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Effect of the extracts of *Excoecaria agallocha* on spore formation and budding in fungi

Mundekkad Deepa[1*] and C. K. Padmaja[2]

[1]*SBST, VIT University, Vellore, Tamil Nadu, India*
[2]*Dept. of Botany, Avinashilingam University for Women, Coimbatore, Tamil Nadu, India*

ABSTRACT

Excoecaria agallocha is a mangrove plant used in traditional medicines for curing a wide variety of diseases. Research on the effect of the extracts on different organisms has yielded varying results. It was understood from literature studies that the extracts have wonderful healing effects and are used against many human disease conditions. Also, it was found to be effective in controlling a variety of plant pathogenic microorganisms. In the present study, an attempt was made to test the antifungal effects of Excoecaria agallocha L. by the inhibition of spore formation in Fusarium oxysporum, Helminthosporium oryzae, Alternaria tennuis and budding in Yeast (Saccharomyces cerevisiae). It was proved that the extract was capable of bringing about the desired antifungal effect in a concentration dependent manner.

Keywords: Antifungal, *Fusarium oxysporum, Helminthosporium oryzae, Alternaria tennuis, Saccharomyces cerevisiae.*

INTRODUCTION

Most of the plant diseases are caused by insects, bacteria, fungi and viruses. Among these, fungal infections have posed particular threat to agriculturists worldwide as they formed one of the principal causes of crop loss [1]. Evidences show that fungal diseases are emerging as infectious diseases [2] raising alarm as it extended and crossed all taxonomic and geographical barriers. Fungal infections have adversely affected the protected habitats and even contributed to serious biodiversity loss [3][4].

Various techniques like crop rotation, breeding fungus resistant cultivars of crops and use of chemical fertilizers were employed for controlling the fungal infections and thus reduce crop loss. But the chemicals designed with the aim to promote agriculture led to the degradation of environment. The resistance of fungi to these chemicals remained a major concern for agriculturists. Moreover, these chemicals persist in the soil and water posing major ecological threat [5]. A second green revolution based on environmentally safe practices and technology is the need of the hour. Safer, less expensive but effective alternatives to chemical control of these microbes are desirable. An integrated, multidisciplinary approach is needed to curb the problem. It may be noted that minimizing the risks is often more important than maximizing profits. In such a challenging situation, antifungal agents from plants could be the best solution.

It is at this juncture that control of fungal diseases through biological means gained wide acceptance [6]. The bio-fungicides substituted chemical fungicides and are being preferred over chemicals [7]. They are successfully utilized

by farmers and agriculture extension workers as an effective remedy to these problems all over the world. Extracts from plants are sources of novel compounds that are found to be biologically active against many micro organisms like fungi, bacteria, *etc.* [8][9]. Garlic and turmeric extracts have proven records as effective anti-fungal pesticides in day-to-day life of Indians. It is a known fact to many Indians that growing thulasi (*Ocimum sanctum*) in the courtyard will keep away common pests (including common bacterial and fungal infectious agents) to an extent. Such experiences prompt researchers to search for more and more sources for effective fungicides. In the present study, an attempt was made to test the antifungal effects of *Excoecaria agallocha* L. by testing the efficacy of the extract in controlling different test fungi like *Fusarium oxysporum, Helminthosporium oryzae, Alternaria tennuis* and Yeast (*Saccharomyces cerevisiae*)

Excoecaria agallocha is the temple tree of Chidambaram (the Sourth Arcot district in Tamil Nadu, India) and stand as an environmental heritage. The plant, belonging to Euphorbiaceae, is a small poisonous evergreen tree with white highly acrid latex that is injurious to human eyes and is therefore known as “the blinding tree” [8]. Medicinally it is a tree of great importance, but its potential is less exploited. Its latex can be applied to obstinate ulcers and used in preparation of rheumatism, leprosy and paralysis [9][10]. It is also a drastic purgative and abortifacient. The poisonous nature of the tree has contributed to a variety of inhibitory effects on different organisms. This property is particularly checked in the present study, which involves the ability of the extracts in inhibiting the spore germination in *Fusarium oxysporum, Helminthosporium oryzae, Alternaria tennuis* and budding in yeast.

MATERIALS AND METHODS

Collection of plant materials and processing

Fresh stems, leaves and roots of *Excoecaria agallocha* were collected from Parangipettai, a coastal area near Chidambaram- the South Arcot District of Tamil Nadu. The plant parts were washed under running water to remove soil particles and air dried to process further.

Preparation of extract

For the extraction procedure, an 80 per cent solution of the extract (ethanol-methanol mixture) was prepared in the ratio 5.6:1 [11] in sufficient quantities as required for the experiment.

Extraction of the plant tissues

The method described by Mahadevan and Sridhar was followed for the extraction of plant tissues [12]. A quantity of 5g of freshly cut and chopped plant part was separately boiled with 25ml of the extract over a water bath for 10 minutes. The extract was decanted and stored. The residue was ground to homogeneity in a mortar and pestle with a pinch of acid washed sand and 10 ml of extract. The slurry was strained through the layers of cheese cloth and the extracts were pooled. The volume was made up to 50 ml with the extract and stored in amber colored vial in a refrigerator till further use.

Determination of dry weight

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

Estimation of total phenolics

The total phenolics in the plant extracts were determined following [13]. In brief, 1ml of the plant extract was taken in a clean test tube and 1ml of 1N Folin Ciocatteau reagent and 2ml of 20 per cent sodium carbonate solution were added. The solution was boiled over a water bath for one minute keeping appropriate control. After cooling, the volume was made up to 25 ml with double distilled water. The intensity of color developed was read along with appropriate control at 725nm using a spectrophotometer. By referring to the standard curve prepared with pyrocatechol, the quantity of total phenolics in the sample was estimated.

Determination of *Ortho*- dihydroxy phenolics

The method described by [12] was followed for the determination of *Ortho*- dihydroxy phenolics. One ml of the 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 up to 25ml with double distilled water and the intensity of color developed was read using a spectrophotometer at 520nm. The values were compared with a standard and the quantity of *Ortho*- dihydroxy

phenolics present in the sample were determined from the standard curve obtained from the standard graph prepared with pyrocatechol.

Processing the extracts

To exactly 50 ml of the different plant extracts (stem, leaves and roots), 2ml of 1N HCl was added and the entire amount was transferred to a separating funnel. 50 ml of pre-chilled diethyl ether was added to this solution in the separating funnel and was shaken well [14]. 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 re-extracted from the aqueous phase for a second time with 25ml of pre-chilled diethyl ether. The extracts were pooled and dried in vacuum. The residue was dissolved in 5ml of *n*-propanol. Thus the extracts of stem, leaves and roots were prepared and stored in 4°C till further use.

Bioassay of the extracts

The assessment of the effect of the various extracts on the test organisms was done according to [15].

Fungal culture

Pure cultures of the test fungi namely, *Fusarium oxysporum*, *Helminthosporium oryzae*, *Alternaria tennuis* and a spore suspension of Yeast (*Saccharomyces cerevisiae*) were collected from the Department of Agricultural Microbiology, TNAU, Coimbatore and maintained on PDA medium in the Department of Botany, Avinashilingam University, Coimbatore, India.

Preparation of spore suspension

A spore suspension of the test fungi was prepared by adding 10-15 loop-full of spores of the test fungi from the pure culture to 10 ml of double distilled water [16] and maintained at room temperature.

Construction of petri dish moist chamber

A petri dish moist chamber was constructed to find the percentage of spore germination [17]. A glass rod bend in the bent in the form of 'V' was placed in a petri dish and sterilized in an autoclave. A clean cavity slide was placed over the sterile 'V' bridge and sterile water (10-15ml) was poured into the petri dish chamber to keep the chamber moist.

Testing the effect of the extracts on spore germination/budding of the test organisms

A quantity of 100µl of the cell suspension of *Fusarium oxysporum / Helminthosporium oryzae / Alternaria tennuis* / *Saccharomyces cerevisiae* was added through the micropipette to each cavity of the slide. Test solutions (the solvent extracted and partially purified leaf / stem / root extracts) were added through a micropipette over the cell suspension in each cavity of the micro-slide and incubated for 6-24 hours. Various dilutions of the extracts like 1:5, 1:10, 1:25, 1:50 and 1:100 were added in the same way along with control.

After sufficient incubation, the percentage of spore germination was calculated by observing at random the number of spores germinated in five microscopic fields (10X) in each sample. The percentage of inhibition of the spore was calculated using the formula

I = [C-T/C] * 100 where I= per cent inhibition, C = spore germination in control and T = spore germination in treatment [18].

RESULTS

Percentage of moisture content in the plant tissues

The moisture content of the various parts of *Excoecaria agallocha* was determined and the results are represented in Table I.

Table I. Percentage of moisture content in the plant tissues

Sample	Fresh weight (in g)	Dry weight (in g)	Percentage of moisture
Leaf	5	0.98	80.4
Stem	5	2.22	55.6
Root	5	3.10	38.0

Estimation of total phenolics and *Ortho*- dihydroxy phenolics

Though the variation was not very prominent, the root extract showed more phenolics content than stem or leaf extracts. The presence of *Ortho*- dihydroxy phenolics was observed to be more in the case of leaf extract compared to root and stem extracts (Table II).

Table II: Estimation of total phenolics and *Ortho*- dihydroxy phenolics

Plant extract	Amount of phenol (mg/ml)	Amount of *Ortho*- dihydroxy phenolics (mg/ml)
Leaf	0.34	0.68
Stem	0.42	0.38
Root	0.48	0.18

Effect of plant extracts on spore germination/budding of various test fungi

The spore germination/budding in various test fungi *Helminthosporium oryzae, Fusarium oxysporum, Alternaria tennuis* and yeast (*Saccharomyces cerevisiae*) was tested in presence of the extracts from *Excoecaria agallocha.* It was found that the extracts were capable of bringing about a concentration dependent inhibition on the spore germination in all the tested organisms. A model of the nature of inhibition in the test fungi when treated with 1:5 dilution of leaf extract is represented in Figure I. The figure clearly depicts the antifungal effect of the extracts from *Excoecaria agallocha.*

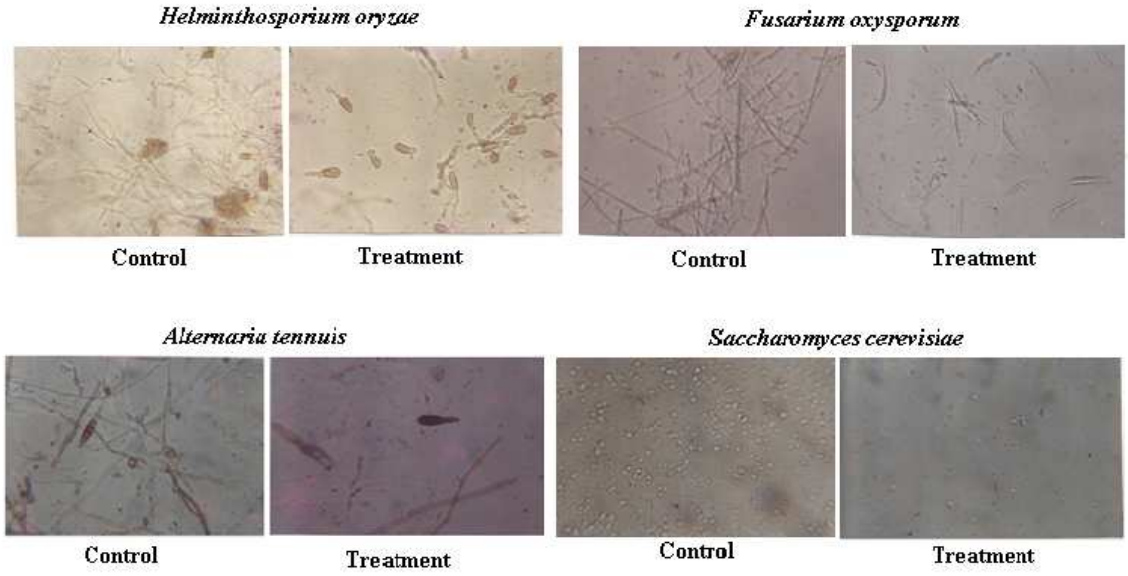

Fig. I. A representative figure to show the effect of the leaf extract of *Excoecaria agallocha* on the germination/ budding of various test organisms

Helminthosporium oryzae was most affected by the leaf extract with a maximum inhibition of spore germination (76.8%) at a dilution of 20%. A minimum inhibition percentage of 6 was observed at 1:100 dilution of root extract against the same fungus (Fig. IIa). When *Fusarium oxysporum* was treated with the various extracts, it was observed that the 20% dilution of stem extract brought about 87.1 per cent inhibition of spore germination (Fig. IIb). Leaf extract was not as efficient as other extracts in inhibiting the germination in *Fusarium oxysporum* whereas in *Alternaria tennuis*, it was the leaf extract that was efficient in bringing down spore germination to 92.7 per cent. The stem extract inhibited 61.7% and root extract inhibited 72.5 % of spore germination in *Alternaria tennuis* (Fig. IIIa). Budding in yeast was also found to be inhibited by the extracts. Among the three extracts, the one from root inhibited budding (81%) of yeast (Fig. IIIb) at a dilution of 20 per cent.

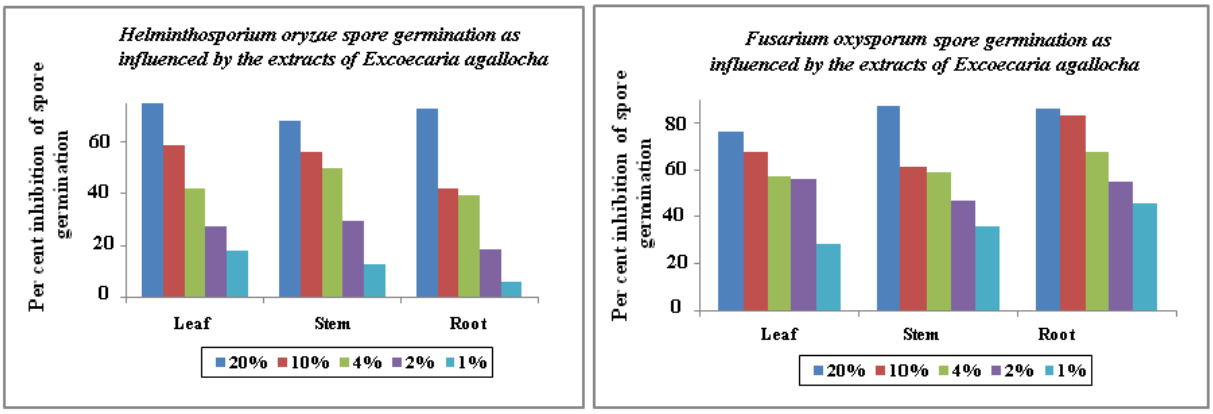

Fig. II. Per cent inhibition of spore germination of a) *Helminthosporium oryzae* and b) *Fusarium oxysporum* by the extracts of *Excoecaria agallocha*

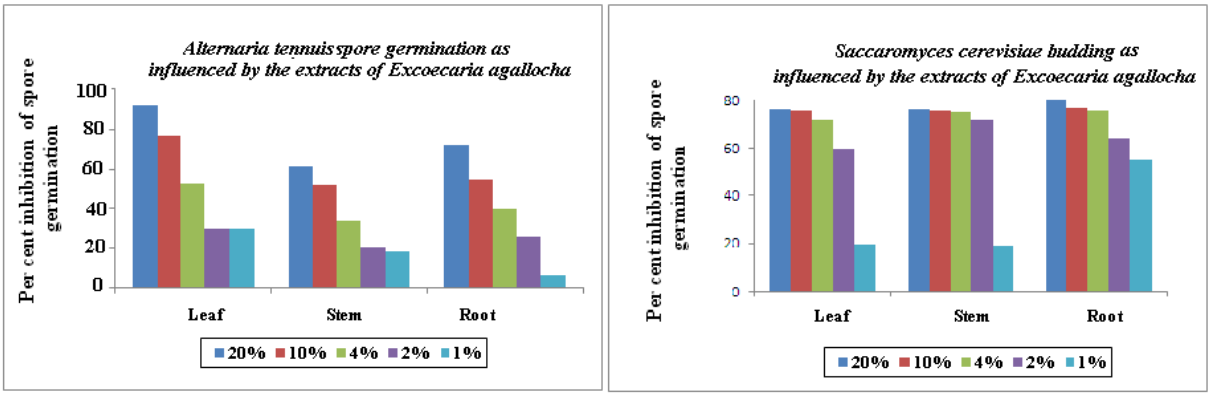

Fig. III Per cent inhibition of a) spore germination of *Alternaria tennuis* and b) budding in *Saccharomyces cerevisiae* by the extracts of *Excoecaria agallocha*

DISCUSSION

Fungi pose a severe threat to the animal and plant life equally and have lead to a number of infectious diseases in the natural population [4]. The fungus from *Fusarium spp.* causes serious diseases such as damping off of seedlings, root rot, wilting of several plants and rots of fruits and vegetables [7]. The various fungicides and botanicals had inhibitory effect on the fungal mycelial growth and spore germination [19]. The effect of plant extracts against fungi was studied by many researchers [20][21]. The fungitoxic effects of *Vinca rosea and A. indica* against *Fusarium oxysporum* were studied [22]. The efficacy of aqueous fractions of certain plant extracts against *Fusarium oxysporum* by spore germination method and found that the extracts showed inhibition of spore germination and mycelial growth in varying degrees [23]. A study conducted on the effect of the extracts of *C. zylanicum*, *M. piperita*, *A. sativum*and *A. hirtifolium* on the growth rate and spore germination of *F. oxysporum* showed that the extracts had considerable inhibitory effect [24]. The toxic effects of extracts of *V. rosea* and *A. indica* against the chilli fruit rot pathogen *Alternaria tenuis* were already reported [25]. It was found that the extract was capable of inhibiting the growth of the organism in a dose dependent manner. Oxyberberine, an alkaloid isolated from *Argemone mexicana* Linn, inhibited 100% spore germination of *Bipolaris* sp. and *Curvularia* sp. [26].

It could well be assumed that the differential behavior of the test organisms towards different extracts of *Excoecaria agallocha* could be due to the presence of chemical compounds in the extract. The same observations were made by [27] where they observed that the differential effect of the extracts of *M. piperata* on different fungi could be attributed to the presence of menthone, neomenthol, menthol and carvone in the extracts. It was observed that many of these activities were also concentration dependent [28]. The same could be said about the effect of the extracts of

Excoecaria agallocha on the test fungi too – it could be due to the presence of phenolics and alkaloids present in the sample.

CONCLUSION

In conclusion, it was understood from the present study that the extracts from *Excoecaria agallocha* had the ability to control the spore formation of fungi and can serve as a good source of fungicide. This plant could be recommended for use against the plant pathogenic fungi. Furthermore, the observations of the present study add credence to the illustrious nature of this plant. The study brings forth the importance of harnessing more such sources of compounds to be used as fungicides and pesticides in the future and it is hoped that the results presented in this study could be used to that effect.

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