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MANANGEMENT OF *SCLEROTIUM ROLFSSII* THROUGH METHANOLIC LEAF EXTRACT OF *ALSTONIA SCHOLARIS* (L.) R. Br. and *AZADIRACHTA INDICA* L.

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ABSTRACT

Sclerotium rolfsii is a polyphagous soil borne fungus infecting more than 500 plant species across the world that is causing vast losses. Although the fungus is soil and seed borne, soil borne inoculum is most vital in infection-causing and syndrome development. Treating soil borne pathogens with fungicides is not reasonable due to very high costs. Environmental hazards are also involved. Therefore, integrated management of pathogens using biological controlling agents is the paramount alternative. Extracts of higher plants have demonstrated a wide range of activity against plant pathogenic organisms. The present research work was carried out to manage the pathogen and disease in vitro by using plant extracts. The antifungal activity of the methanolic leaf extract of two medicinally important indigenous plants, *Alstonia scholaris* and *Azadirachta indica*, against the fungal pathogen *S. rolfsii* was evaluated. In vitro antifungal bioassay was conducted against the *S. rolfsii* using different concentrations (0, 1, 2 and 5) of the methanolic leaf extract of *A. scholaris* and *A. indica* using malt extract (ME) broth as a culture medium. Different concentrations of *A. indica* leaf extract appreciably reduced the fungal biomass growth up to 76% as compared to the control. In the same manner, various concentrations of the leaf extract of *A. scholaris* significantly decreased fungal biomass up to 70% as compared to the control. Higher fungal growth was reduced by a 2% concentration of both plants. The present study concludes that the methanolic extract of *A. indica* has more active antifungal components and can be effectively used to manage phyto-pathogens.

Keywords: Antifungal, *S. rolfsii, A. indica*, Phyto-pathogen, Fungicides.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. In customary medicines, plants were used to treat various serious ailments due to the presence of potent bioactive compounds. In the 20th century, researchers favored the use of commercial drugs over natural medicines for curing several diseases. However, the use of plants and plant based products to cure different infectious diseases is gaining importance as synthetic drugs have numerous side effects on human health (Awal *et al*., 2004; Jiang *et al*., 2006). Naturally occurring products of therapeutic plants are known to be chemically balanced, effectual and have none or very minor side effects in comparison to synthetic medicines (Maffei, 2003).

The potential of medicinal plants in disease anticipation or control has been attributed to most active bio-molecules that

possess antioxidant activity (Ivanova *et al*., 2005). Each part of the medicinal plant contains very active biochemical agents which produce a specific curing physiological action in the therapy of several illnesses in mankind and other organisms. Multi-factorial and health beneficial activity of these plants has been ascribed to the most potent antimicrobial, antioxidant, anticancer, anti-ulcerative and antidiabetic properties. Anti-oxidants have commonly been classified as one of the major health beneficial biological compounds from different species of medicinal plants and are sources of substitute medicines (Daniel, 2005).

These substances are known as secondary metabolites. Secondary metabolites in plants may be alkaloids, steroids, tannins, saponins, ubiquinone, ascorbic acid, phenolic compounds, etc., that are produced and accumulated in defined parts or in all parts of the plant (Tripathi and Tripathi, 2003). A wide range of plants and animals produce antifungal compounds such as proteins, peptides, (Vasilevskii *et al*., 2007) and fungi (Wang and Ng, 2004). In plants, various tissues, including, fruits, bulbs, leaves, seeds, roots and tubers synthesize antifungal proteins and peptides (Lin *et al.*, 2009). The present work is carried out to evaluate the antifungal potential of methanolic extracts of selected medicinal plants (*Azadirachta indica*, *Alstonia scholaris*) against *Sclerotium rolfsii.*

Sclerotium rolfsii Sacc. is a soil-borne phyto-pathogen that generally occurs in the tropics, subtropics, and other temperate areas of the world. *S. rolfsii* has a great host range. Almost 500 species from 100 families are at risk to this fungus. Most common plant hosts are crucifers, legumes and cucurbits (Aycock, 1966). Due to the wide host range, it has been referred to as an almost Omni pathogenic organism (Talukder, 1974). *S. rolfsii* is a facultative parasite that can sustain the continuity of development under unfavorable situations through the production of sclerotia (Ahmed, 1980).

The pathogen damages a great variety of horticultural and agricultural crops including flowers, cereals, vegetables, weeds and forage crops. In some hosts like tomato, peanuts, tobacco and chrysanthemum, the pathogen causes root rot and foot rot (Anahosur, 2001). The fungus induces a wide range of symptoms including seedling blight, seed rots, stem rot, collar rot and wilt in different host plants (Arunasri, 2011).

Chemical fungicides are widely used to manage *S. rolfsii* (Khattabi *et al*., 2001). Use of the synthetic fungicides in agriculture has led to damaging effects on human health and the environment, and developing resistance in pathogens to fungicides. These chemicals also destroy the non-target organisms because of their wide-ranging actions (Haas *et al.*, 2000). Scientists are now searching for natural products, instead of the agro-chemicals, for the management of fungal diseases in plants. One alternative to control these pathogens that is becoming popular is the use of crude and purified plant extracts (Jabeen *et al.*, 2011; Kanwal *et al.*, 2011; Javaid *et al.*, 2012). Plant extracts and their essential oils were found to be effective against a wide range of fungi (Abd-Alla *et al.*, 2001). Plant extracts are biologically degradable (Devlin and Zettel, 1999) and their application in crop protection is a nearly sustainable substitute. Their use decreases environmental adulteration and health risks (Grange and Ahmed, 1988).

Higher plants have been systematically searched for their antifungal activity. Recent studies revealed that plant extracts inhibit the spore germination and mycelial growth in various fungal species

(Singh and Dwivedi, 1987). Alkhail (2005) found inhibitory potential in the aqueous extracts of some plants *viz*., *Carumcarvi, Allium sativum, Cymbopogon proxims, Eugenia caryophyllus* and *Azadirachta indica* had potent antifungal efficacy against *Botrytis cinerea, Rhizoctonia solani* and *Fusarium oxysporum*. Ahmad and Abdelgaleil (2005), reported that the extracts of *Magnolia grandiflora* L. showed effective antifungal activity against *Helminthosporium* spp., *Fusarium culmorium, F. oxysprum, Rhizoctonia solani* and *Alternaria alternatae*. According to Bajwa *et al.* (2001) the aqueous extracts of three Asteraceous allelopathic species showed strong antifungal activity against *Aspergillus niger.*

The genus *Alstonia* belongs to the family Apocynaceae.This family is represented by 89 species in India. Most species of *Alstonia* are tropical, found in several parts of Africa and South Asia (Ramalingam *et al.,* 2010). *Alstonia scholaris* (L.) R. Br. is an evergreen tree of medium to large height--about 40 m (Pratap *et al.*, 2013). Many species of *Alstonia* are rich in alkaloids, flavonoids, steroids and phenols. Alkaloids are the major phyto-constituents (Arulmozhi *et al.,* 2007). *A. scholaris* has great medicinal value and has been used in various traditional systems of medication for the management of various human diseases. Different biological activities have been reported, such as antimicrobial, antioxidant, anti-asthmatic, and anti-inflammatory (Dhar *et al.,* 1977; Patil *et al.,* 1999; Gandhi and Vinayak, 1990; Keawpradub *et al.*, 1999).

Azadirachta indica (L.) is known as Indian lilac or Margos. It belongs to family Meliaceae. Woody and non-woody products of neem are exploited in different ways (Girish and Shankara, 2008). It is a deciduous tree, native to the Indian sub-continent. It is well known in Pakistan and its neighboring countries (Bahuguna, 1997). It has a wide range of climatic, topographic and edaphic factors and is well adapted to stressful conditions. It is referred to as the "Tree for solving global problems" (Abudulai *et al.,* 2013). Many active phyto-constituents of *A. indica* (azadirachtin, salanin, meliantriol, nimbin, etc.) have been identified. The most active component is reported as azadirachtin (Koul, 1990). It is an ancient plant having medicinal importance. Extracts of the leaf and bark contain antioxidant activity (Ghimeray *et al.,* 2009). According to various scientific studies, extracts of this plant are used as an antiviral, anticancer, antifungal, anti-malarial and antibacterial (Rana, 2008). The fruit, seeds, leaves, roots and bark are used for medicinal purposes. This plant contains more than one hundred active ingredients that are used for fungus control (Nahak and Sahu, 2010).

The objective of the present research work was "to assess the antifungal potential of methanolic leaf extracts of *A. indica* and *A. scholaris* against in vitro growth of *S. rolfsii"*.

MATERIALS AND METHODS

The present research work was performed at the Institute of Agricultural Sciences of Punjab University, Lahore (Biofertilizers and Biopestisides Lab).

Collection of Plant Material

Fresh and mature leaves of *Alstonia scholrais* and *Azadirachta indica* were collected during October, 2013 from Govt. Post Graduate College Samanabad, Lahore. Leaves were thoroughly washed and air dried. Dried leaves were crushed and ground to fine powder using a grinder. The powdered leaves were weighed to 200 grams and preserved in plastic jars for further investigation.

Procurement of Fungus

Cultures of *S. rolfsii* were obtained from the National Agriculture Research Council (NARC) Islamabad, Pakistan. Identification of this fungal culture was reconfirmed from the Fungal Culture Bank of Pakistan (FCBP).

Solvent Extraction of Samples by Using Methanol

The crude extracts of the powdered leaves were prepared in methanol by using the maceration method (10 to 15 days). About 200g of each plant leaves powder was extracted with 1000 mL of methanol.

After 15 days, leaves were filtered with muslin cloth then passed through filter paper. Filtrates were evaporated under a vacuum in a rotary thin film evaporator at 45 °C to yield 19.10 and 17.5 g of crude methanolic leaf extract. The dried extracts were preserved in pre-weight beakers until assessed for their antifungal activity.

Bioassay with Methanolic Extract

Methanolic leaf extracts (9 g) of both plants were mixed with 5 mL dimethyl sulfoxide (DMSO) followed by the addition of 15 mL distilled water to prepare 20 mL of stock solution.

In the same way, the control solution was prepared by adding 5 mL DMSO into 15 ml distilled water. Malt extract (ME) broth (3000 mL) was autoclaved in 1000 mL conical flasks and cooled at room temperature. Six concentrations viz. 0, 1, 2, and 5g $100mL^{-1}$ were prepared by adding 0, 1, 2, 3, 4, 5 mL stock solution and 5, 4, 3, 2, 1, 0 mL of control solution, respectively, to each flask to make the total volume of the medium 80 mL. The 80 mL medium of each treatment was divided into four equal portions in 100 mL conical flasks to be used as replicates. For the control treatment, 20 mL of control solution was added to 60 mL of malt extract broth and the medium was divided into four equal replicates (Rauf and Javaid, 2013).

Mycelial discs of *S. rolfsii* were prepared from the tips of fresh fungal culture using a sterilized 5 mm diameter cork borer and shifted to each 100 mL conical flask. Each treatment was replicated four times. Flasks were incubated at 27±2 °C for 4 days. Thereafter, fungal biomass from each flask was filtered and dried at 50 °C to a persistent weight in the hot air oven.

Statistical Analysis

In both laboratory bioassays, standard errors of means of replicates were calculated on the computer software, Microsoft Excel. All the data was evaluated by analysis of variance followed by mean separation through the LSD Test using the computer software Statistics 8.1.

RESULTS

Antifungal Activity of Methanolic Leaf Extract of Azadirachta indica

The effect of different concentrations of the methanolic leaf extract on the biomass of *S. rolfsii* is illustrated in Figure1. All the concentrations of the methanolic leaf extract significantly suppressed the growth of *S. rolfsii*. There was up to a 76% reduction in fungal biomass due to different concentrations of the methanolic leaf extract of *A. indica*. Fungal biomass was reduced using different (1%, 2%, and 5%) concentrations by 73.1%, 76%, 72.5%, 68.2%, 51.6% respectively as compared to control in which 100% growth was seen.

Antifungal Activity of Methanolic Leaf Extract of Alstonia scholaris

The antifungal effect of different concentrations of methanolic leaf extract of *A. scholaris* on the biomass of *S. rolfsii* is illustrated in Figure 2. All the concentrations of the methanolic leaf extract considerably reduced the growth of *S. rolfsii*. There was up to a 70% reduction in fungal biomass due

to different applied concentrations of the methanolic leaf extract of *A. scholaris*. Fungal biomass was reduced using different (1%, 2%, and 5%) concentrations by 68.7%, 70%, 65.3%, 33.6%, 17.3% respectively as compared to control, in which 100% growth was seen.

Effect of *A. indica* leaf extract on *S. rolfsii* biomass

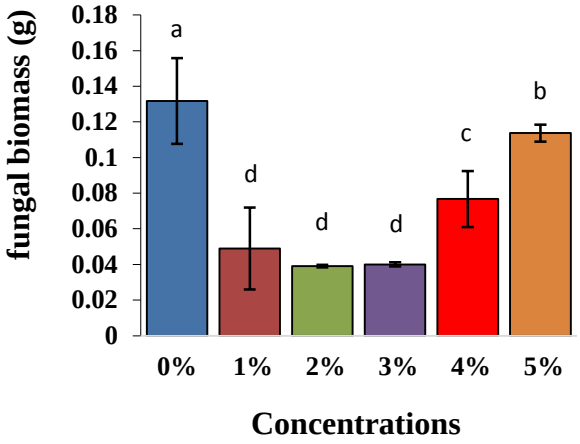

Figure 1: Effect of different concentrations of methanol leaf extract of *Azadirachta indica* on biomass of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as established by LSD Test.

CONCLUSION AND FUTURE PROSPECTS

The present study concludes that both *A. indica* and *A. scholaris* plants have an antifungal potential against *S. rolfsii*. The greater fungal biomass was reduced by using the methanolic leaf extract of *A. indica* because it has more potent antifungal agents than *A. scholaris*. Results showed that *A. indica* has active antifungal components and can be used for the management of tested fungus. On the basis of the present study, it is concluded that the leaf extract of the studied plants were found to be effective against the fungus due to the antifungal potential. These extracts can be further screened for the management of fungal diseases.

Effect of *A. scholaris* leaf extract on *S. rolfsii* biomass

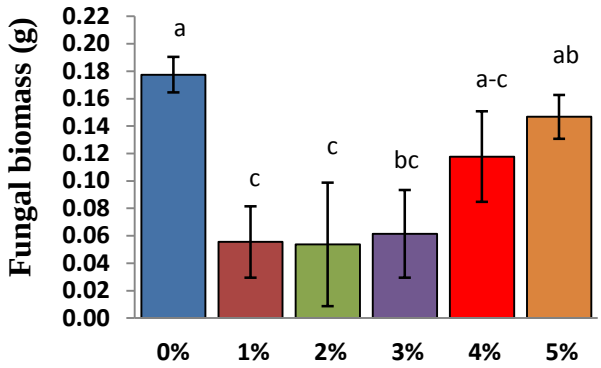

Figure 2: Effect of different concentrations of methanol leaf extract of *Alstonia scholaris* on biomass of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

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