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Fungicidal potential of selected plant extracts against *Fusariumoxysporum*

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Abstract
The rationale of the present study was to examine selected medicinal plants *in vitro* for anti-fungal potential. Four medicinal plants were selected and tested for anti-fungal properties against the fungi, *Fusariumoxysporum*. Acetone extracts of the plant materials were prepared. Anti-fungal potential assay followed by Minimum inhibitory concentration (MIC) of the most potent extracts was ascertained. Significant anti-fungal properties were detected in the acetone extract of the leaves of*Albezialebbeck* and *Syzygium cumini*against the fungus.

Keywords: Fungi, Fungicidal potential, *Fusariumoxysporum*, Minimum inhibitory concentration, Plant extracts

INTRODUCTION

The incidence of diseases caused by fungi in plants has drastically increased in last two decades (Fisher et al., 2012). Use of chemical fungicides against the fungal diseases is one of the most effective control practice. However, use of synthetic chemicals is associated with negative implications to environment and human health concerns (Surapuram et al., 2014). In order to overcome the challenges posed by these chemicals, there has been a growing need for better fungicides with less environmental and human health hazard. Naturally-occurring compounds are potential source of lead molecules for new agrochemicals, although being natural compounds does not necessarily guarantee being better fungicides. Presence of different bioactive secondary metabolites in plants opens endless possibility for identification and development of new safe anti-fungal products (Arif et al., 2009). Different plant extracts have been tested till date for antifungal activity against different fungal pathogens in different crops (Adnew et al., 2022 and Mohammadi et al., 2014).

Fusariumoxysporum is a serious soil born pathogenic fungi causing diseases in several economically important crops throughout the world (Cia at al., 2022). It is amongst the most important and diverse phytopathogenic fungi infecting almost 150 plant species (Joshi, 2018). This fungus has become a dominant species in soil and can become a major threat as main infectious fungus in the future (Cia et al., 2022). Considering the economic importance of the fungus and the crop loss incurred, the present study was carried out which investigated four selected medicinal

plants for anti-fungal properties against the *F. oxysporum*. Antifungal assay followed by Minimum inhibitory concentration (MIC) assay of the most potent extracts was performed.

MATERIAL AND METHODS

Fresh leaves of four medicinal plants, *Syzygiumcumini, Delonixregia, Melia azedarach,* and *Albizialebbeck* were collected from the campus of Sharda University, Greater Noida, India. These medicinal plant leaves were washed three times with distilled water and then washed with 1% mercuric chloride for 20 seconds followed by washing with distilled water three times. The samples were dried in hot air oven at 45-50°C followed by crushing in electric grinder to make a powder.

Preparation of Acetone Plant extracts

10 grams of dried plant leaf powder of each plant was added with 100 ml acetone solvent in a conical flask and kept into shaker for continuous shaking for 48 hours. These were filtered with whatman filter paper and evaporated without heat. The extracts were collected and stored for further use.

Test microorganism

Fusariumoxysporum (ITCC-8111) was obtained from the Division of Plant Pathology, ICAR-Indian Agricultural Research Institute. The growth of fungal mycelia was prepared in buffered peptone water (TM MEDIA, TM307). A loop of fungal culture was added with buffered peptone water in the aseptic condition under laminar air flow by sterilized inoculating loop and was then mixed well. It was incubated in BOD incubator at 25°C for 7 days to get vigorous growth of fungal mycelia.

Anti-fungi activity assay

The anti-fungal assay of the acetone extracts was done using the punch well method (Stokes, 1975). The plates were prepared by dispensing 20 ml of nutrient agar into sterile petri plates and allowed to set. A 4 mm corkborer was used to punch holes in the medium. Four wells were made on each Petri plate, adequately spaced out after inoculation. About 0.2 ml of the different concentrations of the extracts was introduced into each well. The petri plates were incubated at a temperature of 37 °C for 24 h after which zone of inhibition was measured. A standard anti-fungal (carbendazim) was used as positive control.

Minimal inhibitory concentration determination

The extracts that exhibited anti-fungi activity were selected for the minimal inhibitory concentration determination. This was determined by broth dilution methods. 5 ml of nutrient broth was dispensed into each test tube. 0.5 ml of the fungi suspension was inoculated in each test tube containing the broth. This was followed by the introduction of different concentrations (300 $\mu g.ml^{-1}$, 600 $\mu g.ml^{-1}$, 900 $\mu g.ml^{-1}$, 1200 $\mu g.ml^{-1}$, 1500 $\mu g.ml^{-1}$, 3000 $\mu g.ml^{-1}$,6000 $\mu g.ml^{-1}$and 9000 $\mu g.ml^{-1}$) of the crude extract into the test tubes. The MIC was determined by the lowest concentration of the extract that inhibited visible growth.

RESULTS AND DISCUSSION

Antifungal potential assay

Table 1 shows the results of antifungal potential assay. Among those tested, extracts from two plants showed significant zone of inhibition against *F. oxysporum*. The highest zone was inhibited by acetone extract of *A.lebbeck followed by S. cummini.*Carbendazim, the standard fungicide, utilized in this work as control exhibited highest inhibition. Ibrahim and Hafeez, 2023 also examined that acetone extract of *A. lebbeck* exhibited antifungal potential against *F. oxysporum*. In terms of antifungal properties, the extracts from *M. azedarach*and *D. Regia*seemed to be

particularly ineffective in controlling *Fusarium* growth even at the highest concentration. Our results are in line with the findings of some earlierworkers reporting antibacterial/antifungal effects of theseacetoneplant extracts against many fungal species such as *Ascochytarabiei*(Jabeen et al., 2011), *Aspergillus flavus*,*Diaporthephaseolorum* var. meridionales, *F. oxysporum*, *F. solani*, *F. verticillioides*and *Sclerotiniasclerotiorum* (Carpinellaetal., 2003). Neyceeetal.,2012has also shown that *M. azedarach* leaf extract effect on *Sclerotium* spp., *F. oxysporum*and Rhizoctoniasolani were not significant.Gupta et al., 2015 also exhibited antifungal properties by *S. cumminia*gainst *F.oxysporum*and *Aletrnariaalternata.*

Table 1*. Anti-fungi potential of selected medicinal plants against *F. oxysporum

Plant extract/p.control	**Diameter of the zone of inhibition (mm) against test organisms**
Delonixregia	0.00
Melia azedarach	21.85
Albizialebbeck	38.95
Syzygiumcumini	34.33
Carbendazim (P.control)	39.25

The values are mean ± SE of 5 replicates. p. control implies positive control

Determination of minimum inhibitory concentration

The least MIC value was seen in the acetone extract of *Albezialebbeck against F. oxysporum* (300 $\mu g.ml^{-1}$) as indicated in Table 2.The different antimicrobial activities of plant extracts could be attributed to the presence of phytochemical compounds such as phenolics, flavonoids, alkaloids, tannins, saponins, steroids, and triterpenes, which have antimicrobial properties and cause damage of the cell membrane, leading to cell death (Khare et al., 2021). Several studies have demonstrated that medicinal plants have the antimicrobial potential as bactericidal, bacteriostatic, fungicidal, or fungistatic agents against microbial pathogens which is well established in the study as well (Kebede et al., 2021).

Table 2. MIC of the active plant extracts against test organism, *F. oxysporum*

Plant Extract/p.control	**MIC (µg / ml)**
Delonixregia	-
Melia azedarach	-
Albizialebbeck	300
Syzygiumcumini	600
Carbendazim (P.control)	46.2

– Represents not tested; MIC – minimal inhibitory concentration; p. control implies positive control

CONCLUSION

The reason for the fungicidal potential of selected plant extracts against *F.oxysporum*can be attributed to them containing active metabolic compounds, especially alcoholic and alkaloid, and this confirms the validity of previous studies that showed that phenolic and alkaloid compounds have inhibitory activity against laboratory-isolated fungi. Plant extracts such as these need to be further purified through anti-fungal activity guided fractionation to isolate and identify the compounds responsible for biological potency. The mechanisms that are the reason behind the efficiency of medicinal plant extracts in inhibiting microorganisms differ and depend on the presence of these compounds.

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