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### FIRST REPORT OF BIOCONTROL OF PHYTOPATHOGEN *CURVULARIA* IN *EMBLICA OFFICINALIS* BY PLANT EXTRACTS IN RAJASTHAN

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#### ABSTRACT

*Emblica officinalis* is a member of Euphorbiaceae family, a valuable medicinal plant with enormous source of Vitamin C. It is used for curing diseases like cancer, diabetes, hypertension and high cholesterol. In agriculture, synthetic fungicides are used to control the fungal pathogens and diseases but causes harm to the environment, soil, water and plant quality. Research has been focused on the bio-control of fungal pathogens by plant extracts. Till date, bio-control of any disease from plant extract has not been done on *Emblica officinalis* from Rajasthan state. The objective of the research work focuses on the evaluation of medicinal plant extracts against *Curvularia* spp. on leaves of *Emblica officinalis*. The study investigated the antifungal properties of aqueous and ethanol extracts of *Nerium indicum* and *Calotropis procera* respectively by disc diffusion method. The results obtained were fully disease resistant when treated with the disc of plant extracts. Botanical aqueous extracts of *Nerium indicum* and ethanol extracts of *Calotropis procera* showed fully resistant properties against fungal growth of *Curvularia* species. The results concluded from the study that medicinal plant extracts have broad range of antifungal activity and could be useful in control on fungal diseases of plants.

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## INTRODUCTION

Pathogenic fungi of plants have devastating impact on agricultural sector for many years [1]. Global concern has been raised against the pathogenic microorganisms that harm the crops and their productivity every year. Modern research has been focused on the biocontrol of pathogenic diseases of crops by medicinal plant extracts as raw drugs [2]. In today's active concerned world, strict rules have been set on removal of chemicals from the market [3]. Fungi are found in environment and fungal pathogens on crops has become a common problem for farmers. The genus *Curvularia* species is widely distributed and most commonly found fungi in plants.

Genus *Curvularia* is a facultative pathogen of many species of plants. They are generally present in tropical and a few temperate regions. It is a dematiaceous hyphomycetes which is wooly, pale brown or black in color with cylindrical or slightly curved conidia. About 40 *Curvularia* species are pathogenic in plants and colonize soil, vegetation by air borne spores [4]. Microscopic studies reveal dark pigmented hyphae with branched conidiophores, ends holding brown conidia which are usually four celled. The central cell generally larger than the other cells which gives a specific feature of identification of the genus *Curvularia*. Conidia is solitary, simple, often curved, clavate, ellipsoidal, fusiform, obovoid or pyriform with 3 or more transverse septa [5].

Medicinal plant extracts have been investigated to act as botanical fungicides and protect the plants and environment by harmful effects of synthetic fungicide chemicals. In the present study, *Nerium indicum* and *Calotropis procera* has been taken for study against *Curvularia* species on leaves of *Emblica officinalis*. The aqueous extract of *Nerium indicum* plant parts like stem, leaves and flowers has shown outstanding results.

The objective of the research work was to evaluate in vitro the antifungal activity of medicinal plants against *Curvularia* species by the measurement of zone of inhibition (in mm). The plant extracts of *Nerium indicum* and *Calotropis procera* showed inhibition properties. *Emblica officinalis* is an important and useful medicinal plant. Plant parts like Fruits, leaves and stem of *Emblica officinalis* has been attacked by number of fungi. [6] isolated pre- harvest fruit disease in *Emblica officinalis* from various regions of India.

The present study was done to investigate low cost, efficient and highly effective method for high healthy yield of *Emblica officinalis*. Botanical extracts have been studied and investigated for eradication of fungal disease in *Emblica officinalis*.

## MATERIALS AND METHODS

### Plant materials

Infected leaf parts of *Emblica officinalis* were collected from different localities of Ajmer city. Microscopic study of the infected plant part was carried out and observed under compound light microscope. The slides were stained with freshly prepared cotton blue dissolved in lactophenol.

### Surface sterilization and Inoculation

A small portion of infected leaf was surface sterilized with 0.1% (w/v) mercuric chloride solution [7]. Sterilized plant part was inoculated into sterilized petri dishes poured with PDA media. To avoid growth of bacterial pathogen, streptomycin sulfate 2.5 (ug/ml) was used. Petri dishes were incubated at 28C for 5-7 days.

### Subculture

The fungal colonies were sub cultured for purification and identification. Sterilized PDA broth was prepared which was inoculated with *Curvularia* for antifungal sensitivity test.

### Plant extracts

Sterilized plant parts were weigh two grams and dried overnight in oven at 30C. They were processed in 10ml organic solvents like ethanol and aqueous and homogenized well with help of mortar and pestle. They were allowed for centrifugation for 15 minutes (1000 rpm). The supernatant was taken and known volume was made. They were stored at a cool place for antifungal sensitivity test.

### Antifungal sensitivity test

Disc diffusion method [8], was taken for the screening of antifungal activity. Small disc of equal sizes was autoclaved and treated with plant extracts. These discs were tested against the fungal pathogen *Curvularia* species which was identified from the PDA culture of *Emblica officinalis*. Sterilized Petri dishes were poured with sterilized PDA media and allowed to solidify. The PDA fungal broth was evenly spread over the media. The Petri dishes were divided into four chambers with the help of marker pen at outer base of the Petri dishes. The disc was placed in each chamber pre-labelled with plant extracts. The plates were incubated at 28C. The Petri dishes were observed after 24 hrs. and 48 hrs. After the incubation period, Petri dishes were observed for the zone of inhibition around the disc which indicated the antifungal activity. The zone of inhibition was measured (in mm).

## RESULTS AND DISCUSSIONS

### Antifungal activity effect of some medicinal plants against *Curvularia* species on leaves of *Emblica officinalis*

Antifungal activity of two medicinal plant extract was studied by Disc diffusion method. The results obtained showed significant reduction on the growth of fungal pathogen against the plant extracts.

Among the plant extracts examined, the aqueous extracts of *Nerium indicum* and *Calotropis procera* showed maximum antifungal activity. Aqueous extract of *Nerium indicum* leaf and ethanol extract of *Calotropis procera* flower showed 100% antifungal activity results. The results of aqueous extracts of *Nerium indicum* flower and ethanol extracts of *Calotropis procera* stem showed minimum zone of inhibition (3-10 mm). The stem plant extract of *Nerium indicum* and leaf extract of *Calotropis procera* showed no antifungal activity against *Curvularia* species.

Kiran et al., 2011[9] studied the Antifungal activity of aqueous and other solvent extracts of *Psoralea corylifolia* against seed borne fungi of maize. *Curvularia lunata* was one of the fungal disease identified from maize. The fungi showed 86.0% of inhibition in aqueous extract. Plant extracts for biocontrol of fungal pathogens have proved to have antifungal activity. Aqueous extracts have wide scope in agricultural sectors with low cost investment and environment friendly. This extracts have simple methods of extraction with less time investment and strong potential for future agribusiness industries and productions. *Curvularia* species as leaf spot disease of rice in Peninsular Malaysia was studied. 33 isolates were studied based on Morphological and molecular characterization [10]. *Emblica officinalis* is a ayurvedic medicinal plant. It's plant parts like fruits, leaves and stem are edible and used for curing many diseases. The plant is itself used as an antifungal agent. Shubha et al., 2016 [11], used the aqueous extract of *Emblica officinalis* as an antifungal agent against *Candida albicans* by agar well diffusion method. The aqueous extract of the plant caused 99% fungal inhibition at 200 mg/ml and exposure time of 60 min.

**Table 1: Effect of Antifungal activity of Medicinal plants against *Curvularia* spp.**

S. No.	Plant	Part	Solvent	Zone of inhibition diameter (in mm)	Sensitive/ Resistant
1.	<i>Nerium indicum</i>	leaf	Aqueous	No fungal growth	Resistant
2.	<i>Nerium indicum</i>	flower	Aqueous	7	Less Resistant
3.	<i>Nerium indicum</i>	Stem	Aqueous	Nil	Sensitive
4.	<i>Calotropis procera</i>	flower	Ethanol	No fungal growth	Resistant
5.	<i>Calotropis procera</i>	Stem	Ethanol	3	Less Resistant
6.	<i>Calotropis procera</i>	leaf	Ethanol	Nil	Sensitive



A. *Curvularia* spp. on PDA



B. Microscopic study



C. Antifungal test.

## CONCLUSION

The research work conducted on *Emblica officinalis* made us to conclude that biocontrol of Phytopathogen *Curvularia* spp can be done by plant extracts. The phytopathogen is reported for the first time from Rajasthan in *Emblica officinalis* and the biocontrol by plant extracts. The results obtained opens wide scope for botanical fungicides with cheap, time consuming and efficient methods.

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