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IN VITRO ANTIFUNGAL EVALUATION OF *DENDROPHTHOE FALCATA* LEAF EXTRACT AGAINST PHYTOPATHOGENS

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ABSTRACT: The aqueous and methanol leaf extract of *Dendrophthoe falcate* was investigated for its antifungal activities against twelve phytopathogens viz. *Alternaria alternata*, *Aspergillus fumigatus, Aspergillus niger, Colletotrichum falcatum*, *Curvularia lunata, Fusarium moniliforme*, *Fusarium udum*, *Helminthosporium oryzae, Phytophthora infestans, Pyricularia oryzae, Pythium debaryanum* and *Rhizoctonia solani*, using Agar dilution method . The methanol extract was found to be the most effective and showed significant antifungal activity against the test organisms. The extract of *Dendrophthoe falcate* seems promising since it showed fungitoxicity against the phytopathogens.

KEYWORDS: *Dendrophthoe falcata,* phytopathogens, extract, fungitoxic**.**

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1. INTRODUCTION

India is very rich in biodiversity, where diverse types of plants grow in different parts of the country. Thousands of plant species are known to have medicinal value. Different parts of the medicinal plants have been used successfully to cure specific ailments since ancient times. Traditionally plants have been well exploited by man for the treatment of human diseases, but not much information is available on the exploitation of plant wealth for the management of plant diseases, especially against phytopathogenic fungi. The potential of higher plants as a source of new fungicides is still largely unexplored. In the last few decades have seen a major increase in the use of synthetic antimicrobial drugs for the management of plant diseases. The indiscriminate use of synthetic antimicrobial drugs has developed multiple drug resistance in bacteria and other pathogens

[10], [26]. In addition, these drugs also create adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [1], [14]. This situation forced scientists to develop new, natural & safe antimicrobial drugs. Nowadays much attention is being focused on the alternative methods of plant disease control. In the past few years, a lot of work has been done on plant-derived compounds [24].Naturally occurring biologically active compounds from plants and their products (extracts) are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease control agents with no adverse side-effect on humans [24], [5], [6], [28], biodegradable, nonpollutive and therefore environmentally safe alternative to commercial antimicrobial drugs [25],[30]. They are effective in the treatment of plant diseases and at the same time mitigating the side effects that are often associated with synthetic antimicrobial drugs [15].The genus *Dendrophthoe falcata*(family: Loranthaceae) is a medicinal plant. It is an evergreen, shrubby, aerial hemi-parasite and abundantly distributed In Gorakhpur & adjacent districts of Uttar Pradesh (India). It is locally called as "Briksch bhakscha" (i.e. Plant eater). The branches on which it parasitizes do not bear flowers & fruits. The genus *Dendrophthoe* comprises about 20 species, out of them 7 species are distributed in India. The plant has high medicinal value. It is reported to have anticancer activity [20], antidiabetic [22] and antihypertensive [4]. It is also used in asthma, swelling wounds, ulcers, pulmonary tuberculosis, and menstrual disorders. It is used as an antifertility agent. However much work on the fungitoxic evaluation of the plant, especially against phytopathogens has not been done. Therefore, study was undertaken to investigate the fungitoxic activity of leaf extracts of *Dendrophthoe falcata,* a hemiparasite on mango (plate: 1).

Plate :1 ***Dendrophthoe falcata*** **(A) on** ***mangifera indica*** **(B)**

2. MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of *Dendrophthoe falcata*, a hemi parasite on mango plant were collected from Gorakhpur district, Uttar Pradesh (India), washed thoroughly under running tap water and finally with sterile water, air dried, and then ground to fine powder and stored in airtight bottles. Powder is

kept for extraction. A voucher specimen of the plant is deposited in the herbarium of Botany Department, St. Andrew's College, Gorakhpur, U. P.

Preparation of extracts

Aqueous extract

Hundred grams of dried powder of plant material was extracted in sterile distilled water for 6 h at slow heat. Every 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected. This procedure was repeated twice. The supernatant collected was filtered through Whatman No. 1 filter paper and heat sterilized in an autoclave at 121 ºC for 30 min. The extract was preserved aseptically in a bottle at 4 ºC for further use.

Solvent extracts

Hundred grams of dried powder of plant material was suspended in petroleum ether and kept at room temperature overnight for removal of all fatty substances. The supernatant was discarded and the residue was dried at room temperature. The residue was further suspended in 200 ml of methanol in sterile conical flasks and kept at 4º C overnight. The methanolic phase was separated using separator funnel and through Whatman filter paper No. 1.The filtrate was dried and preserved aseptically in a bottle at 4 ºC for further use. All the extracts were subjected to antifungal activity assay.

Phytopathogenic fungi tested

The fungal strains *Alternaria alternata* (MTCC 1884), *Aspergillus fumigates* (MTCC 2483), *Aspergillus niger* (MTCC 1344), *Colletotrichum falcatum* (MTCC 2106), *Curvularia lunata* (MTCC 2030), *Fusarium moniliforme* (MTCC 6576), *Fusarium udum* (MTCC 2204), *Pyricularia oryzae* (MTCC 1477) and *Rhizoctonia solani* (MTCC 4633) were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh (India). The fungal strains *Helminthosporium oryzae, Phytophthora infestans* and *Pythium debaryanum* were obtained from Natural Fungicide Laboratory, Department of Botany, St. Andrew's College, Gorakhpur, U. P. (India).

Fungitoxic activity assay

The fungitoxic activity assay of aqueous and methanol extracts was determined by Agar dilution method [8], [18], with minor modifications. Potato Dextrose Agar (PDA) medium is autoclaved and poured into presterilized Petri dishes (19 ml each).Different concentrations of the extract were prepared by adding 20µg, 40µg, 60µg, 80µg and 100µg separately in 1 ml of the sterile medium. 1 ml of the prepared extracts was added to each medium to make final volume and spread uniformly. The medium is then allowed to cool. After complete solidification of the medium, 5 mm disc of 7-day-old culture of the test fungi were placed aseptically in the centre of each assay plate and incubated at 28 ± 2^0C for six days. Colony diameters in mutual perpendicular directions were measured on the seventh day in assay plates. Fungitoxicity was recorded in terms of the percent inhibition of mycelial growth and calculated using the following formula [29]:

$$\text{Percent Inhibition} = \frac{dc - dt}{dc} \times 100$$

Where, dc = average diameter of fungal colony in control sets.

dt = average diameter of fungal colony in treatment sets.

The experiments were repeated twice and each set contained four replications. The PDA media devoid of the extract served as control. Four replicates were maintained for each concentration.

3. RESULTS AND DISCUSSION

The methanol and aqueous extracts of *Dendrophthoe falcata* were tested for its antifungal activity against twelve phytopathogens viz. *Alternaria alternata*, *Aspergillus fumigatus, Aspergillus niger, Colletotrichum falcatum*, *Curvularia lunata, Fusarium moniliforme*, *Fusarium udum*, *Helminthosporium oryzae, Phytophthora infestans, Pyricularia oryzae, Pythium debaryanum* and *Rhizoctonia solani*, using Agar dilution method. Results obtained in the present study clearly indicate that the methanol extract had higher fungitoxic activity against *Alternaria alternata*, *Aspergillus fumigates, Aspergillus niger, Fusarium moniliforme*, *Fusarium udum, Helminthosporium oryzae and Pyricularia oryzae* than the aqueous extract at 100 µg / ml concentration. The methanol extract showed highest inhibition zone of 19 mm for *Aspergillus fumigates, Aspergillus niger* and *Helminthosporium oryzae* (Table - 1) whereas the aqueous extract showed comparatively lesser inhibition at 100 µg / ml concentration (Table - 2). Both methanol and aqueous extracts showed lowest inhibition zone of 05 mm against *Rhizoctonia solani* at 100 µg / ml concentration (Table - 1 & 2).

Table 1: Fungitoxic activity of methanol extract of *Dendrophthoe falcata* on different phytopathogenic fungi

Phytopathogenic fungi	Zone of inhibition in mm					
	Concentration of extract (µg/ml)					
	20	40	60	80	100	Control
Alternaria alternata	06 ± .32	10 ± .33	13 ± .20	17 ± .21	18 ± .23	00
Aspergillus fumigatus	05 ± .22	09 ± .22	12 ± .31	16 ± .33	19 ± .33	00
Aspergillus niger	04 ± .21	08 ± .20	13 ± .21	15 ± .66	19 ± .20	00
Colletotrichum falcatum	02 ± .12	04 ± .20	06 ± .32	07± .21	10 ± .22	00
Curvularia lunata	03 ± .56	06 ± .33	09 ± .20	12 ± .22	16 ± .66	00
Fusarium moniliforme	04 ± .22	08 ± .21	11 ± .22	16 ± .21	18 ± .22	00
Fusarium udum	05 ± .33	08 ± .33	10 ± .33	16 ± .33	18 ± .20	00
Helminthosporium oryzae	06 ± .22	09 ± .57	12 ± .66	15 ± .22	19 ± .20	00

Phytophthora infestans	03 ± .33	07 ± .20	10 ± .22	13 ± .66	16 ± .57	00
Pyricularia oryzae	06 ± .22	09 ± .57	12 ± .66	15 ± .23	18 ± .20	00
Pythium debaryanum	03 ± .66	10 ± .33	08 ± .20	10 ± .33	11 ± .33	00
Rhizoctonia solani	02 ± .23	09 ± .22	04 ± .20	04 ± .33	05± .33	00

Table 2:Fungitoxic activity of aqueous extract of *Dendrophthoe falcata* on different phytopathogenic fungi

Phytopathogenic fungi	**Zone of inhibition in mm**					
	Concentration of extract (µg/ml)					
	20	**40**	**60**	**80**	**100**	**Control**
Alternaria alternata	01 ± .22	04 ± .22	05 ± .33	10 ± .22	16 ± .22	00
Aspergillus fumigatus	03 ± .20	05 ± .33	08 ± .22	13 ± .33	17 ± .20	00
Aspergillus niger	04 ± .22	06 ± .20	09 ± .21	11 ± .21	16 ± .22	00
Colletotrichum falcatum	01 ± .33	02 ± .22	04± .22	07 ± .33	08 ± .33	00
Curvularia lunata	03 ± .20	06 ± .21	09 ± .33	11 ± .57	14 ± .20	00
Fusarium moniliforme	04 ± .22	07 ± .21	11 ± .57	14 ± .20	17 ± .33	00
Fusarium udum	03 ± .32	06 ± .22	09 ± .57	13 ± .21	16 ± .22	00
Helminthosporium oryzae	04 ± .20	08 ± .33	11 ± .22	13 ± .22	17 ± .66	00
Phytophthora infestans	04 ± .33	07 ± .20	09 ± .33	11 ± .22	14 ± .54	00
Pyricularia oryzae	03 ± .22	05 ± .22	07 ± .33	10 ± .33	15 ± .22	00
Pythium debaryanum	01 ± .33	02 ± .22	04± .22	06 ± .33	07 ± .33	00
Rhizoctonia solani	02 ± .23	02 ± .22	03 ± .20	03 ± .33	04± .33	00

Medicinal plants are important sources of potentially useful structures for the development of new chemotherapeutic agents. The use of plant extracts in the treatment of diseases caused by various bacteria, viruses and fungi had been reported. Antifungal properties of plant extracts are widely recognized [9], [13], [17], [21]. Extracts obtained from many medicinal plants have recently gained popularity and scientific interest for their antifungal activities [19], [3], [2] [27]. Bansal and Sobti, demonstrated the antifungal activity of neem extracts against *Aspergillus niger* infection in groundnut [7].The antifungal activities of *Allium cepa, Eucalpytus rostrata* and *Capsicum frutescens* extracts were noticed against spore germination and vegetative growth of *Alternaria solani* and *Saprolegnia parasitica* [16]. Rawal and Thakore [25] reported the inhibitory effect of leaf extracts (20%) of *Datura stramonium* against mycelial growth of *Fusarium solani*, which causes *Fusarium* rot of sponge gourd. Harish *et al*. [12] have shown the inhibitory property of *Nerium oleander* and *Pithecellobium dulce* leaf extracts against mycelial growth (77.4%, 75.1% reduction, respectively)

and spore germination (80.3%, 80.0% reduction, respectively) of *Bipolaris oryzae in vitro*. The plant extracts and their active principles are frequently used for the disease control in human beings [11]. However, only a few reports are available on the exploitation of antifungal property of plants for developing commercial formulations for the protection of crop diseases. In the present study the methanol extract of *Dendrophthoe falcata* showed strong antifungal activity against phytopathogens viz. *Alternaria alternata*, *Aspergillus fumigates, Aspergillus niger, Fusarium moniliforme*, *Fusarium udum, Helminthosporium oryzae and Pyricularia oryzae***.** Therefore the extract can be used as an alternative drug for controlling diseases in plants.

4. CONCLUSION

This study clearly indicates that the methanol extract possessed antifungal activity. Being natural the extract is ecofriendly and non phytotoxic, therefore the extract can serve the purpose of a natural fungicide to control phytopathogens. Also, further work is needed to identity the antifungal compound present within the extract as well as to determine its mechanism of action. Nevertheless, any suggestion regarding the large scale practical utility of the extract on the commercial basis, must await the results of *in vivo* investigations.

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CONFLICT OF INTERST

Authors have no conflict of interest.

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