rosa borbaniana* against the opportunistic pathogen, *Candida albicans*. The aqueous extracts of leaves, roots, fruits and flowers of these plants were found to inhibit the fungus.

Fruit rot disease caused by *Curvularia tuberculata* and *Alternaria alternata* could be effectively kept under control using aqueous leaf extracts of *Calotropis procera, Azadirachta indica, Lantana camara* and *Ocimum basilicum* (Srivastava and Lal, 1997). Philip and Sharma (1997) noted that the leaf extracts of plants like *Azadirachta indica*, *Pongamia glabra* etc. inhibited the mycelial growth, spore formation and spore germination of *Fusarium oxysporum.*

Gardini *et al.* (1997) evaluated the antifungal activity of hexanal on *Aspergillus niger* as dependent on its vapour pressure. They concluded that the effectiveness of the volatile hexanal depends on vapour pressure - that is, increasing temperature increased the antifungal activity of hexanal due to its effect on vapour pressure.

Extracts from *Allium* and *Capsicum species* and essential oils from *Cymbopogon martini, Cinnamomum zeylanicum* and *Eugenia caryophyllata* showed antifungal activity against *Botrytis cinerea* (Wilson *et al.*, 1997). Leaf extracts of *Clerodendron viscosum, Lantana camara, Vitex nigundo* and *Citrus aurantifolia* were tested for their fungicidal effect on *Rhizoctonia solani* and was found that its growth and sclerotial production was significantly inhibited (Shylaja *et al.*, 1997).

Extracts from *Cymbopogon citratus, Eucalyptus citriodora, Lippia multiflora* and *Ocimum americanum* were tested against *Aspergillus flavus* and *Candida albicans* and were found to inhibit these fungi (Baba *et al.*,

1997). Root extracts of *Moringa olerifera* showed maximum inhibition of the mycelial growth of *Rhizoctonia solani* (Dubey, 1998) whereas, *Ficus religiosa* and *Eclypta alba* showed comparatively less inhibition.

2.3. PLANT EXTRACTS AGAINST *FUSARIUM* AND *ALTERNARIA SPECIES*

Most of the plant extracts tested were reported to show pronounced antifungal activity, especially against *Fusarium* and *Alternaria species*. The natural and synthetic compounds of garlic oil exhibited inhibitory effect on mycelial growth and spore germination of *Fusarium* and *Alternaria* (Murthy and Amonkar, 1974).

Garlic Clove Juice (GCJ) inhibited the spore germination and mycelial growth of *Fusarium oxysporum f. sp. niveum,* the causal pathogen of watermelon wilt, similar to the effect of some fungicides (Mona *et al.*, 1985).

Root exudates and extracts of Chickpea cultivar was found to suppress the spore germination and germ tube growth of *Fusarium oxysporum* significantly (Chowdhury and Sinha,1989). Evaluation of the hexane extracts of *Azadirachta indica, Valeriana officinalis* and *Tamarindus indica* for their antifungal properties revealed 100 per cent inhibition of *Alternaria alternata* (Kazmi *et al.*, 1993).

Plants which are said to be highly medicinal like *Mentha spicata* serve as excellent fungicides with their leaf extracts having absolute inhibition for mycelial growth of *Fusarium oxysporum* even at 1:2 dilution (Singh *et al.*, 1994). At this dilution, the extract was found to be fungitoxic.

It also exhibited a broad spectrum of activity against other fungi.

Ouf *et al.*, (1994) were of the opinion that the effectiveness of the extracts of any plant part largely depended upon the solvent, plant species, organ and also the test fungus. They supported their view adding that petroleum - ether extracts were ineffective as fungistatics while the methanol extracts possessed a high inhibitory effect towards spore germination of *Fusarium oxysporum.*

The effect of garlic extract on the germination and mycelial growth of *Alternaria alternata* was studied. It was found to be affected at 250 ppm (Barros *et al.*, 1995). Leaf extracts of *Azadirachta indica, Calotropis gigantia and Eucalyptus spp.* were evaluated for their antifungal activity against *Fusarium oxysporum.* The result showed that 1:5 dilution of leaf extracts of *C.gigantia* and *A. indica* were most effective against the fungus where the mycelial inhibition was by 78.5 per cent and 73.2 per cent respectively. (Gupta *et al.*, 1996).

Pandey *et al* . (1996) stated that the latex from *Jatropha gossypifolia* was highly toxic and inhibited the conidial germination of *Alternaria brassicicola.* The toxicity of the plant extract and latex is such that even after 25 fold of dilution, they were found to be toxic against *A. brassicicola.*

Extracts from different plant species like *Annona glabra, Citrus reticulata, Ricinus communis and Swietenia mahagoni* were tested for their antifungal activity against *Fusarium oxysporum f. sp. lycopersici, Alternaria solani* and *Colletotrichum gloeosporioides.* The inhibition was found to be greater than 40 per cent (Hidalgo and Fernandez, 1996).

The essential oils of aegle, citronella, geranium, lemon grass, orange, palmarosa and patchouli were tested for their antifungal activity and was found inhibitory towards *Fusarium oxysporum* and *Alternaria citri* (Pattnaik *et al.*, 1996). Eucalyptus and peppermint oils were also found to be effective against these fungi.

Quercetin, the flavanoid obtained from *Clerodendron infortunatum* showed good inhibition of spore germination of *Fusarium lini and Alternaria alternata* (Roy *et al.*, 1996). The xanthones isolated from the fruit hulls of *Garcinia mangostana* inhibited the growth of *Fusarium oxysporum f. sp. vasinfectum and Alternaria tennius* (Geetha *et al.*, 1997).

The fungitoxic effects of leaf and oil cake extracts of neem *(Azadirachta indica)* and karanj *(Pongamia glabra)* were tested on the mycelial growth, spore production and spore germination of *Fusarium oxysporum* and *Fusarium solani.* Both the extracts were found to be the most effective against these fungi (Philip and Sharma, 1997).

The antifungal activity of aromatic constituents of essential oils (citral and geraniol) were evaluated and it was found that they inhibited *Fusarium oxysporum, Alternaria citri* and also *Helminthosporium compactum* (Pattnaik *et al.*, 1997).

Ethanol leaf extracts of *Allium cepa, Allium sativum, Azadirachta indica, Calotropis procera* etc. were found to be toxic against many fungi like *Fusarium oxysporum* and *Alternaria brassicicola* (Asha *et al.*, 1997). Ethanol extracts of certain plants were most fungitoxic against *Fusarium* and also *Alternaria* with pronounced efficacy at 1000 µg/ml (Shivpuri and Sharma, 1997).

Sas and Piotrowski (1997) were of the opinion that alcohol + acetone extract from species of Polygonaceae were effective against *Fusarium oxysporum.*

2.4. EFFICACY OF THE PLANT EXTRACTS AGAINST *HELMINTHOSPORIUM* AND *ALTERNARIA SPECIES*

Leaf extracts of many plants like *Allium cepa* L., *Allium sativum* L. and *Acacia loculata* Willd. were found to be 100 per cent inhibitory to *Helminthosporium oryzae and Alternaria tennuis* (Shekhawat and Prasada, 1971). The mycelial growth and spore germination of *Helminthosporium oryzae and Alternaria alternata* were inhibited by natural garlic oil (Murthy and Amonkar, 1974). Egawa *et al.* (1977) reported the inhibitory activity of the leaf extracts of *Eucalyptus spp.* on the growth of *Alternaria solani.* The inhibitory effect of *Parthenium hysterophorus* on *Alternaria alternata* was revealed by the studies of Tripathi *et al.* (1978).

The leaf extracts of *Vernonia cinerea* and *Beta vulgaris* were also found to inhibit these fungi significantly. Garlic extracts have been found to inhibit the mycelial growth of the air borne plant pathogenic fungi *Helminthosporium turcicum* (Srivastava and Singh, 1982). Extracts from the leaves of certain plant species were found inhibitory towards the spore germination of *Helminthosporium oryzae* (Jagannathan and Narasimhan, 1988).

Even the extracts made from some local plants like *Lantana camara, Cassia fistula, Rhododendron arboreum and Acacia arabica* possessed high fungitoxic activity as they showed maximum inhibition of conidial germination of *Alternaria species* (Sundriyal, 1991).

Leaf extract and powder of *Vernonia amygdalina* (Del.) were evaluated for antifungal activity against *Cochliobolus lunatus (Helminthosporium lunatus)*. It caused 66.2 per cent inhibition of spore germination and 9.8 per cent inhibition of sporulation (Ekpo, 1991).

Water extracts of leaves of *Vinca rosea* showed maximum inhibition of mycelial growth and spore germination of *Helminthosporium oryzae* whereas extracts of *Datura metel* showed lowest antifungal activity (Ganguly, 1994). Ganesan and Krishnaraju (1995) tested the antifungal properties of some plant extracts like *Leucas aspera, Polygonum chinense* and *Spermacoce articularis* against *Helminthosporium oryzae.* Most of the plant species tested showed inhibition of spore germination.

The experiments of Chatterjee *et al.* (1996) revealed total inhibition of spore germination of *Helminthosporium oryzae* and *Alternaria solani* by the extracts of *Coriaria nepalensis*. According to the studies conducted by Roy *et al.* (1996), plants used in traditional medicine as vermifuge and expectorant were found to exhibit good inhibition of spore germination of *Helminthosporium oryzae* and *Alternaria carthami.* They reported a flavonoid, cabruvin, isolated from the roots of *Clerodendron infortunatum* and stated that this flavonoid exhibited good inhibition of spore germination of these fungi.

Latex from *Jatropha gossypifolia* inhibited conidial germination of *Helminthosporium oryzae and Alternaria brassicicola* by 100 per cent (Pandey *et al.*, 1996). Observations made by Dushyent and Bohra (1997) on the effect of the extract of some halophytes on the growth of *Alternaria solani* showed that upto 96 hours, the plant extracts showed steady antifungal activity.

2.5. *EXCOECARIA AGALLOCHA* - A WONDERFUL MEDICINAL PLANT OF THE COASTAL AREAS

Excoecaria agallocha L. belongs to the Family Euphorbiaceae. It is a mangrove tree seen abundantly in the coastal areas like Chidambaram, Cochin and Travancore. It is the temple tree of Chidambaram and stand as an environmental heritage.

Excoecaria agallocha is a small poisonous evergreen tree with white, highly acrid latex that is injurious to human eyes and therefore, is known as "the blinding tree." (Bulletin Botanical Survey of India, 1960). This tree is of immense potential. Medicinally it is of great value but is a less exploited tree. Its latex can be applied to obstinate ulcers and in preparations of rheumatism, leprosy and paralysis. It is also a drastic purgative and abortifacient.

The leaves of *Excoecaria agallocha* are alternate, thickly coriaceous, elliptic and acuminate. The flowers are minute, fragrant, yellowish green.. Male flowers are sessile and female flowers, pedicellate. Fruit is a capsule with deep lobes and seeds are smooth and subglobose (Kirtikar and Basu, 1993).

2.6. PROPERTIES OF *EXCOECARIA AGALLOCHA*

Prakash *et al.* (1983) described the occurrence of a piperidine alkaloid from the stem wood of *E. agallocha.* He also described the occurrence of a chalcone from the same. Wiriyachitra *et al.* (1985) studied the various

PLATE 1

EXCOECARIA AGALLOCHA L.

1.a DISTANT VIEW

1.b CLOSE VIEW OF A TWIG

EXCOECARIA AGALLOCHA L.

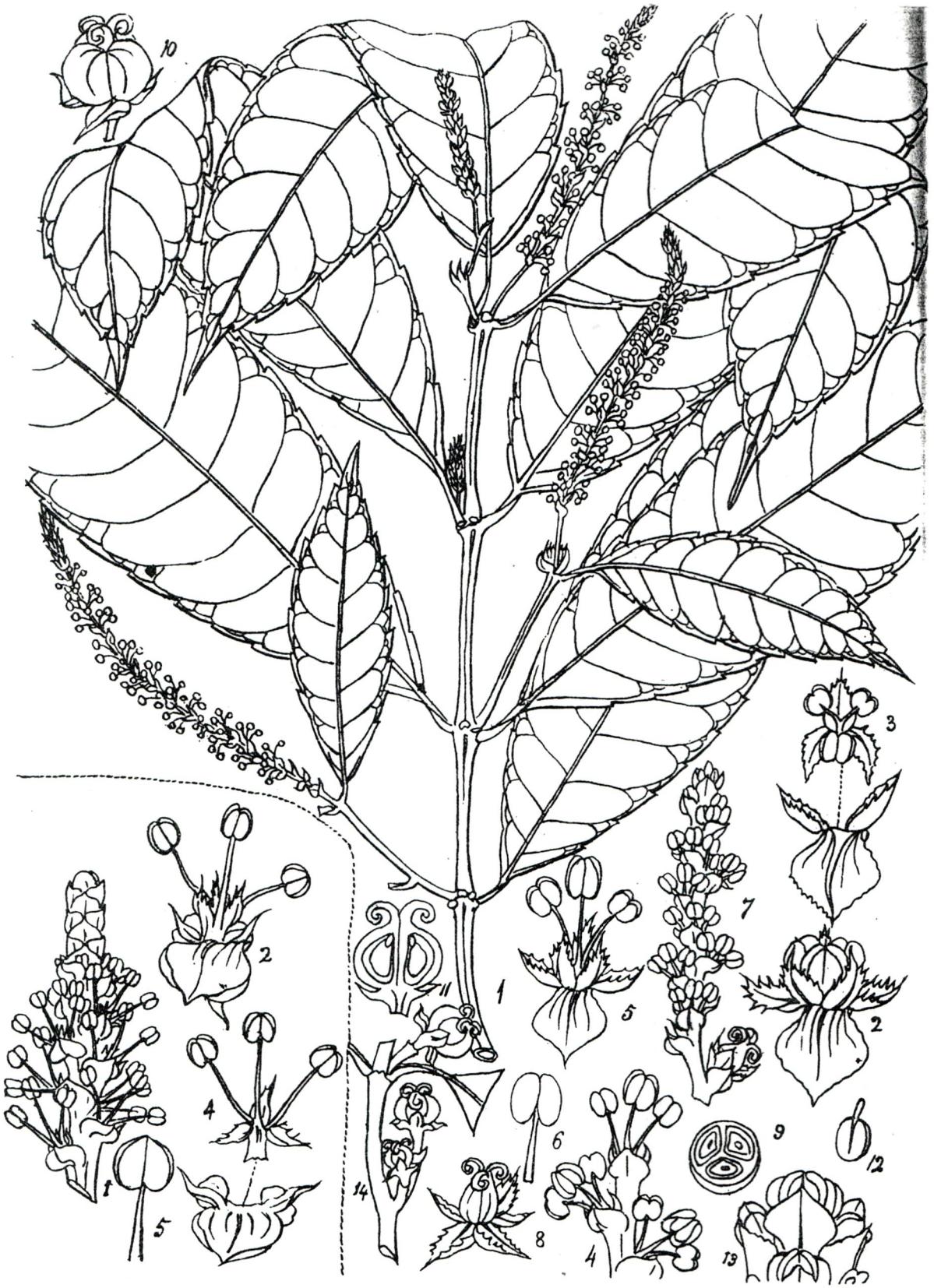
(F. Euphorbiaceae)

HABIT	:	Tree
STEM	:	Shiny, smooth, grey coloured. soft wood is white.
LEAF	:	Alternate, coriaceous, elliptic, acuminate, leaf base acute, glabrous and petiolate.
FLOWERS	:	Fragrant, yellowish green.
MALE FLOWER	:	Sessile, numerous, arranged in catkin like spikes.
FEMALE FLOWER	:	Pedicellate, arranged in racemes.
CALYX	:	Sepals broadly ovate and acute.
COROLLA	:	Ovate and concave.
ANDROECIUM	:	3 free stamens.
GYNOECIUM	:	3 lobed, style recurved.
FRUIT	:	Deeply lobed capsule.
SEED	:	Globose and smooth.

MEDICINAL USES.

ROOTS	:	Aphrodisiacal tonic.
LEAVES	:	Epilepsy, ulcers.
LATEX	:	Irritant poison.
BARK AND LEAVES	:	Used to treat a variety of ailments.

(Courtesy-CAS in Marine Biology, Parangipettai, Chidambaram)



EXCŒCARIA AGALLOCHA, *LINN.*

1. A twig showing male and female flowers
2. Single female flower
3. Axis of the female flower
4. Upper imperfect female flowers
5. A female flower showing prominent stigma and long style
6. Stigma
7. A raceme of female flowers
8. A female flower
9. T.S. of ovary
10. A female flower
11. Section of gynoecium showing ovary and ovules
12. L.S. of ovary
13. Calyx of female flower
14. A twig with female flower

INSET

1. A spike of male flowers
2. A single male flower
4. Axis of male flower
5. Stamen

medicinal plants of Euphorbiaceae and Thymelaeaceae occurring and used in Thailand and found that certain free or cryptic skin irritants of the diterpine ester type from the latex of *Excoecaria agallocha, E. oppositifolia* and *E. bicolor* were widely used in local medicine. They also stated that, though they were being widely used in local medicines, long- term exposure to the extracts containing these irritant factors should be avoided as they may promote tumours.

Biotoxicity studies of *Excoecaria* latex showed that certain species of crabs, gastropods, bivalves, fishes and prawns were most sensitive to the latex and within a few minutes, death of these organisms were noticed in treatments (Kathiresan *et al.*, 1987). It even caused some abnormal changes in these marine organisms.

Results on the light induced effect of the latex of *E.agallocha* revealed that it has high inhibition towards phytoplankton productivity and the inhibition was found to be counteracted by all the light treatments, remarkably by Red. The interesting feature about the effect of light is that Green light aggravated the negative effect of latex on productivity (Kathiresan *et al.*, 1987).

Exposure to the latex and extract of the leaves of *Excoecaria agallocha* resulted in symptoms of nerve poisoning such as excitation and convultions followed by paralysis (Kathiresan and Thangam, 1987). The toxicity experiments carried out by them have demonstrated that the latex and leaf extracts of *E. agallocha* have the larvicidal activity against salt marsh mosquito *Culex sitiens*. The symptoms became apparent within a few hours of treatment and mortality was observed after 6 hours of treatment. These results are indicative of larvicidal compounds in the latex

and shoot. The toxic effect, however, was found to be counteracted by Red, Yellow and Blue light treatments.

In another experimental work, Thangam and Kathiresan (1988) tested the acetone extract of *E. agallocha* and some other mangrove plants and found that they were toxic to the 4th instar larvae of *Anopheles stephensi.*

Data given by Kathiresan and Ravi (1990) on the monthly variation of gallotannin contents in the leaves of *E. agallocha* showed the highest gallotannin contents during the monsoon period from October to December. The total range of tannin content over all species and seasons was 2.14 - 21.42 mg / g dry weight.

So (1990), through his studies, have revealed the excellent antifungal property of *E. agallocha* and several other mangrove species against several facultative plant parasites of local plants. His studies were based upon the antifungal property of this plant on *Alternaria dauci and Fusarium moniliforme.* He reported that *E.agallocha* root revealed highest percentage of inhibition. *Fusarium moniliforme* was inhibited by all the plant extracts whereas *Alternaria dauci* was the least affected. He further stated that these mangrove species should be exploited commercially for their antifungal property.

Bark and fruit of *E. agallocha* extracted in acetone were tested for the mosquito larvicidal activity against *Aedes aegypti* (Thangam and Kathiresan, 1992). It showed satisfactory results. Mosquito coil formulations from the leaves of *E. agallocha* and some other mangrove plants were tested against the biting of female of *Culex quinquefasciatus*. Among the various samples

tested, the smoke from the coil of *E. agallocha* was found to be the most effective against mosquito biting (Thangam and Kathiresan, 1992).

Similarly, another experiment was carried out to test the smoke repellency and killing effect of the same plant species against the mosquito *Aedes aegypti Linn.* But here, *E. agallocha* was not found to be as effective as against *Culex quinquefasciatus* (Thangam *et al.*, 1993). Studies on the repellency of *E. agallocha* leaves against the biting of *Aedes aegypti* on human skin too yielded positive results (Thangam and Kathiresan, 1993).

Karalai *et al.* (1994) revealed the skin irritant nature of excoecarins from the latex of *E. agallocha.* By an extremely mild separation procedure, the highly irritant mixture A1, A2 and A3 (the three Excoecaria factors) was obtained directly. They represent the 'free' Excoecaria factors, the natural constituents of the latex of *E. agallocha,* responsible for its bioactivity.

The wound healing properties of the latex of *E. agallocha,* the arborescent halophyte of the mangrove flora of Tamil Nadu, were investigated using an animal model and in man (Balu and Madhavan, 1995). The raw latex increased the wound healing compared with a formulation containing latex in an emulsion base. In rabbits, the wound healing properties of the latex were comparable with that of a standard medicine, furacin ointment. With the latex, the wound healed in 18-22 days and with the ointment, the wound healed in 19-20 days.

A new phorbol ester was isolated from the bark, leaves and stems of *E. agallocha* (Erickson *et al.*,1995). Its structure was determined by spectral means and it was found that it inhibited the replication of HIV- I (Human Immunodeficiency Virus type-I) in vitro. Sil *et al.* (1995)

concluded that the protein extracted from mature leaves of *E. agallocha* could be used for feeding fish. Their further experiments proved that with maturity, the moisture, protein, soluble carbohydrates and lipid contents in the leaves decreased.

Krishnamurthi *et al.* (1995) observed that the latex of *E. agallocha* were highly toxic to the larvae of fresh water prawn, *Macrobrachium lamarrei* with 100 per cent mortality observed at 0.1-1.0 ppm within 2 hours. Konishi *et al.* (1996) established the structures of Excoecarins A, B and C -the three labdane type diterpenes from the wood of *E. agallocha* on the basis of spectroscopic data and chemical evidence. They stated that these could be used as fish poison.

The petroleum ether and acetone extracts of *E. agallocha* were studied with pyrethrum for its synergistic larvicidal activity and was found to exhibit synergism against the larvae of *Culex quinquefasciatus* (Thangam and Kathiresan, 1997). Extracts of 16 mangrove species were screened for antiviral activity against tobacco mosaic virus (TMV) by Padmakumar and Ayyakkannu (1997). Among these, 62.5 per cent inhibited TMV. The leaves of *Excoecaria agallocha* showed significant antiviral activity (greater than 70 per cent inhibition).

To search for possible antineoplastic agents, 17 diterpenes isolated from the resinous wood of *Excoecaria agallocha* were screened using an in vitro synergistic assay system (Konishi *et al.,* 1998). It was found that some of these diterpenes exhibited remarkable antitumor promoting activity.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. COLLECTION OF PLANT MATERIALS

Fresh stem, leaves and roots of *Excoecaria agallocha L.* were collected from Parangipettai, a coastal area near Chidambaram--the South Arcot District of Tamil Nadu.

3.2. PREPARATION OF EXTRACTANT

80 per cent solution of the extractant (ethanol - methanol mixture) was prepared in 5.6:1 ratio (Shekhawat and Prasada, 1971) in sufficient quantities as required for the experiment.

3.3. EXTRACTION OF THE PLANT TISSUES

For the extraction of plant tissues, the method given by Mahadevan and Sridhar (1974) was followed. A quantity of 5g of freshly cut, chopped plant part was separately boiled with 25 ml of the extractant over a water bath for 10 minutes (Gupta and Singh, 1983). The extract was decanted. The residue was ground to homogenecity in a mortar and pestle with a pinch of acid washed sand and 10 ml of the extractant was added. The slurry was strained through the layers of a cheese cloth and the extracts were pooled. The volume was made upto 50 ml with the extractant and stored in an amber coloured vial in a refrigerator till further use.

3.4. DETERMINATION OF DRY WEIGHT OF THE PLANT TISSUE

A quantity of 5 g of the fresh plant tissues was weighed accurately and taken in a petridish. The petri dishes were placed in a hot air oven at 105° C. After drying for 3 hours, the tissues were reweighed and the loss of moisture was calculated.

3.5. ESTIMATION OF TOTAL PHENOLICS

The total phenolics in the plant extracts were estimated as per the method given by Bray and Thorpe (1954).

A quantity of one ml of the plant extract was taken in a clean test tube and one ml of 1N Folin- Ciocatteau Reagent and 2 ml of 20 per cent Sodium carbonate solution were added. The solution was boiled over a waterbath for one minute keeping appropriate control. After cooling, the volume was made upto 25 ml with distilled water. The intensity of colour developed was read along with an appropriate control at 725 nm using spectrophotometer. By referring to a standard curve prepared with Pyrocatechol, the quantity of total phenolics in the sample was estimated.

3.6. PREPARATION OF STANDARD CURVE

A standard curve was prepared using Pyrocatechol for the estimation of total phenolics in the sample tested. A quantity of 10 mg of Pyrocatechol was dissolved in 10 ml of distilled water and serial dilutions were made with distilled water. The colour intensity developed in each case was read along with an appropriate control at 725 nm in a spectrophotometer. The

value obtained were plotted on a graph and the standard curve was obtained. From this, the total phenolics in each sample was calculated.

3.7. DETERMINATION OF *ORTHO*- DIHYDROXY PHENOLICS

The method described by Mahadevan and Sridhar (1974) was followed for the estimation of *Ortho*-dihydroxy phenolics. One ml of the alcohol extract was transferred to a test tube and one ml of Arnow's reagent was added to it and mixed well. The volume in each test tube was made upto 25 ml with distilled water and the colour intensity developed in each solution was read at 520 nm using an appropriate control.

The values were compared with a standard curve prepared from Pyrocatechol as the standard and the quantity of *Ortho*- dihydroxy phenolics in the sample was calculated.

3.8. PROCESSING OF THE EXTRACTS

The processing of the extracts was done according to Biehn *et al.* (1968). To exactly 50 ml of the plant extract, 2 ml of 1N HCl was added and the entire amount was transferred to a separating funnel. A quantity of 50 ml of pre- chilled diethyl ether was added to the solution in the separating funnel and was shaken well.

The mixture in the separating funnel was shaken well for 2-3 minutes. The heavy aqueous layer was drained off into a separate beaker and the light organic phase was collected in a conical flask. The organic phase was again collected by reextracting the aqueous phase for a second time with 25 ml of diethyl ether.

The pooled ether extracts were transferred to a 250 ml beaker and dried in vacuum. The residue was dissolved in 5 ml of *n* - propanol in a clean glass vial. This was stored under low temperature (5° C) until further use.

3.9. BIOASSAY OF THE EXTRACTS

According to the method followed by Gupta and Singh (1983), the bioassay of the extracts was done. This consists of a quantitative assessment of the effect of the plant extracts on the test fungi constitute the bioassay.

3.9.1. FUNGAL CULTURE

Pure culture of the test fungi namely *Fusarium oxysporum, Helminthosporium oryzae, Alternaria tennuis* and a spore suspension of *Saccharomyces cerevisiae* were collected from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore and maintained on Potato Dextrose Agar medium in the Department of Botany, Avinashilingam University, Coimbatore.

3.9.2. PREPARATION OF SPORE SUSPENSION

A spore suspension of the test fungi was prepared by adding 10-15 loopful of the spore of the test fungi from the pure culture to 10 ml of distilled water (Sheikh and Agnihotri,1972). This was maintained at room temperature.

3.9.3. CONSTRUCTION OF PETRI DISH MOIST CHAMBER

To find the percentage of spore germination, the petri dish moist chamber was constructed (Anonymous, 1943).

A glass rod bent in the form of 'V' is placed in a petri dish and the petri dish chamber was sterilised in an autoclave. A clean cavity slide was placed over the 'V' bridge and sterile water (10-15 ml) was poured into the petri dish chamber to keep the chamber moist. A quantity of 0.1 ml of the cell suspension of *Saccharomyces cerevisiae or Alternaria tennuis or Helminthosporium oryzae or Fusarium oxysporum* was added through a micro pipette to each of the cavity of the slide.

Test solution, namely the partially purified leaf, stem and root extracts of *E. agallocha* was added through a micropipette over the cell suspension contained in the cavity of the slide. Suitable dilutions of the original extracts like 1:5, 1:10, 1:25,1:50 and 1:100 were prepared well before the assay. The petri dishes with the dilutions and control were incubated for 6-24 hours.

3.9.4. SPORE GERMINATION COUNT

After incubating sufficiently for 24 hours, the percentage of spore germination was calculated by observing the number of spores germinated in five microscopic field (10 x) on each plate at random. The percentage of inhibition of the spore was calculated using the formula (Vincent, 1947),

$$I = \frac{C - T}{C} \times 100$$

where I = per cent inhibition

C = spore germination in control

T = spore germination in treatment.

3.9.5. ANALYSIS OF THE RESULT

The data was analysed and based on the results, conclusions were drawn.

EXPERIMENTAL RESULTS

CHAPTER IV

EXPERIMENTAL RESULTS

Results pertaining to the antimicrobial activity of *E. agallocha* L. based on the investigation carried out are presented in this chapter.

4.1. ESTIMATION OF TOTAL PHENOLICS

The results on the determination of total phenolics in *E. agallocha* are presented in Table 1.b.

The data indicate that among the three tissues, root sample contained more phenolics than stem or root samples. However, the variation is not very prominent.

4.2. ESTIMATION OF *ORTHO* -DIHYDROXY PHENOLICS

The results on the *Ortho*-dihydroxy phenolics in *E. agallocha* are presented in Table 1.c.

The results indicate that leaf tissue contained a higher amount of *Ortho*-dihydroxy phenolics than in root or stem tissues.

4.3. BIOASSAY OF THE PLANT EXTRACTS

The petri dish moist chamber was constructed as described under materials and methods.

TABLE 1

1.a PERCENTAGE OF MOISTURE IN PLANT TISSUE

Sample	Fresh Weight (gm)	Dry Weight (gm)	Percentage of Moisture
Leaf	5	0.98	80.4
Stem	5	2.22	55.6
Root	5	3.10	38.0

1.b ESTIMATION OF TOTAL PHENOLICS

Plant Extract	Amount of Phenol (mg/gm)
Leaf	0.34
Stem	0.42
Root	0.48

(Mean of two estimations)

1.c ESTIMATION OF *ORTHO* - DIHYDROXY PHENOLICS

Plant Extract	Amount of *Ortho*-Dihydroxy Phenolics (mg/gm)
Leaf	0.68
Stem	0.38
Root	0.18

(Mean of two estimations)

4.3.1. ASSAY OF SPORE GERMINATION

Assay of the spore germination of the various test fungi was done as described under materials and methods. The per cent inhibition of spore germination of the test fungus was calculated for different dilutions of the plant extract.

4.3.2. SPORE GERMINATION OF *HELMINTHOSPORIUM ORYZAE* AS INFLUENCED BY THE EXTRACTS

The results showing the per cent inhibition of the spore germination of *Helminthosporium oryzae* are set out in Table 2.a.

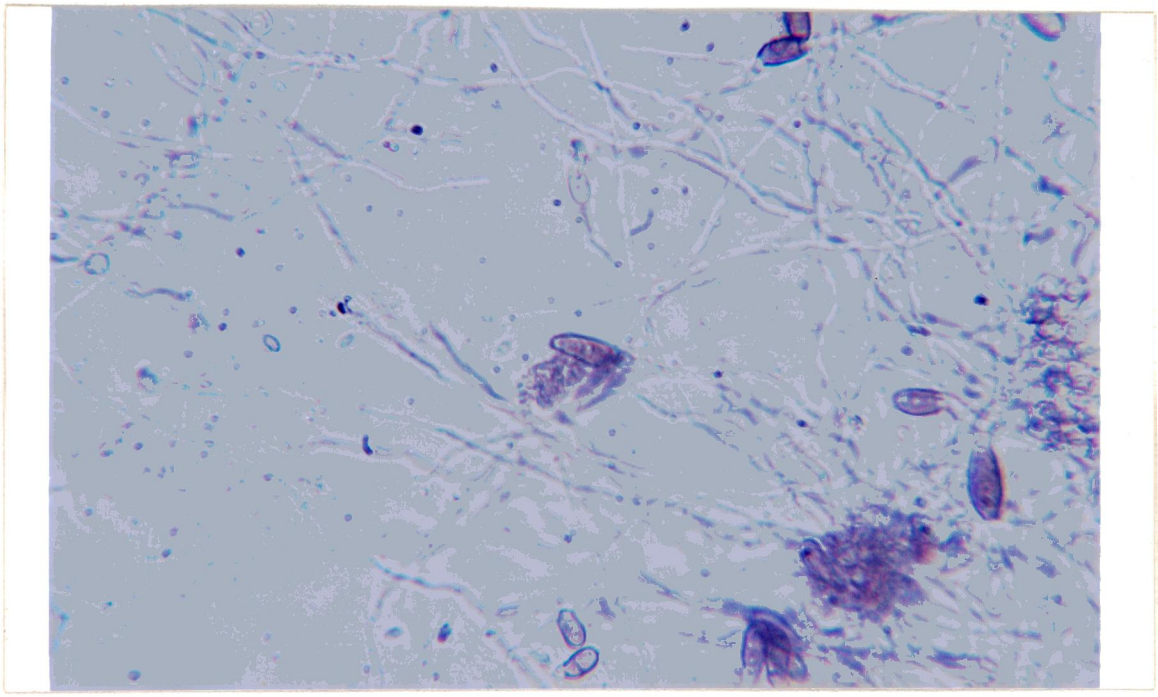
Helminthosporium oryzae was found to be affected most by the leaf extract than that of either root or stem extracts. The extract showed a maximum inhibition of 76.8 per cent at the dilution 1:5. The minimum inhibition was however, 6 per cent shown for root extract of 1:100 dilution. This is the lowest inhibition per cent observed.

4.3.3. SPORE GERMINATION IN *FUSARIUM OXYSPORUM* AS INFLUENCED BY THE PLANT EXTRACTS

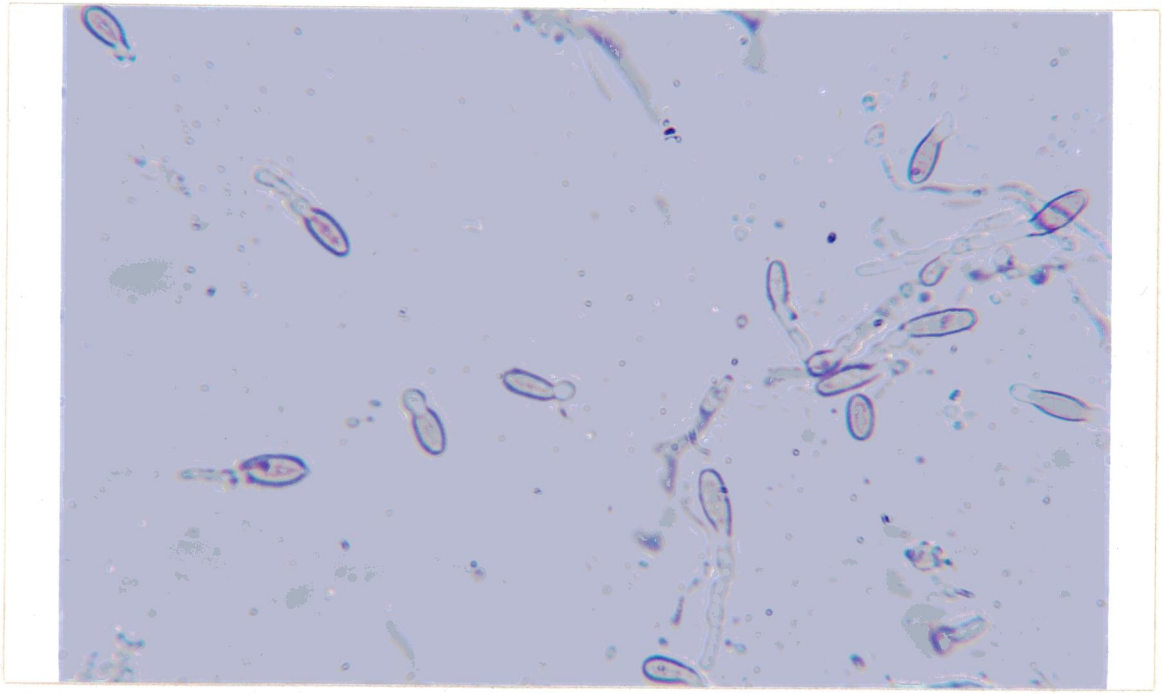
The result of the spore germination of *Fusarium oxysporum* as influenced by the plant extracts are presented in table 2.b.

The results indicated that the stem extract of 1:5 dilution showed more inhibition of spores (87.1 per cent) than that of leaf or root extracts. The minimum inhibition was shown by the leaf extract of 1:100 dilution - it showed 28.2 per cent inhibition of spore germination.

PLATE 2
HELMINTHOSPORIUM ORYZAE
AS INFLUENCED BY THE PLANT EXTRACT

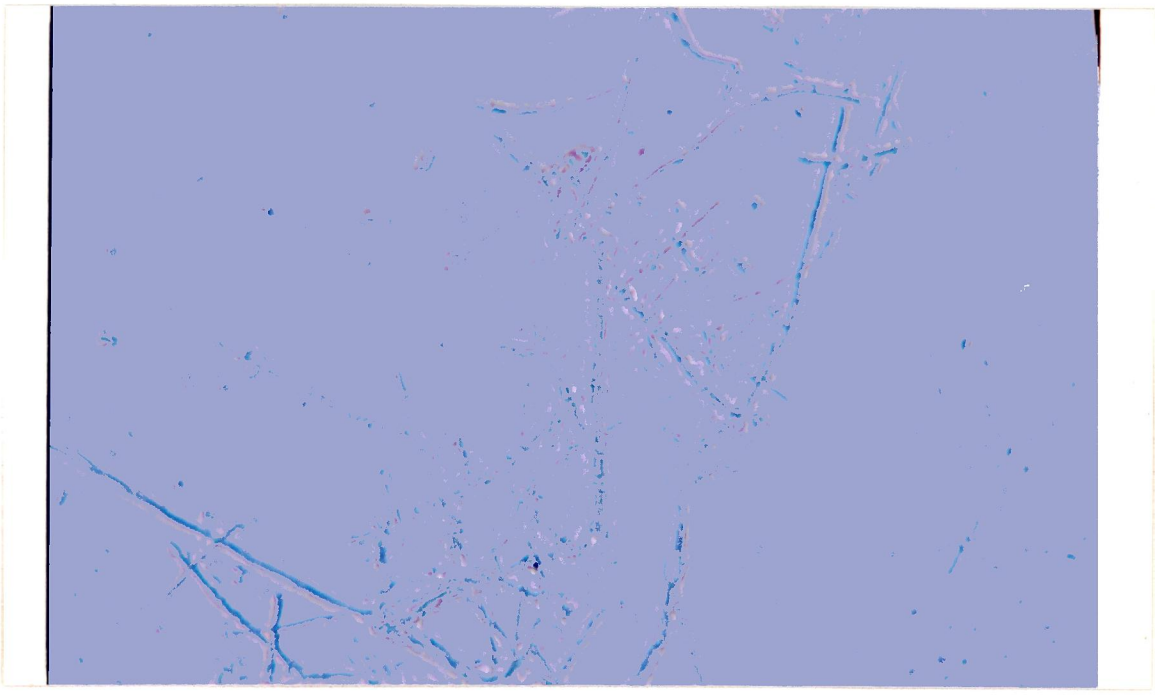


2.a CONTROL

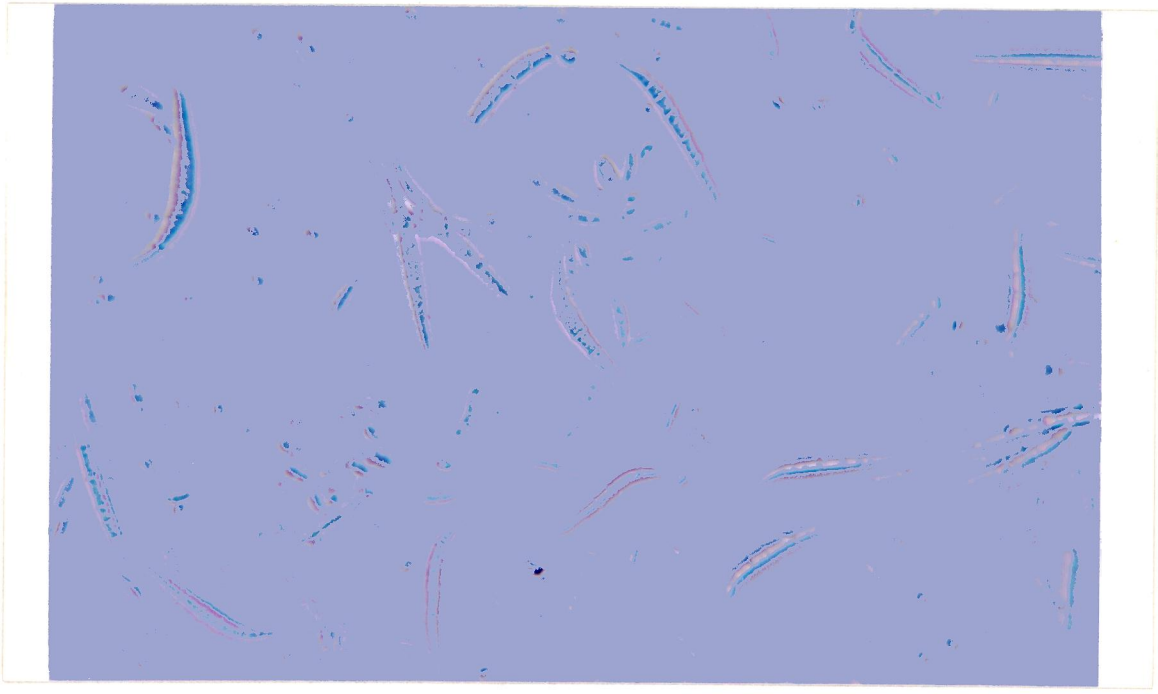


2.b TREATMENT

PLATE 3
FUSARIUM OXYSPORUM
AS INFLUENCED BY THE PLANT EXTRACT



3.a CONTROL



3.b TREATMENT

TABLE 2

2.a SPORE GERMINATION IN ***HELMINTHOSPORIUM ORYZAE*** AS INFLUENCED BY THE PLANT EXTRACTS

Dilution	Per cent inhibition of spore germination in extracts of		
	Leaf	Stem	Root
1:05	76.8	68.2	72.8
1:10	58.9	56.3	41.7
1:25	42.1	49.7	39.1
1:50	27.5	29.5	18.5
1:100	17.9	12.6	6.0

(Data represents mean of five determinants)

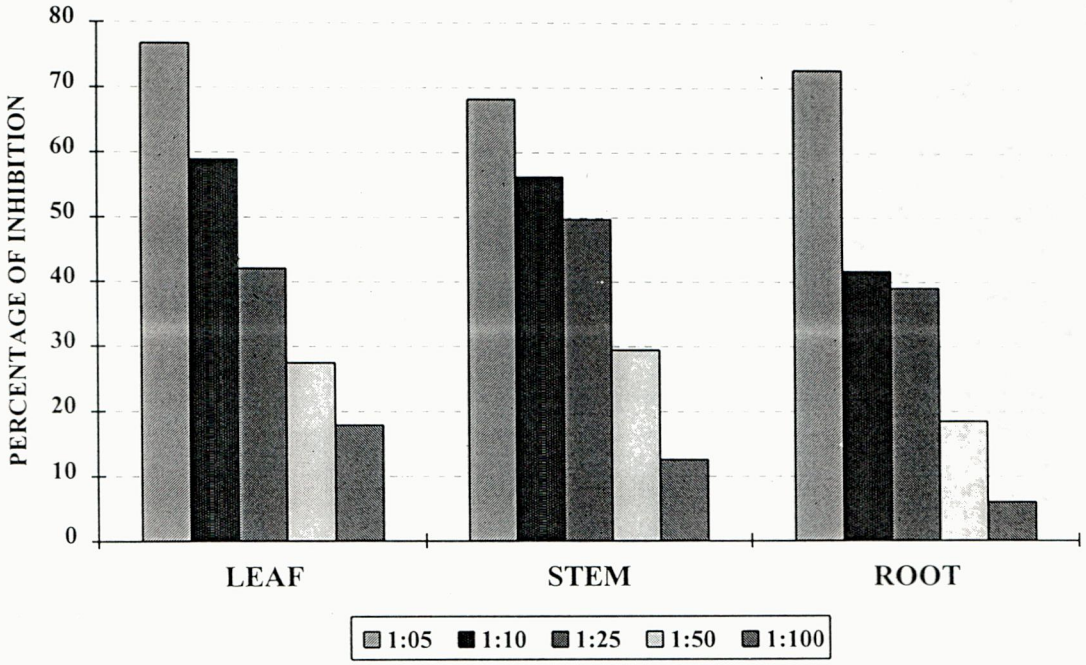
2.b SPORE GERMINATION IN ***FUSARIUM OXYSPORUM*** AS INFLUENCED BY THE PLANT EXTRACTS

Dilution	Per cent inhibition of spore germination in extracts of		
	Leaf	Stem	Root
1:05	76.3	87.1	86.0
1:10	67.5	60.5	83.1
1:25	57.3	58.7	67.5
1:50	55.6	46.7	54.8
1:100	28.2	36.0	45.5

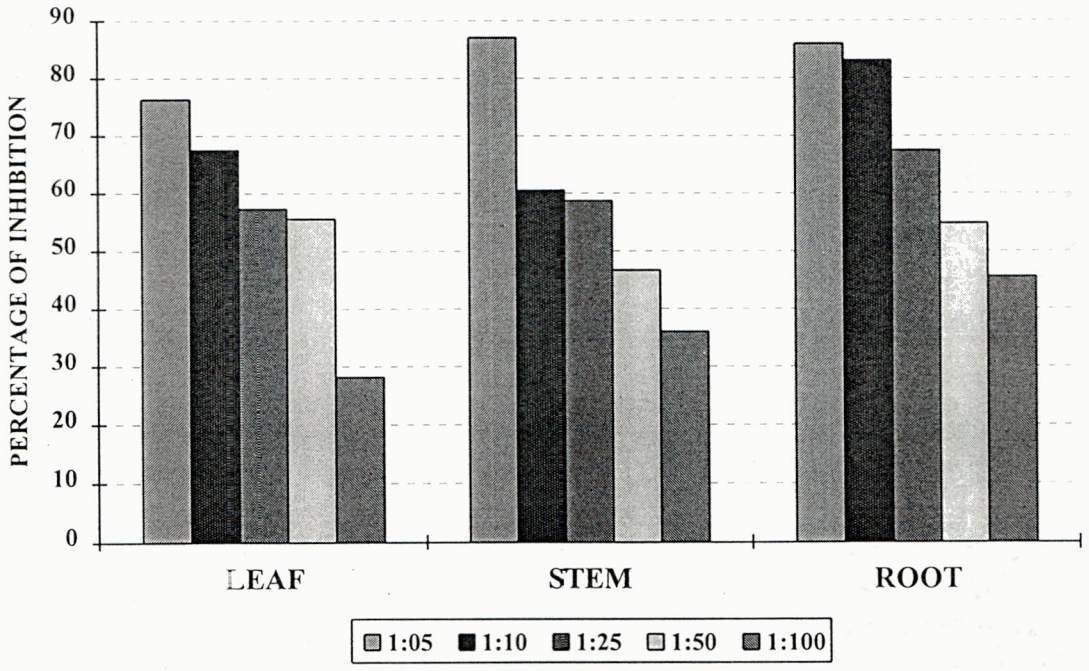
(Data represents mean of five determinants)

FIGURE 1

1.a SPORE GERMINATION OF ***HELMINTHOSPORIUM ORYZAE*** AS INFLUENCED BY THE EXTRACTS OF ***EXCOECARIA AGALLOCHA*** L.



1.b SPORE GERMINATION OF ***FUSARIUM OXYSPORUM*** AS INFLUENCED BY THE EXTRACTS OF ***EXCOECARIA AGALLOCHA*** L.



4.3.4. SPORE GERMINATION OF *ALTERNARIA TENNUIS* AS INFLUENCED BY THE PLANT EXTRACTS

The results on the per cent inhibition of spore germination of *Alternaria tennuis* are shown in Table 3.a.

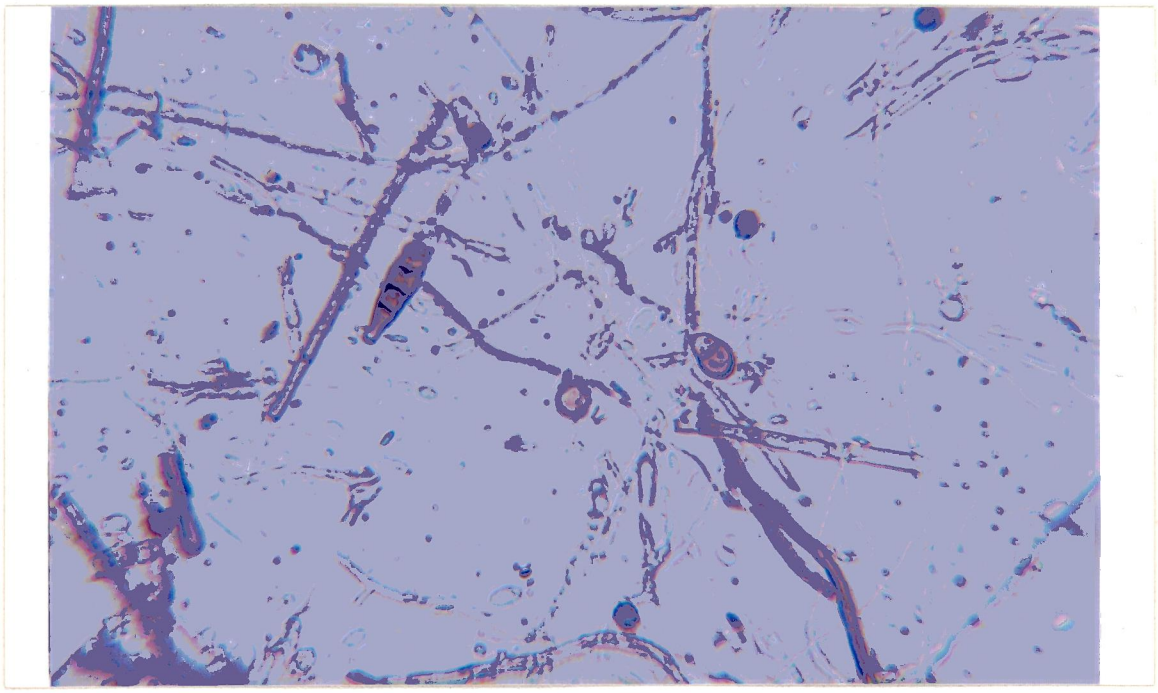
Among the three extracts, the leaf extract was found to exhibit maximum inhibition of the spore germination of *Alternaria tennuis*.It showed 92.7 per cent inhibition of spore germination at 1:5 dilution- the highest inhibition per cent shown among the three extracts. The lowest inhibition per cent (6.7) was shown by the root extract of 1:100 dilution.

4.3.5. BUDDING IN *SACCHAROMYCES CEREVISIAE* AS INFLUENCED BY THE PLANT EXTRACTS

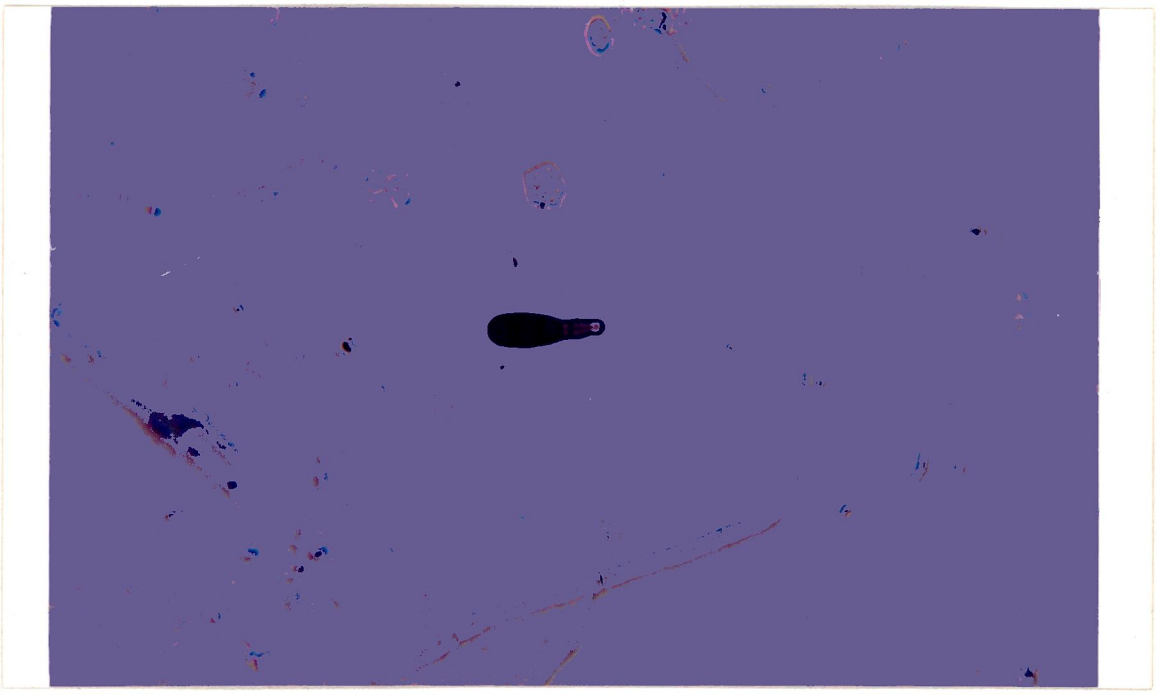
Per cent inhibition of budding in *Saccharomyces cerevisiae* as influenced by the various concentrations of the plant extracts is shown in Table 3.b.

From the results, it was seen that among the three extracts, the one from root inhibited budding more than the other two extracts. It showed 81.0 per cent inhibition at the dilution 1:5. The least inhibition of budding was 18.9 per cent shown by stem extract of 1:100 dilution.

PLATE 4
ALTERNARIA TENNUIS
AS INFLUENCED BY THE PLANT EXTRACT

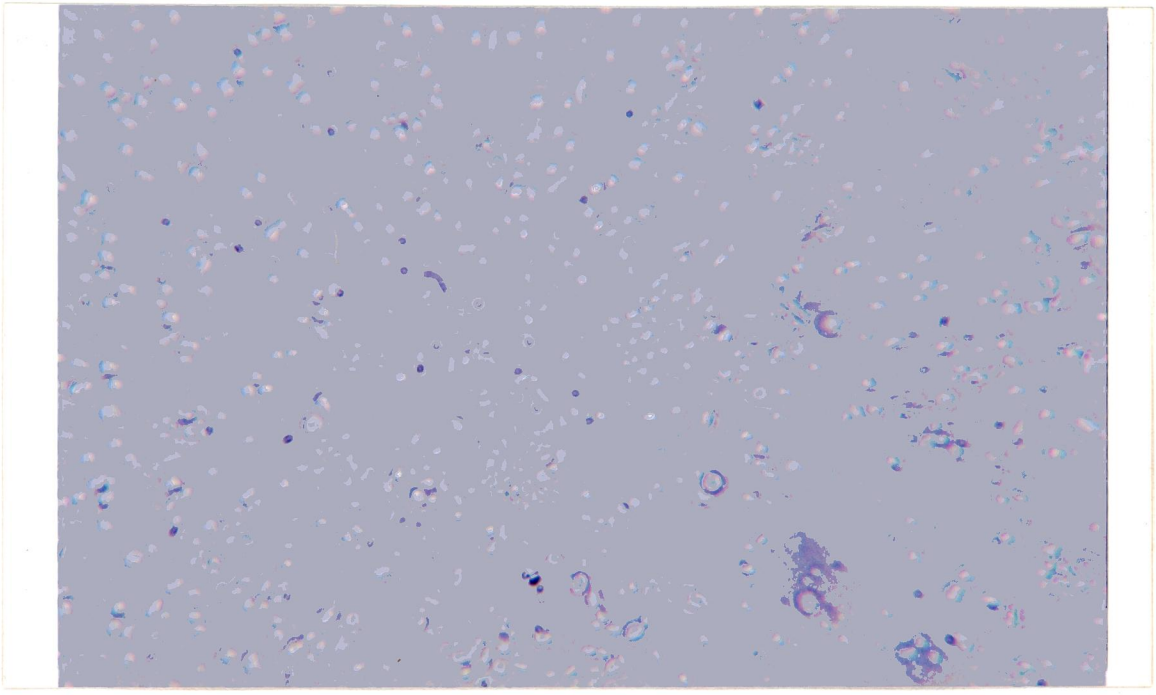


4.a CONTROL

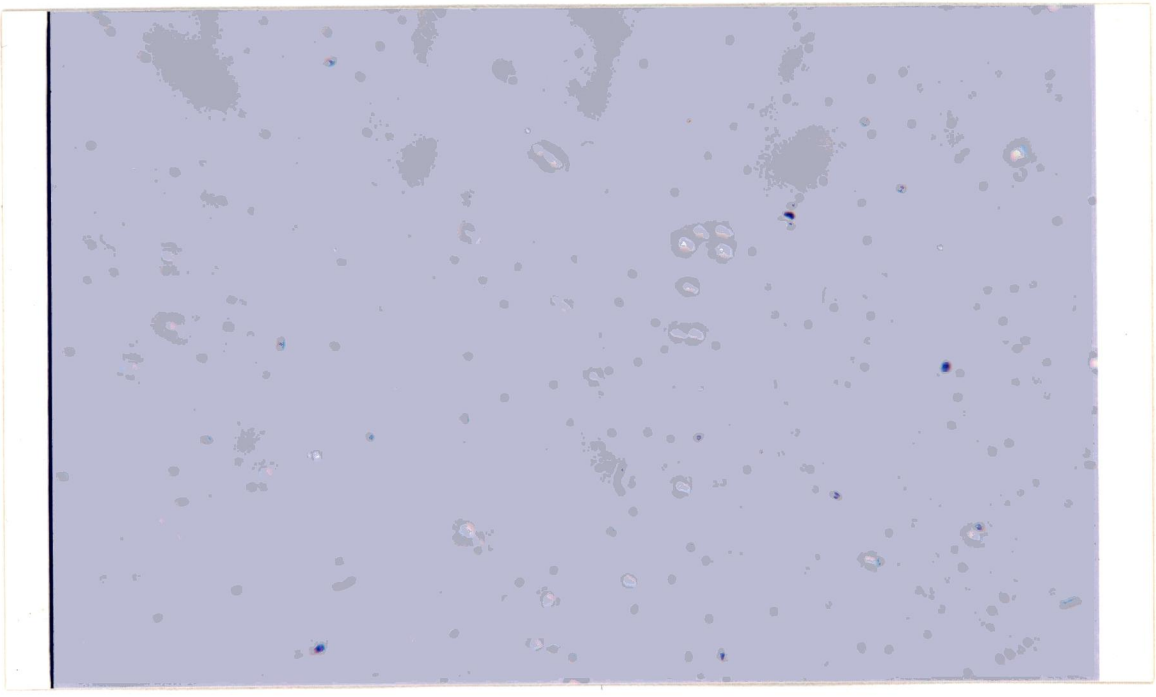


4.b TREATMENT

PLATE 5
SACCHAROMYCES CEREVISIAE
AS INFLUENCED BY THE PLANT EXTRACT



5.a CONTROL



5.b TREATMENT

TABLE 3

3.a SPORE GERMINATION OF *ALTERNARIA TENNUIS* AS INFLUENCED BY THE PLANT EXTRACTS

Dilution	Per cent inhibition of spore germination in extracts of		
	Leaf	Stem	Root
1:05	92.7	61.7	72.5
1:10	77.2	52.3	54.9
1:25	52.8	34.2	40.4
1:50	30.5	20.7	25.9
1:100	30.0	18.7	6.7

(Data represents mean of five determinants)

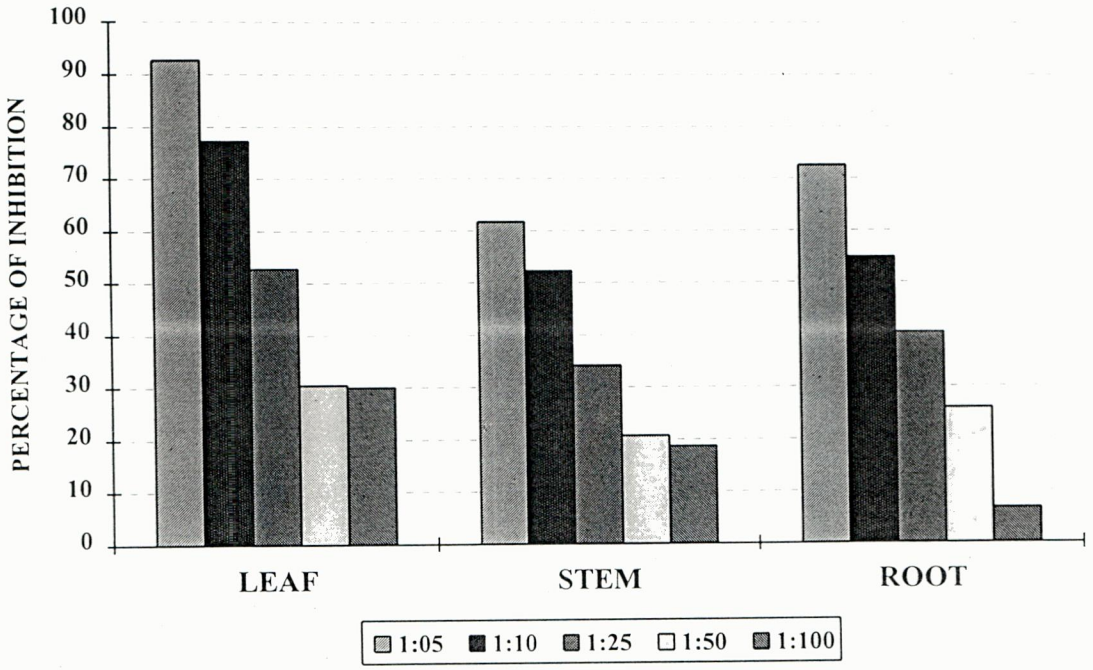
3.b BUDDING IN *SACCHAROMYCES CEREVISIAE* AS INFLUENCED BY THE PLANT EXTRACTS

Dilution	Per cent inhibition of spore germination in extracts of		
	Leaf	Stem	Root
1:05	76.2	75.8	81.0
1:10	75.4	75.4	76.4
1:25	71.9	75.0	75.4
1:50	59.9	71.5	64.2
1:100	19.8	18.9	55.1

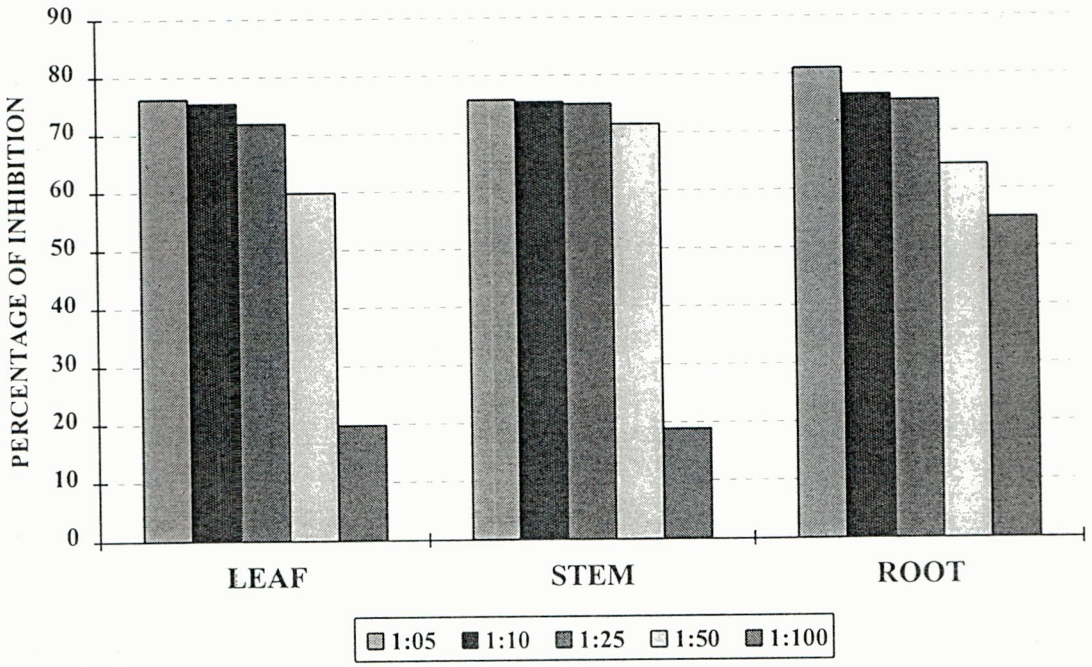
(Data represents mean of five determinants)

FIGURE 2

2.a SPORE GERMINATION OF ***ALTERNARIA TENNUIS*** AS INFLUENCED BY THE EXTRACTS OF ***EXCOECARIA AGALLOCHA*** L.



2.b BUDDING IN ***SACCHAROMYCES CEREVISIAE*** AS INFLUENCED BY THE EXTRACTS OF ***EXCOECARIA AGALLOCHA*** L.



DISCUSSION

CHAPTER V

DISCUSSION

The results on the antimicrobial properties of E. agallocha and the probable inhibitory principles are discussed in this chapter.

5.1. USEFULNESS OF BIOLOGICAL FUNGICIDES

For use in agriculture, synthetic crop protection chemicals were developed from about 1940 onwards. A disadvantage of these chemicals is the innate capacity of target organisms to acquire resistance. This condition urged agrochemical industry to develop chemicals with new modes of action. Such chemicals can be developed by using natural bioactive products. These bioactive compounds could be obtained from the extracts of various plant parts like leaves, stem, root, flower, seeds etc.

The undesirable side effects of chemical pesticides on humans make biological pesticides / fungicides more useful in crop protection (Jespers and De Waard, 1993). These biological fungicides are inherently regarded degradable in the natural environment, since they are primarily of bacterial, fungal or plant origin.

5.2. PLANT EXTRACTS AS PESTICIDES

Medicinal plants are known for many centuries and the extracts obtained from these plants were used in folklore and modern medicine. Many of the plant extracts have inherent qualities to control pests and diseases. 'Pyrethrum', obtained from the flower heads of *Chrysanthemum*

spp. is still used as an effective insecticide. Extracts obtained from *Azadirachta indica* are used for medicinal as well as insecticidal purposes (Jespers and De Waard, 1993). The inhibitory property of these extracts are attributed to the presence of certain organic compounds like phenolics and *Otho*-dihydroxy phenolics. These plant secondary metabolites possess very high toxicity towards plant pathogenic microbes.

5.3. CHEMICAL COMPOUNDS AND THEIR DISTRIBUTION IN PLANTS

Earlier studies of extracts of many plants revealed the presence of various compounds which contributes to their antimicrobial action.

Machado *et al.* (1948) reported the presence of compounds like volatile Sulphur compounds and alicin from garlic to be inhibitory to *Fusarium oxysporum.* Prakash *et al.* (1983) have described a piperidine alchaloid and a chalcone from the stem wood of E. agallocha which is poisonous to fish.

Mona *et al.* (1985) have revealed the spore germination and mycelial growth inhibition of *Fusarium oxysporum* f. sp. *niveum* by garlic and clove juice at 75 per cent dilution.

Phenol level in root exudates of chickpea cultivar was higher in resistant varieties than in susceptible ones (Chowdhury and Sinha, 1989). In the present investigation, it is seen that stem extract had poor inhibition of spore germination as compared to both leaf and root extracts which had good inhibition of spore germination of *Alternaria tennuis*. The differential

behaviour of the extract towards the inhibition of spore germination of the test organism may be due to its phenol content.

Kathiresan and Ravi (1990) reported that the leaves of E. agallocha contained the highest quantity of gallotannin. Karalai *et al.* (1994) have described the occurrence of a highly irritant mixture- Excoecaria factors A1/A2/A3 from *E. agallocha.*. These "free" Excoecaria factors belong to the poly functional Diterpene ester (DTE) group which are the natural constituents of the latex responsible for its bioactivity.

Flavonoids were determined in the leaves of *E. agallocha* by Oswin and Kathiresan (1994). A novel phorbol ester was isolated from the bark, leaves and stem of *E. agallocha* which was found to inhibit the replication of HIV-1 (Erickson *et al.*, 1995).

Total inhibition of spore germination of *Helminthosporium oryzae* was recorded by the extract of a medicinal plant- *Coriaria nepalensis* by Chatterjee *et al* .(1996). Koketsu *et al.* (1996) has reported the occurrence of an antifungal compound-aspidistrin- in the methanol extract of the plant *Aspidistra elatiori* Blume. It showed activity against the food borne fungus *Saccharomyces cerevisiae* at 2.5 fg/ml.

Polyphenols and tannins were reported from the leaves of *E. agallocha* by Basak *et al.* (1996). Two flavonoids isolated from the plant *Clerodendron infortunatum* roots - Cabruvin and Quercetin - showed inhibition of spore germination of *Alternaria carthami, Helminthosporium oryzae* and *Fusarium lini* (Roy *et al.*, 1996).

Another antimicrobial compound - a diterpene (Chalcone) - isolated from the plant *Alpinia galanga* showed antifungal activity against *Candida albicans* (Haraguchi *et al.*, 1996).The volatile oil of the plant *Fimbristylis junciformis* reduced the growth of *Fusarium oxysporum* significantly (Singh *et al.*, 1996).

Some essential oils like cinnamon and clove oil were also found to show high antifungal activity. Wilson *et al.* (1997) attributed the antifungal property of these oils to their constituents like D- limonene, cineole, alpha-pinene etc. They exhibited high activity against the fungus *Botrytis cinerea.*

The evaluation of antifungal activity of Xanthones from the fruit hulls of *Garcinia mangostana* by Geetha *et al.* (1997) revealed that the Xanthones inhibited the growth of *Alternaria tennuis* and *Drechslera oryzae* (*Helminthosporium oryzae*). Phytocassane E, a diterpene phytoalexin from rice was found to inhibit the spore germination of the dreadful blast pathogen *Magnoporthe grissea* (Koga *et al.*, 1997).

Acetophenones found in the leaves of *Rhododendron dauricum* was found to exhibit activity against the growth of *Fusarium oxysporum* (Aoyama *et al.*, 1997). Padmakumar and Ayyakkannu (1997) stated that the antimicrobial property of *E. agallocha* may be due to certain lipophilic metabolites in the plant extracts. It may also be due to the presence of tannin like substances.

A triterpenoid glycoside obtained from the bark of the tree *Pithecellobium racemosum* was found to inhibit the growth of *Saccharomyces cerevisiae* at 12.5 fg / ml (Khan *et al.* 1997).

In the present investigation, the total phenolics and *Ortho*-dihydroxy phenolics present in the leaf, stem and root of *E. agallocha* have been estimated. It may be concluded that the probable inhibitory activity of these extracts are due to the total phenolics and *Ortho*-dihydroxyphenolics.

These observations bring forth the importance of harnessing these sources of compounds as biofungicides and pesticides in the future.

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

The antimicrobial properties of *Excoecaria agallocha* were evaluated and it was found that the plant possessed excellent antimicrobial properties.

- The plant was found to inhibit the spore germination of *Helminthosporium oryzae*. The leaf extract showed maximum inhibition - 76.8 per cent - of spore germination.

- The spore germination of *Fusarium oxysporum* was found to be effectively kept under control by the extracts of the plant. The highest inhibition observed was 87 per cent.

- Spore germination in *Alternaria tennuis* was also found to be affected by the plant extracts. Here 92.7 per cent was the maximum inhibition observed.

- The single celled food borne fungus *Saccharomyces cerevisiae* was also found to be inhibited by the plant extracts. The budding in this fungus was inhibited by 81 per cent.

- Phenolics and *Ortho*-dihydroxyphenolics were estimated as the probable inhibitory principles.

- On the basis of the experiment conducted, it is concluded that the extracts of *E. agallocha* can serve as a good fungicide and can be recommended for use against the plant pathogenic fungi.

BIBLIOGRAPHY

BIBLIOGRAPHY

Anonymous, 1943. The slide germination method of evaluating protectant fungicides. Committee on standardisation of fungicide tests. *American Phytopathological Society*. pp. 863.

Aoyama, M., Mori, M., Okumura, M., Doi, S. and Anetai, M. 1997. Antifungal activity of 2', 6'-dihydroxy-4'- methoxyacetophenone and related compounds. *J. Japan Wood Research Society. 43(1)*:108-111.

Apisariyakul, A., Vanittanakom, N. and Buddhasukh, D. 1995. Antifungal activity of turmeric oil extracts extracted from *Curcuma longa* (Zingiberaceae). *J. Ethnopharmacology. 49(3)*:163-169.

Asha, S., Sharma, O. P. and Jhamaria, S.L. 1997. Fungitoxic properties of plant extracts against pathogenic fungi. *J. Mycol. Pl. Pathol. 27(1)*: 29-31.

Azcon-Aguilar, C. and Barea, J.M. 1996. Arbuscular mycorrhizas and biological control of soil borne plant pathogens- an overview of the mechanisms involved. *Mycorrhiza. 6(6)*: 457-464.

Baba, M.F., Koumaglo, K., Ayedoun, A., Akpagana, K., Moudachirou, M. and Bouchet, P.1997. Antifungal activity of essential oils extracted in the African states of Togo and Benin. *Cryptogamie, Mycologie. 18(2)*:165-168.

Balu, S. and Madhavan, S.1995. Wound healing property of *Excoecaria agallocha* L. *J. Econ. Taxon. Bot. 19(3)*: 571-575.

Barros, S.T., Oliveira, N. T. DE. and Maia, L.C. 1995. Effect of garlic *(Allium sativum)* bulb extract on mycelial growth and spore germination of *Alternaria* spp. *Summa Phytopathologica. 21(2)*: 168-170.

Basak, U. C., Das, A. B. and Das, P. 1996. Chlorophylls, carotenoids, proteins and secondary metabolites in leaves of 14 species of mangrove. *Bull. Mar. Sci. 58(3)*:654-659.

Bhowmick, B.N. and Vardhan, V. 1981. Antimycotic activity of leaf extracts of some medicinal plants on *Curvularia lunata. Indian Phytopath. 34*: 385-386.

Biehn, W. L., Kric, J. and Williams, E. B. 1968. Accumulation of phenols in resistant plant fungi interactions. *Phytopathology.58*:1255-1264.

Bray, H. G. and Thorpe, W. V. 1954. Analysis of phenolic compounds of interest in metabolism. *Meth. Biochem. Anal.1*:27-57.

Bulletin Botanical Survey of India.1960. *Medicinal uses of plants.2*:180.

Chatterjee, K. R. and Chowdhury, A. 1995. Antifungal activity of extract of *Clerodendron siphonenthus. J. Mycopathological Research. 33(1)*:67-70.

Chatterjee, M. R., Chatterjee, M. and Chowdhury, A. 1996. Antifungal activities of some fractions of extracts from *Coriaria nepalensis* on growth of three pathogenic fungi. *J. Mycopathological Research. 34(1)*:53-57.

Chowdhury, A. K. and Sinha, A. K. 1989. Differential effects of root exudates and extracts from chick pea cultivar on vascular wilt pathogen, *Fusarium oxysporum* f. sp. ciceri. *Indian Phytopathol. 42*:309.

Dubey, S. C. 1998. Evaluation of different fungal antagonists, plant extracts and oil cakes against *Thanatephorus cucumeris* causing banded blight of rice. *J. Mycol. Pl. Pathol. 28(3)*:266-269.

Dushyent, G. and Bohra, A. 1997. Effect of extracts of some halophytes on the growth of *Alternaria solani. J. Mycol. Pl. Pathol. 27(2)*:223

Egawa, H. , Tsutsui, O., Tatsuyama, K. and Hatta, T. 1977. Antifungal substances found in leaves of *Eucalyptus spp. Experientia. 33*:889-890.

Ekpo, E. J. A. 1991. Antifungal activity of leaf powder and extract of *Vernonia amygdalina* (Del.) on maize seed isolates of *Curvularia lunata* (Wakker) and *Fusarium semitectum* (Berk. and Rau.). *Zimbabwe J. Agric. Research. 29(2)*:129-132.

Erickson, K. L., Beutler, J. A., Cardellina, J. H. 11., Mc Mahon, J. B., Newman, D. J. and Boyd, M. R.1995. A novel phorbol ester from *Excoecaria agallocha. J. Nat. Prod. 58(5)*:769-772.

Ganesan, T. and Krishnaraju, J. 1995. Antifungal properties of wild plants. *II Advances in Plant Sciences. 8(1)*:194-196.

Ganguly, L. K. 1994. Fungitoxic effect of certain plant extracts against rice blast and brown spot pathogen. *Environment and Ecology. 12(3)*:731-733.

Gardini, F., Lanciotti, R., Caccioni, D. R. L. and Guerzoni, M. E. 1997. Antifungal activity of hexanal as dependent on its vapour pressure. *J. Agric. Food Chem. 45(11)*:4297-4302.

Geetha, G., Banumathi, B. and Suresh, G. 1997. Evaluation of the antifungal activity of natural xanthones from *Garcinia mangostana* and their synthetic derivatives. *J. Nat. Prod. 60(5)*:519-524.

Gupta, R. P. and Singh, A. 1983. Effect of certain plant extracts on Teliospore germination. *Indian J. Mycol. Pl. Pathol. 13(1)*:116-117.

Gupta, V. P., Govindiah. and Dutta, R. K. 1996. Plant extracts: a non-chemical approach to control Fusarium diseases of mulberry. *Curr. Sci. 71(5)*: 406-409.

Haraguchi, H., Kuwata, Y., Inada, K., Shingu, K., Miyahara, K., Nagao, M. and Yagi, A. 1996. Antifungal activity from *Alpinia galanga* and the competition for incorporation of unsaturated fatty acids in cell growth. *Planta Medica. 62(4)*:308-313.

Hidalgo, L. and Fernandez, A. 1996. Antifungal activity of plant extracts of different species of Cuba. *Revista- de- Proteccion- Vegetal. 11(1)*:19-23.

Jagannathan, R. and Narasimhan, V. 1988. Effect of plant extracts/ products on two fungal pathogens of finger millet. *Indian J. Mycol. Pl. Pathol. 18(3)*:250-254.

Jespers, M. B. K. and De Waard, M. A. 1993. Natural products in plant protection. *Neth. J. Pl. Path. 99(3)*:109-117.

Karalai, C., Wiriyachitra, P., Opferkuch, H. J. and Hecker, E.1994. Cryptic and free skin irritants of the daphnane and tigliane types in latex of *Excoecaria agallocha. Planta Medica. 60(4)*:351-355.

Kathiresan, K., Bose, K. S. and Sivakumar, A. 1987. Light induced effect of *Excoecaria agallocha* L. on phytoplankton productivity in Pichavaram mangroves. *Proc. Natu. Sem. Estaurine Management*, Trivandrum. 514-515.

Kathiresan, K. and Ravi, A. V. 1990. Seasonal changes in tannin content of mangrove leaves. *Indian Forester. 116(5)*:390-392.

Kathiresan, K. and Thangam, T. S. 1987. Biotoxicity of *Excoecaria agallocha* L. latex on marine organisms. *Curr. Sci. 56(7)*:314-315.

Kathiresan, K. and Thangam, T. S. 1987. Light induced effects of latex of *Excoecaria agallocha* L. on salt marsh mosquito *Culex sitiens* L. *J. Mar. Biol. Ass. India. 29(1-2)*:378-380.

Kazmi, A. R., Niaz, I. and Jilani, G. 1993. Evaluation of some plant extracts for antifungal properties. *Pakistan J. Phytopathol. 5(1-2)*:93-97.

Khan, I. A., Clark, A. M. and Mc Chesney, J. D. 1997. Antifungal activity of a new triterpenoid glycoside from *Pithecellobium racemosum* (M.). *Pharmaceutical Research.14(3)*:358-361.

Koga, J., Ogawa, N., Yamauchi, T., Kikuchi, M., Ogasawara, N. and Shimura, M. 1997. Functional moiety for the antifungal activity of phytocassane E, a diterpene phytoalexin from rice. *Phytochemistry. 44(2)*:249-253.

Koketsu, M., Kim, M. and Yamamoto, T. 1996. Antifungal activity against food borne fungi of *Aspidistra elatior* Blume. *J. Agric. Food Chem. 44(1)*:301-303.

Konishi, T., Azuma, M., Itoga, R., Kiyosawa, S., Fujiwara, Y. and Shimada, Y. 1996. Three new labdane- type diterpenes from wood, *Excoecaria agallocha. Chemical and Pharmaceutical Bulletin. 44(1)*:229-231.

Konishi, T., Kiyosawa, S., Konoshima, T. and Fujiwara, Y. 1996. Chemical structures of excoecarin A, B and C : three new labdane- type diterpenes from wood, *Excoecaria agallocha. Chemical and Pharmaceutical Bulletin. 44(2)*:2100-2102.

Konishi, T., Takasaki, M., Tokuda, H., Kiyosawa, S. and Konoshima, T. 1998. Antitumor promoting activity of diterpenes from *Excoecaria agallocha. Biological and Pharmaceutical bulletin. 21(9)*:993-996.

Krishnamoorthy, P., Maruthanayagam, C. and Subramanian, P. 1995. Toxic effect of mangrove plant (*Excoecaria agallocha* L.) latex on the larvae of fresh water prawn *Macrobrachium lamarrei. Environment and Ecology. 13(3)*:708-710.

Machado, P. A., Duran, M. G., Cross, J. D. and Santos, D. C. 1948. Garlicin, a new antibiotic. *Abstracts World Med. 5*:27.

Mahadevan, A. and Sridhar, R. 1974. *Methods in physiological plant pathology.* Shivakashi Publications. Madras.pp.316.

Mona, A. El. S., Fadl, F. A., Tawfick, K. A., Sirry, A. R. and El Zayat, M. M.1985. Antifungal property of *Garlic Clove Juice* compared with fungicidal treatments against Fusarium wilt of watermelon. *Egypt. J. Phytopathol. 17(1)*:55-62.

Murthy, N. B. K. and Amonkar, S. V. 1974. Effect of a natural insecticide from Garlic(*Allium sativum* L.) and its synthetic form(Diallyl-disulphide) on plant pathogenic fungi. *Indian J. Exp. Biol. 12*:208-209.

Nenoff, P., Haustein, U. F. and Brandt, W. 1996. Antifungal activity of the essential oil of *Melaleuca alternifolia* (tea tree oil) against pathogenic fungi in vitro. *Skin Pharmacology. 9(6)*:388-394.

Oswin, S. D. and Kathiresan, K. 1994. Pigments in mangrove species of Pichavaram. *Ind. J. Mar. Sci.23(1)*:64-66.

Ouf, S. A., Hady, F. K. A., Elgamal, M. H. and Shaker, K. H. 1994. Isolation of antifungal compounds from some *Zygophyllum spp.* and their bioassay against two soil borne plant pathogens. *Folia Microbiologica. 39(3)*:215-221.

Padmakumar, K. and Ayyakkannu, K. 1997. Antiviral activity of marine plants. *Indian J. Virology. 13(1)*:33-36.

Pandey, A., Maity, B. R. and Samaddar, K. R. 1996. Antifungal activity of plant latex towards certain fungal organisms. *J. Mycopathological Research. 34(1)*:35-40.

Pattnaik, S., Subramanyam, V. R., Bapaji, M. and Kole, C. R. 1997. Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios. 89(358)*: 39-46.

Pattnaik, S., Subramanyam, V. R. and Kole, C. 1996. Antibacterial and antifungal activity of ten essential oils in vitro. *Microbios. 86(349)*:237-246.

Perez, C. and Suarez, C. 1997. Antifungal activity of plant extracts against *Candida albicans. American J. Chinese medicine. 25(2)*:181-184.

Philip, T. and Sharma, D. D. 1997. In vitro evaluation of leaf and oil cake extracts of *Azadirachta indica* and *Pongamia glabra* on mulberry root rot pathogens. *Indian J. Sericulture. 36(2)*:150-152.

Prakash, S., Khan, H. and Zaman, A. 1983. A piperidine alkaloid from *Excoecaria agallocha. Phytochemistry. 22(8)*:1836-1837.

Quasem, J. R. and Abu-Blan, H. A. 1995. Antifungal activity of aqueous extracts from some common weed species. *Annals of Applied Biology. 127(1)*:215-219.

Rajiv, K. and Sachan, S. N. 1979. Effect of some plant extracts on the conidial germination of *Curvularia pallescens. Indian Phytopath. 32*:489.

Ravi, A. V. and Kathiresan, K. 1990. Seasonal variation in gallotannin from mangroves. *Ind. J. Marine Sci. 19(3)*:224-225.

Roy, R., Pandey, V. B., Singh, U. P. and Prithiviraj, B. 1996. Antifungal activity of the flavonoids from *Clerodendron infortunatum* roots. *Fitoterapia. 67(5)*:473-474.

Sarvamangala, H. S., Govindaiah. and Datta, R. K. 1993. Evaluation of plant extracts for the control of fungal diseases of mulberry. *Indian Phytopath. 46(4)*:398-401.

Sas- Piotrowska, B. and Piotrowski, W. 1997. Evaluation of fungicidal action of plant extracts on damping-off fungi in sugarbeet. *Biutelyn Instytutu Hodowli i Aklimatyzacji Roslin. 202*:253-258.

Sheikh, R. A. and Agnihotri, J. P. 1972. Antifungal properties of some plant extracts. *Ind. J. Myco. Pl. Pathol. 2(1)*:143-146.

Shekhawat, P. S. and Prasada, R. 1971. Antifungal properties of some plant extracts . 1. Inhibition of spore germination. *Ind. Phytopathol. 24(4)*:800-802.

Shivpuri, A., Sharma, O.P. and Jhamaria, S. C. 1997. Fungitoxic properties of plant extracts against pathogenic fungi. *J. Mycol. Pl. Pathol. 27(1)*:29-31.

Shrivastava, A. K. and Singh, K. V. 1982. Bio-assay of essential oils for antifungal activity. *Med. Aromat. Pl. Abstr. 4*:209.

Shylaja, M., Ranakausar., Reddy, S. M., Srivastava, H. P., Purohit, D. K. and Ram Reddy, S. 1997. Antifungal activity of some leaf extracts on the growth and sclerotial production of *Rhizoctonia solani. Microbial Biotechnology.* Scientific Publishers., Jodhpur. 143-144.

Sil, H., Krishna, R., Kaviraj, A. and Ray, K. 1995. Nutritive values of leaf and leaf litters of two mangroves of the Sundarbans for prawn and fish culture. *National Academy Science Letters. 18(1-2)*:43-46.

Singh, J., Dubey, A. K. and Tripathi, N. N. 1994. Antifungal activity of *Mentha spicata. Int. J. Pharmacog. 32(4)*:314-319.

Singh, P. R., Mishra, K. B. and Prasad, B. K. 1996. Volatile antifungal activity of *Fimbristylis junciformis* root bits on the growth of *Fusarium oxysporum. Neo-Botanic. 4(1)*:31-32.

Singh, U. P., Singh, K. P., Tripathi, V. K. and Pandey, V. B. 1994. Antifungal activity of some naturally occurring plant alkaloids. *Int. J. Trop. Pl. Diseases. 12(2)*:209-212.

So, M. L. 1990. Antifungal activities of mangrove plants. Proceedings of the 3rd International Conference on Plant Protection in the Tropics. Kaula Lumpur (Malaysia). *Malaysian Plant Protection Society. 2*:95-98.

Srivastava, A. K. and Lal, B. 1997. Studies on biofungicidal properties of leaf extracts of some plants. *Ind. Phytopathol. 50(3)*:408-411.

Sundriyal, R. C. 1991. Fungitoxic properties of flower extracts of some wild plants of Garhwal Himalaya. *Advances in Plant Sciences. 4(2)*:230-234.

Thangam, T. S. and Kathiresan, K. 1988. Toxic effect of mangrove plant extracts on mosquito larvae *Anopheles stephensi* L. *Curr. Sci. 57(16)*:914-915.

Thangam, T. S. and Kathiresan, K. 1992. Smoke repellency and killing effect of marine plants against *Culex quinquefasciatus. Tropical Biomedicine. 9(1-2)*:35-38.

Thangam, T. S. and Kathiresan, K. 1992. Mosquito larvicidal activity of mangrove plant extracts against *Aedes aegypti. International Pest Control. 34(4)*:116-119.

Thangam, T. S. and Kathiresan, K. 1993. Repellency of marine plant extracts against the mosquito *Aedes aegypti. Int. J. Pharmacog. 31(4)*:321-323.

Thangam, T. S. and Kathiresan, K. 1997. Mosquito larvicidal activity of mangrove plant extracts and synergistic activity of *Rhizophora apiculata* with pyrethrum against *Culex quinquefasciatus. Int. J. Pharmacog. 35(1)*:69-71.

Thangam, T. S., Srinivasan, K. and Kathiresan, K. 1993. Smoke repellency and killing effect of mangrove plants against the mosquito *Aedes aegypti* (Linn.). *Tropical Biomedicine. 10(2)*:125-128.

Tripathi, R. N., Panday, D. X., Tripathi, N. N. and Dixit, S. N. 1978. Antifungal activity in pollen of some higher plants. *Nat. Acad. Sci. Lr. 4*:107-109.

Vincent, J. M. 1947. Distortion of fungal hyphae in presence of certain inhibitors. *Nature. 96*:596.

Wannisorn, B., Jarikasem, S. and Soontorntanasart, T. 1996. Antifungal activity of lemon grass oil and lemon grass oil cream. *Phytotherapy-Research. 10(7)*:551-554.

Wilson, C. L., Solar, J. M., El-Ghaouth, A. and Wisniewski, M. E. 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea. Plant Disease. 81(2)*:204-210.

Wiriyachitra, P., Hajiwangoh, H., Boongton, P., Adolf, W., Opferkuch, H. J. and Hecker, E. 1985. Investigations of medicinal plants of Euphorbiaceae and Thymelaeaceae occurring and used in Thailand; II. Cryptic irritants of the diterpene ester type from three *Excoecaria species. Planta Medica. 5*:368-371.