Antifungal activity of *Pithophora oedogonia* against plant pathogens

*Pamela Sukumaran and **Thevanathan, R

*Department of Botany, Women's Christian College, Chennai 600 006. Tamilnadu India
pamelasukumaran@yahoo.com
****Professor*** CAS in Botany, University of Madras, Guindy campus, Chennai 600 025. Tamilnadu India
thevanathan@gmail.com

ABSTRACT: Methanol and n-hexane extracts of the green alga, *Pithophora oedogonia* were tested for antiifungal activity against isolates of two plant pathogenic fungi namely, *Colletotrichum lindemuthianum* and *Dreschlera oryzae*. Hexane extract inhibited the radial mycelial growth of *Dreschlera oryzae* (< 100 ppm) In contrast, methanolic extract promoted the radial growth of the two phytopathogens. Both methanolic and n-hexane extracts of *Pithophora oedogonia* delayed conidial formation in *Colleltotrichum lindemuthianum*. Treatment with hexane extract of the alga delayed conidial initiation by 4 to 6 days. The findings suggest that the 'nuisance alga'. *Pithophora oedogonia*, could serve as a potential source of biologically active compound for agricultural application.
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1.INTRODUCTION

Many marine algae in the past four decades algae have been reported to exhibit antifungal activity (Burkholder et al., 1960; Gauthier, 1965; Matusiak et al., 1965; Fenical et al., 1973, 1974; al., Sim et al., 1975; Codomeir et al., 1977; Pesando et al., 1979; Caccamese et al., 1980, 1981; Shelat, 1980, 1981; Moreau, et al., 1984; Reichelt and Browitzka, 1984; de Campos Takaki et al., 1988; Kellam et al., 1988; Ballestros et al., 1992; Miyazaki et al., 1995; Robles Centeno et al., 1996; Melo et al., 1997, Saito et al., 1997; Atta ur Rahman et al 1997; Khaliq uz Zaman, 1998). Algae that have the potential to act as antifungal agents belong to the Divisions Chlorophyceae, Phaeophyceae, Rhodophyceae and Bacillariophyceae. Among the diatoms, Chaetocheros launderii is shown to be active against the plant pathogen Microsporium gypseum and 3 species of Fusarium (Viso et al., 1987). Antifungal activity of the algae testedhas ben shown to vary with the solvent system used for extraction (Pesando and Caram, 1984; Moreau et al., 1984; Padmakumar and Ayyakkanu, 1986; Padmini Srinivasa Rao, 1986; deCampos-Takaki et al., 1988; Kellam et al., 1988; Tariq, 1991; Manimala and Rengasamy, 1993; Miyazaki et al., 1995). Pithophora oedogonia (Mont.) Wittrock, the 'horse hair' or 'cotton ball alga' is a fresh water green alga of the order Cladophorales. The alga produces free-floating mats of vegetation in static or slow moving bodies of water. Its luxuriant growth in shallow lakes and ponds as thick clumps or mats with profusely branched filaments having rigid, coarse cell walls, biomass production in huge quantities and high degree of resistance to many algicides have placed the alga in a prominent position as a filter clogging or nuisance alga of water systems. Though the alga has been studied as a nuisance alga, its nutritive and bioactive properties are not explored. The present investigation explores the possibilities of exploiting this alga for agricultural applications

2.MATERIALS AND METHODS

Filaments of Pithophora oedogonia were collected from cultures maintained in cement tanks containing Bold basal medium at 27 ± 2°C and a light intensity of 50 m Einsteins m-2 s-1. Harvested filaments (250 g) were shade dried and extracted with n-hexane for 48 hrs and the marc was re-extracted with methanol. Both extracts were dried separately in flash evaporator and the residues were redissolved in known quantities of acetone (1µg/mL,10 µg/mL 100 µg/mL & 1000µg/mL). Antifugal bioassay was carried out using Poisoned food technique. (Ramdas et al., 1998). Ten days old culture of Colletotrichum lindemuthianum and Dreschlera oryzae. grown on czapek Dox Agar were used for bioassay. and the time taken for conidil initiaion was noted The mean mycelial growth (mm), and the percent inhibition calculated.

3.RESULTS

1. Effect on radial mycelial growth

The radial mycelial growth of both Colletotrichum lindemuthianum and Drechslera oryzae in Czapek-Dox Agar medium containing the algal extract at desired concentration(1,10, 100, and 1000 µg/mL) was essentially linear throughout the assay period (240 hours) in all treatments and controls. Nevertheless magnitude of growth rate varied with the type of solvent extract used. The effect of the extracts on the radial mycelial growth of

the test fungi as percent inhibition is given in Figs 1&2.

PERCENT INHIBITION ON TREATMENT WITH THE METHANOLIC EXTRACT OF PITHOPHORA OEDOGONIA

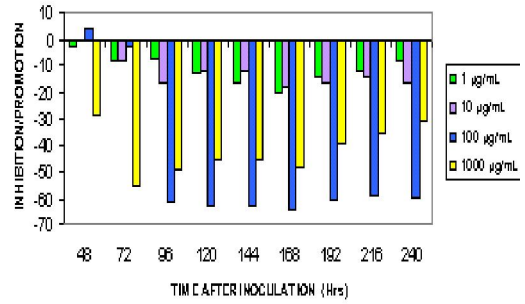


Fig. 1 COLLETOTRICHUM LINDEMUTHIANUM

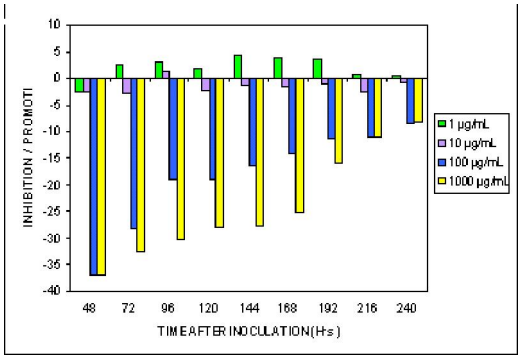


Fig. 2 DRECHSLERA ORYZAE

Methanolic extract of the alga had a promotory effect on the radial mycelial growth of both C.lindemuthianum and D. oryzae and the effect on C.lindemuthianum was visually evident only at 100 and 1000 µg/mL concentrations. In contrast, D. oryzae registered increased radial growth in all concentrations as compared to control (Figs 3 & 4).

EFFECT OF THE METHANOLIC EXTRACT OF PITHOPHORA OEDOGONIA ON THE RADIAL MYCELIAL GROWTH ON CDA MEDIUM

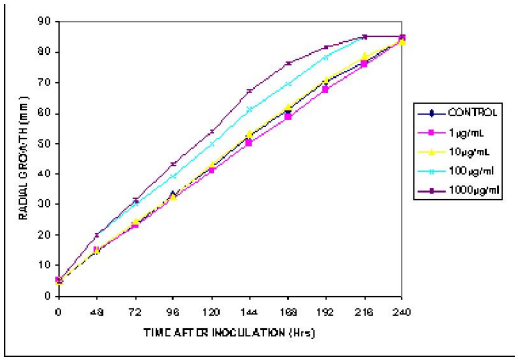


Fig. 3 COLLETOTRICHUM LINDEMUTHIANUM

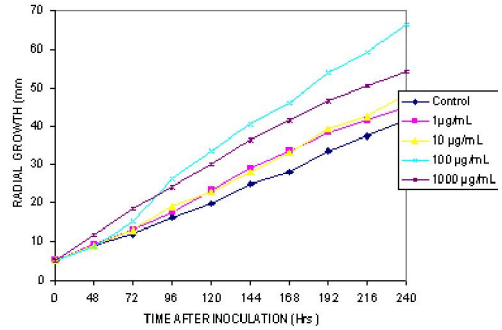


Fig. 4 DRECHSLERA ORYZAE

In similar experiments with hexane extract, only a marginal increase could be observed in the radial mycelial growth of C. lindemuthianum (Fig. 5).

EFFECT OF THE n-HEXANE EXTRACT OF PITHOPHORA OEDOGONIA ON THE RADIAL MYCELIAL GROWTH ON CDA MEDIUM

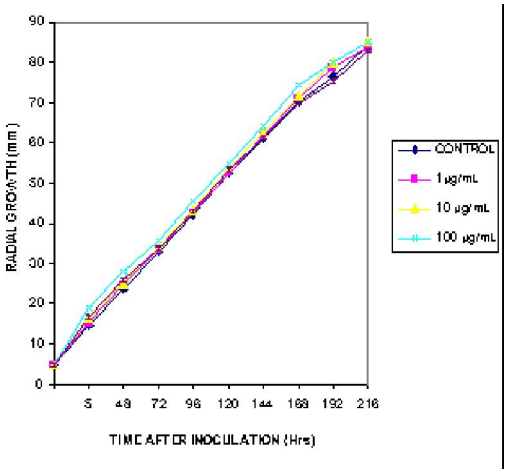


Fig. 5 COLLETOTRICHUM LINDEMUTHIANUM

However, D. oryzae could exhibit increased radial mycelial growth even at 1µg/mL and 10 µg/mL concentration (Fig.6). Treatment of the n-hexane extract at 100 µg/mL and 1000 µg/mL concentrations inhibited the radial growth of D.oryzae to an extent of 22.2% and 15.8% respectively at 48 hours after incubation. However, continued incubation reduced the margin of percent inhibition as compared to control for 100 µg/mL and 1000 µg/mL.The data presented in fig 7&8 would clearly indicate the inhibitory effect of the n-hexane extracts on the radial mycelial growth on D. oryzae.

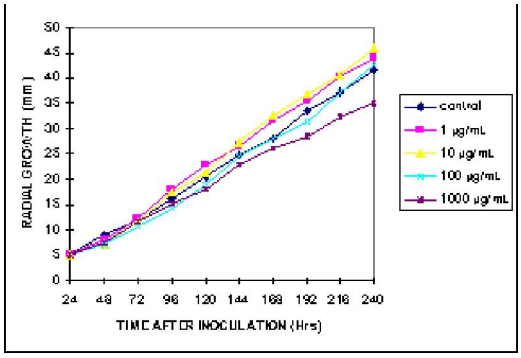


Fig. 6 DRECHSLERA ORYZAE

PERCENT INHIBITION ON TREATMENT WITH n-HEXANE EXTRACT PITHOPHORA OF OEDOGONIA

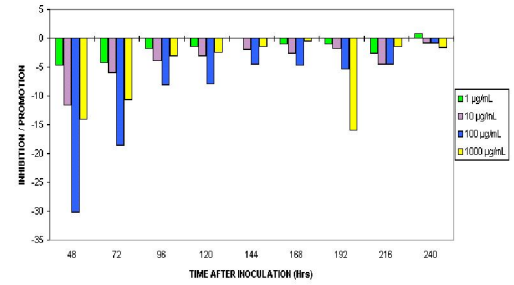


Fig. 7 COLLETOTRICHUM LINDEMUTHIANUM

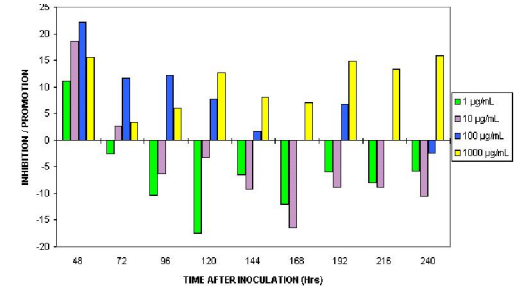


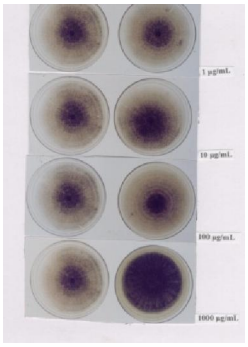
Fig. 8 DRECHSLERA ORYZAE

2. Effect on Conidiation

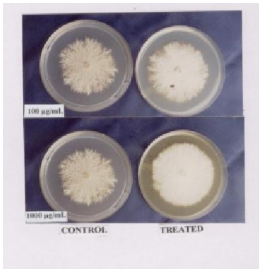
Effect of the solvent extracts on of the alga conidiation of the two test fungi was studied by determining the time taken for the initiation of conidia in Czapek Dox agar amended with 100 µg/mL and 1000µg/mL of the solvent extracts. In control, the production of conidia was observed on the fourth day after inoculation. Conidial formation in C. lindemuthianum was delayed by 6days at 100 µg/mL and by 3 days at 1000 µg/mL concentrations of the methanolic extract (Table 1). Treatment with n-hexane extract delayed conidial formation of C. lindemuthianum by 2–3 days (Table 1). Conidial initiation in D. oryzae was noticed after 10 days in the control plates. Methanolic extracts of the alga did not affect the conidial initiation in D. oryza. while n-hexane extracts delayed the process by aditional 4 days at100 µg/mL and by 6 more days at 1000 µg/mL concentrations.

EFFECT OF METHANOLIC EXTRACT OF PITHOPHORA OEDOGONIA ON RADIAL MYCELIAL GROWTH

COLLETOTRICHUM LINDEMUTHIANUM

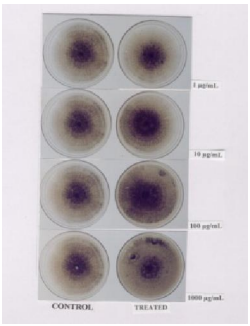


DRECHSLERA ORYZAE



EFFECT OF n- HEXANE EXTRACT OF PITHOPHORA OEDOGONIA ON RADIAL MYCELIAL GROWTH

COLLETOTRICHUM LINDEMUTHIANUM



DRECHSLERA ORYZAE

Table 1 Conidiation of *colletotrichum lindemuthianum* and *Drechslera oryzae* on CDA medium incorporated with *Pithophora oedogonia* extracts

Test Fungi	Time taken for conidiation (days)					
	Methanolic extract			n-hexane extract		
	Control	100 µg/mL	1000 µg/mL	Control	100 µg/mL	1000 µg/mL
C. lindemuthianum	4 ± 0	10 ± 0	7 ± 0.32	4 ± 0	6 ± 0.57	7 ± 0.32
D. oryzae	10 ± 0	10 ± 0	10 ± 0	10 ± 0	14 ± 0.57	16 ± 0.57

4.DISCUSSION

Methanol and n-hexane extracts of Pithophora oedogonia were tested for activity against Colletotrichum lindemuthianum and Drechslera oryzae two of the economically important pathogenic fungi in the region. Visually evident promotion of radial mycelial growth by the methanolic extract of Pithophora oedogonia was recorded for both pathogens (Figs 1 & 2). In contrast n-hexane extracts inhibited the radial mycelial growth of Drechslera oryzae at concentrations above 100 µg/mL. At concentrations below 100 µg/mL, n-hexane extracts also promoted the growth of the pathogenic fungi (Figs 3 & 4). The morphology of the test fungi was significantly altered by the algal extract to produce dense mats (Plate I). Inhibitory effect being marginal, the dense branching of the mycelium in response to treatment with extracts of Pithophora oedogonia at all concentrations should be considered to indicate a promotory effect rather than an inhibitory effect. This kind of promotory effect by the extract of the fresh water alga Pithophora oedogonia on fungal plant pathogens is not known for other fresh water and marine algae.

Compounds that delay conidiation are of importance in management of diseases caused by fungal pathogens. Delayed conidiation will imply prolonged vegetative growth, which means vulnerability of the fungus to disease management techniques and a decreased incidence of infections. Both methanolic and n-hexane extracts of Pithophora oedogonia delayed conidial formation in Colletotrichum lindemuthianum (Table 1). Methanolic extract of the alga did not affect conidial initiation in Drechslera oryzae while n-hexane extract delayed the process by 4 to 6 days. A delay in conidiation by 3 – 6 days in the presence of n-hexane extract at 1000 µg/mL concentration may be significant for consideration in the management of Drechslera oryzae. However, field studies are required to arrive at definite conclusions.

Corresponding Author:
Dr. Pamela Sukumaran
Department of Botany, Women's Christian College
Chennai 600 006. Tamilnadu India
pamelasukumaran@yahoo.com

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