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# Biological control of *Sclerotium rolfsii* through the leaf extract of *Melia azedarach* L. and *Syzigium cumini*

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## Abstract
**Background of study:** *Sclerotium rolfsii* is a destructive plant pathogen causes diseases in plants and many economical important crops. This is a soil born plant pathogen therefore its control using commercial fungicides is not a significant method as these fungicides are non biodegradable and comparatively expensive. Use of plants and their products to manage microbial growth especially fungus has been found effective and safe due to naturally occurring potent plant metabolites. *Syzygium cumini* and *Melia azedarach* are two important therapeutic plants that possess a wide range of biological properties.
**Methodology:** The present study was carried out to manage this fungal pathogen using the two medicinal plant extracts. Methanolic leaf extracts of *Syzigium cumini* and *Melia azedarach* were used to control the fungal growth of *S. rolfsii*. *In vitro* antifungal bioassay against *S. rolfsii* was conducted using different concentrations (0%, 1%, 2%, 3%, 4% and 5%) of plant extracts using malt extract broth as culture media.
**Results:** Results of the present study were found to be significant in reducing the fungal growth. Different concentrations of leaf extracts of *S. cumini* and *M. azedarach* reduced fungal biomass up to 97% and 86% respectively over the control. The high concentrations (4, 5%) of the *S. cumini* leaf extract showed great decrease in fungal biomass production. Similarly, high concentrations of *M. azedarach* found to be effective against the fungal growth than the control.
**Conclusion:** This study concludes that *Sclerotium rolfsii* can be efficiently managed using methanolic leaf extracts of medicinal plants hence it is significant biological method to control *S. rolfsii*

**Keywords:** *Sclerotium rolfsii*, fungal pathogen, plant metabolites, antifungal, biological method, *in vitro* bioassay, non biodegradable, commercial fungicides.

## Introduction
The use of plants and their products has a long history that began with traditional medicine and through the years has been included into traditional and allopathic medicine. Since ancient times, many plants species reported to have pharmacological properties as they are known to contain various secondary metabolites like glycosides, flavonoids, tannins, alkaloids steroids, tirpenes which is therefore should be utilized to fight the disease causing pathogens [4, 6]. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. *Melia azedarach* L., traditionally have been used as anthelmintic, diuretic, astringent and stomachic agent. The activity of higher plants as a mean of new drugs is still largely unknown. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated, phytochemical investigation of a given plant will expose only a very narrow spectrum of its components. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of numerous therapeutic agents [7]. Medicinal plants are rich source of antimicrobial agents.
The wide use of synthetic chemicals especially fungicides, which causes more carcinogenic risk than other chemical pesticides may give rise to adverse biological effects on animals and human beings [2]. Therefore, the development of bio pesticides has been focused as a feasible pest control approach in recent years. One of the major sources of new fungicides is natural products of plants. Plant extracts and essential oils show antifungal activity against a wide range of fungi. The present study is carried out to investigate the antifungal activity of selected plants against the *Sclerotium rolfsii.*

*Sclerotium rolfsii* is an important soil-borne pathogen. It commonly occurs in tropical, sub- tropical and other warm temperate regions of the world. Pathogen attacks more than 500 species of cultivated and natural plants including almost all the agricultural and horticultural crops. Several monocotyledonous species have also been infected, but mostly *S. rolfsii* diseases have been reported on dicotyledonous hosts. Humid weather is favorable to sclerotial germination and mycelial growth, pathogen produces large no of sclerotia that inhibit in soil for long time. The first report of *S. rolfsii* from Pakistan was made by Ahmad in 1984 that isolated it from maize [1]. Diseases caused by *S. rolfsii* continue to receive considerable attention to the development of biological control strategies. *S. rolfsii* survives on dead plant material in the soil as sclerotia, which later germinate and attack host plants, causing necrosis by attacking cell wall [14].

**Syzygium cumini**

*Syzygium cumini* (L.) Skeels belongs to Myrtaceae, commonly known in Brazil as "jambolão" (jambolan or java plum in English). It is a local tree of the tropics, originally from South East Asia and India. It is extensively present in some states of North, Northeast and Southeast Brazil [10]. The bark of the plant is sweet, carminative, stomachic, antibacterial, diuretic, digestive, antihelminthic and constipating. The fruits and seeds are used to treat diabetes and ringworm infection. The plants have acetyl oleanolic acid, triterpenoids, ellagic acid, and myricetin in different concentrations [13]. Most of these compounds have been reported to possess antioxidant and free radical scavenging activities [15].

**Melia azedarach**

*Melia azedarach* L. (Meliaceae) is one of the most useful therapeutic plants like *Azadirachta indica,* member of the family Meliaceae. Every part of *M. azedarach* has some medicinal properties like *A. indica* and thus is commercially usable. During the last twenty years, apart from the chemistry of this plant, considerable progress has been achieved regarding the biological activity and medicinal applications. *M. azedarach* is native to tropical regions. It is wide spread and found in most of the tropics and subtropical countries. It is widely distributed in northern area of Iran [9].

**Objectives of the study**

The main objective of the study is to evaluate antifungal potential of methanolic leaf extract of *Syzygium cumini* and *Melia azadirah,* and to manage the *S. rolfsii* by using these extracts.

**Material and Methods**

Fresh and mature leaves of both plants were collected from Samanabad College. Leaves were washed thoroughly and then dried in oven at 70ºC. Dried leaves were crushed using grinder and 200 grams of leaf powder was soaked into 1000 mL of methanol and left at room temperature for 15 days. After filtration, solvent was evaporated through rotary evaporator to obtain (21 gm) crude extract. Crude extracts (9 g) of both plants were mixed with 5 mL dimethyl sulfoxide (DMSO) then 15 mL of distilled water was added to prepare the 20 mL of stock solution.

In the same way, the control solution was made by mixing 5 mL DMSO into 15 ml distilled water. Malt extract broth (3000 $mL^{-1}$) was prepared. The media was autoclaved and cooled at room temperature. Five concentrations (0% control, 1, 2, 3, 4, 5%) were made by mixing 0, 1, 2, 3, 4, 5 mL stock solution and 5, 4, 3, 2, 1, 0 mL of control solution accordingly, to each flask and rise its volume to 80 mL. The 80 mL medium of each treatment was divided into four equal portions that were used as replicates. Control treatment was made, 20 mL of control solution was mixed to 60 mL of malt extract broth and the medium was divided into four equal parts used as replicates.

Mycelial discs of fungus were made and added into each flask under sterile conditions; flasks were incubated at 27 °C for four days. Then biomass of fungus was harvested and dried at 50 °C in hot air oven [5].

Reduction in fungal biomass was calculated using formula as follows

% of fungal biomass reduction = Control – Treatment/ Control x100

**Results**

**Antifungal activity of methanolic leaf extract of *Syzygium cumini***

The effect of leaf extract concentrations on the biomass of *S. rolfsii* proved to be highly effective as shown in the figure 1. All the higher concentrations of methanolic leaf extract significantly reduce the growth of *S. rolfsii*. The applied concentrations of methanolic leaf extract (0, 1, 2, 3, 4 and 5%) reduced the fungal biomass as 0%, 71%, 77%, 79%, 87% and 97% respectively. But the higher concentrations (3, 4%) found to be very effective against the fungus. The antifungal effect of the extract was dependent on the concentration of the extract.

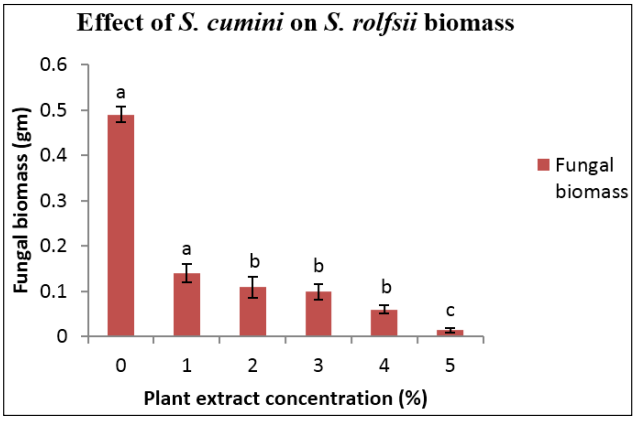


**Fig 1:** Effect of different concentrations of methanol leaf extract of *S. cumini* on biomass of *Sclerotium rolfsii*. Each value is a mean of four replicates with standard deviation. One way ANOVA with Duncan's multiple test range is applied to compare means by using SPSS software.

**Antifungal activity of methanolic leaf extract of *Melia azedarach***

The effect of different concentrations of methnolic leaf extracts on biomass of *S. rolfsii* was found to be significant as shown in figure 2. Different concentrations of methanolic leaf extract had different effect on the growth of *S. rofsii*. Antifungal effect of all the tested concentrations was significant as compare to control. The higher concentrations showed more resistance in the growth of *S. rolfsii* as compare to the lower concentrations of methanolic leaf extract. The used concentrations of methanolic leaf extract (0, 1, 2, 3, 4 and 5%) reduced the fungal biomass as 0%, 43%, 51%, 69%, 85% and 86% respectively.

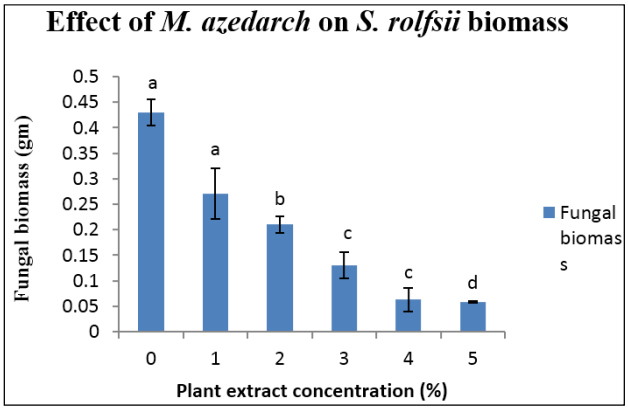


**Fig 2:** Effect of different concentrations of methanol leaf extract of *M. azedarach* on biomass of *Sclerotium rolfsii.* Each value is a mean of four replicates with standard deviation. One way ANOVA with Duncan's multiple test range is applied to compare means by using SPSS software.

## Discussion

In the present investigation methanolic leaf extract of two medicinal plants were evaluated against the fungal pathogen *S. rolfsii*. Different concentrations of methanolic extracts were prepared by using stock solution A and B. These concentrations were applied against the fungal pathogen. The efficiency of the different concentrations against the pathogen varied. The growth of fungus was high in control solution as compared to the experimental solution. The higher concentration (4%, 5%) of methanolic extract were effective against the fungal pathogen. The higher concentrations were proved to be most effective against the fungal pathogen. Earlier studies showed that methanolic extract of allopathic plants are effective in the management of phyto-pathogen.

Previous studies showed that the *Cymbopogon citrates*, *Lantana camara*, *Nerium oleander*, *Ocimum basilicum* and *Olea europaea* leave extracts, with either water or different organic solvent, were prepared to investigate the antifungal activity. The methanol extract of lemon grass, lanta and nerium followed by their ethyl acetate extracts showed the highest activities against *Trichophyton rubrum* [3]. Fungal species such as *Alternaria alternata, A. clamydophor, Aspergillus niger, A. flavus, Rhizopus oryzae, Rhizopus* spp., *Mucor* spp., *Fusarium* spp., *Drechslera australiensis, Penicillium* spp., *Curvularia lunata* and *Cladosporium,* that were isolated from stored grains of wheat, were effectively controlled by the use of Mancozeb [11]. From the literature it have been seen that different plants including *Plantago media subspstepposa, Quercus infectoria, Punic granatum, Thymus otschyana, Zingier officinalis, Rhus angustifolia and Cinnamomum* have high resistance against the fungus. These plants showed maximum activity against the *Candida* fungus [12].

## Conclusion

Present investigation concludes that the medicinal plants naturally possess numerous antifungal compounds that can be used as an alternative source to control fungal pathogens than the synthetic fungicides. This study demonstrates that organic solvent extract of medicinal plants contain fungicidal actions that significantly used to reduce fungus growth. Additional studies are needed to isolate more antifungal agents from these plants. The discovery of new fungicidal compounds may lead to the synthesis of novel fungicides that would be valuable against many economic important plant pathogens.

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