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ANTIFUNGAL ACTIVITY OF METHANOLIC LEAF EXTRACTS OF ALLELOPATHIC TREES AGAINST *SCLEROTIUM ROLFSII* SACC.

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Keywords: Allelopathic trees, Antifungal activity, Leaf extracts, *Sclerotium rolfsii*

Abstract

Laboratory bioassays were carried out to evaluate the antifungal potential of methanolic leaf extracts of three allelopathic trees, namely *Melia azedarach* L., *Mangiferea indica* L. and *Syzygium cumini* (L.) Skeels, against *Sclerotium rolfsii* Sacc., a soil-borne fungal pathogen that causes diseases in more than 500 plant species. Different concentrations of methanolic leaf extracts ranging from 1 to 5% were tested against *in vitro* growth of the fungus using malt extract broth as a growth medium. It was found that fungal biomass was significantly decreased by 36 - 54% due to the effect of different concentration of *M. azedarach* extract. The effect of methanolic leaf extracts of *M. indica* and *S. cumini* were generally insignificant. Methanolic leaf extract of *M. azedarach* was subjected to GC-MS analysis and nine compounds namely phytol (36.041%); 9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z)- (34.78%); hexadecanoic acid, methyl ester (12.307%); cyclopropaneoctanoic acid, 2-[2-[(ethylcyclopropyl) methyl] cyclopropyl] methyl-, methyl ester (2.771%); vitamin E (2.677%); 7-methyl-Z-tetradecen-1-ol acetate (2.481%); hexanedioic acid, dimethyl ester (2.284%) and hexanedioic acid, dimethyl ester (1.336%); 2-heptanol (0.577%) were identified.

Introduction

The omnivorous soil-borne fungal pathogen *Sclerotium rolfsii* Sacc. causes disease on a wide range of agricultural and horticultural crops, including over 270 genera in USA alone (Fichtner 2016). Important agricultural crops attacked by this fungus include wheat (*Triticum vulgare* Vill.), pumpkin (*Cucurbita pepo* L.), sweet potato [*Ipomea batatas* (L.) Lam.], corn (*Zea mays* L.), peanut (*Arachis hypogea* L.), chickpea (*Cicer arietinum* L.) and peppers (*Capsicum annuum* L.). Likewise, horticultural crops susceptible to this fungus are included in the genera *Chrysanthemum, Narcissus*, *Zinnia, Iris* and *Lilium* (Farr *et al.* 1989, Javaid and Iqbal 2014, Javaid and Khan 2016). Great economic losses in different crops associated with *S. rolfsii* infection are due to prolific growth of this fungus, its wide host range, and ability to produce persistent sclerotia (Aycock 1966).

Control of plant diseases caused by *S. rolfsii* and other fungi, still depends mainly on the use of synthetic fungicides (Khan and Javaid 2015). However, development of fungicide-resistant pathogens as well as health and environmental concerns, have accelerated the search for alternative strategies to control plant diseases (Knight *et al*. 1997). One such approach involves the use of natural plant products as fungicides either in their crude forms or as purified compounds (Jabeen *et al.* 2011, Javaid *et al.* 2015). Iqbal and Javaid (2012) documented the substantial fungicidal potential of methanolic extracts of various parts of *Coronopus didymus* against *S. rolfsii.* Moreover, amending soil with dry biomass of *C. didymus* considerably decreased collar rot disease in chilies caused by *C. didymus* (Javaid and Iqbal 2014). Similar effects of methanolic leaf extract of *Melia azedarach* L. against *S. rolfsii* and leaf dry biomass as soil amendment against collar rot disease of chickpea have also been reported (Khan and Javaid 2013, Javaid and Khan 2016). There are reports that many allelopathic plants possess antifungal activity against plant pathogens (Javaid and Shoaib 2013). The contemporary investigation was, therefore, conducted to investigate the antifungal potential of three allelopathic tree species against *S. rolfsii*.

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Materials and Methods

Leaves of three allelopathic trees viz., *Melia azedarach, Mangiferea indica* and *Syzygium cumini* were collected from University of the Punjab Lahore, Pakistan. Oven dried leaves (at 45°C) were crushed. Each thoroughly crushed leaf sample (200 g) was soaked in 2 l methanol for 2 weeks. Thereafter, the soaked materials were filtered through cheese cloth and then Whatman filter papers. Methanol was evaporated on a rotary evaporator at 45°C and 18.28, 22.5 and 23.12 g crude extracts of *M. azedarach, S. cumini* and *M. indica,* respectively, were obtained (Javaid and Iqbal 2014).

Initially the stock solution of solid methanolic extract was made by dissolving 9 g of each plant species extract in 5 ml dimethyl sulphoxide (DMSO) separately. Final volume of stock solution (15 ml) was prepared with autoclaved distilled water. Same quantity of DMSO was mixed with 10 ml distilled water to prepare control solution. Autoclaved malt extract broth (55 ml) was mixed with 1, 2, 3, 4, 5 ml of stock solutions and 4, 3, 2, 1 and 0 ml of control solution to form final concentrations of 1, 2, 3, 4, and 5% and volume 60 ml. Prepared solution of each of five different concentrations was equally divided into 15 ml in 250 ml flask to serve as replicates. In order to make negative control treatment, 5 ml control solution was mixed with 55 ml autoclaved malt extract broth to keep the same amount of DMSO in control and experimental treatments. The fungicidal activity of the plant extract was determined by inoculating 5 mm (diameter) mycelial discs of *S. rolfsii* in each flask under aseptic conditions. After an incubation period of 10 days at 27°C, fungal biomass was filtered, oven dried (at 60°C) and weighed on an electric balance (Javaid *et al.* 2015). Statistical software Statistics 8.1 for Windows was used to analyze the data. All results were presented as mean ± standard errors. Data regarding fungal biomass were analyzed by one way ANOVA followed by separation of treatment means by Tukey's HSD test at 5% level of significance.

GC-MS analysis of crude methanolic leaf extract of *M. azedarach* was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA)

Results and Discussion

Methanolic extract of *M. azedarach* showed the best antifungal activity against *S. rolfsii* causing 36-54% decrease in fungal biomass. Fungal biomass was gradually decreased with an increase in the extract concentration (Fig. 1A and 2). Linear relationship with $R^2 = 0.7508$ was existed for the effect of concentrations of methanolic extract of *M. azedarach* on fungal biomass (Fig. 3A). Earlier, Jabeen *et al.* (2011) reported that a 5% alcoholic extract of *M. azedarach* leaves reduced growth of *Ascochyta rabiei* by 57%. They attributed antifungal activity of the leaf extract to the presence of β-amyrin and other compounds such as ursolic acid and 3,5 dimethoxybenzoic acid. Similarly, Carpinella *et al.* (2003) reported that ethanolic extract *M. azedarach* leaves showed fungicidal activity against *Sclerotium sclerotinia, Fusarium solani, F. oxysporum, F. verticillioides,* and *Aspergillus flavus.* Methanolic leaf extract of this tree also found highly effective in controlling growth of *Macrophoina phaseolina* (Javaid and Rehman 2011).

Methanolic extracts of the other two allelopathic tree species viz. *M. indica* and *S. cumini* showed comparatively lower antifungal activity than the extract of *M. azedarach.* Lower concentrations of methanolic leaf extract of *M. indica* viz. 1% and 2% significantly reduced fungal biomass by 32% and 43%, respectively. In contrast, the effect of higher concentrations of this extract was insignificant against the fungal biomass production (Fig. 1B and 2). Methanolic leaf extract of *S. cumini* showed the least antifungal activity where fungal biomass was gradually increased with increase in extract concentration. The highest extract concentration resulted in 42% more fungal biomass as compared to control (Fig. 1C and 2). By contrast, earlier studies showed

that leaf extract of *S. cumini* significantly suppressed growth of *M. phaseolina* and *A. rabiei* (Jabeen and Javaid 2008, Javaid and Rehman 2011). It indicates that leaf extract of *S. cumini* has selective antifungal activity against different fungi.

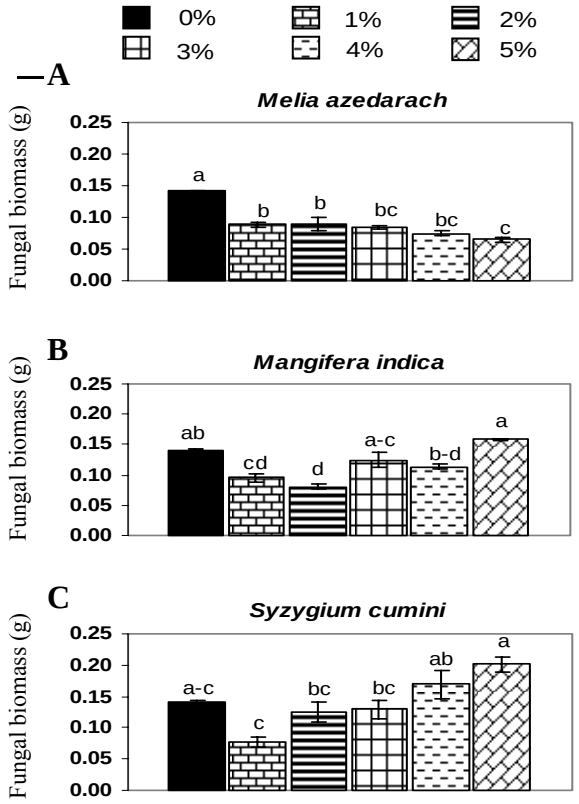

Fig. 1. Effect of different concentrations of methanolic leaf extract of allelopathic trees on the growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by Tukey's HSD test.

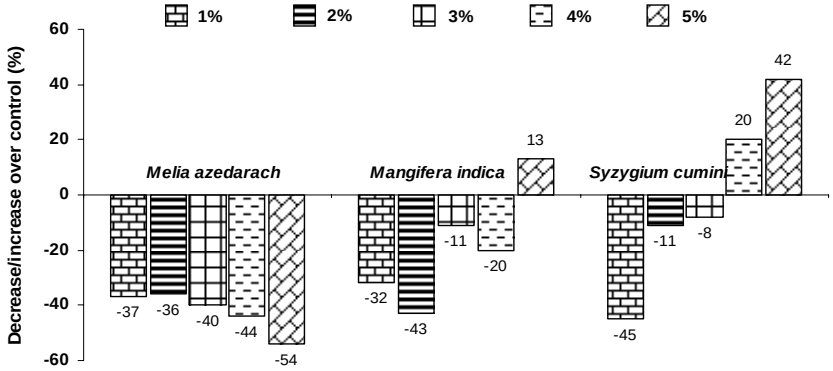

Fig. 2. Percentage decrease/increase in biomass of *Sclerotium rolfsii* due to different concentrations of methanol leaf extract of allelopathic trees over control.

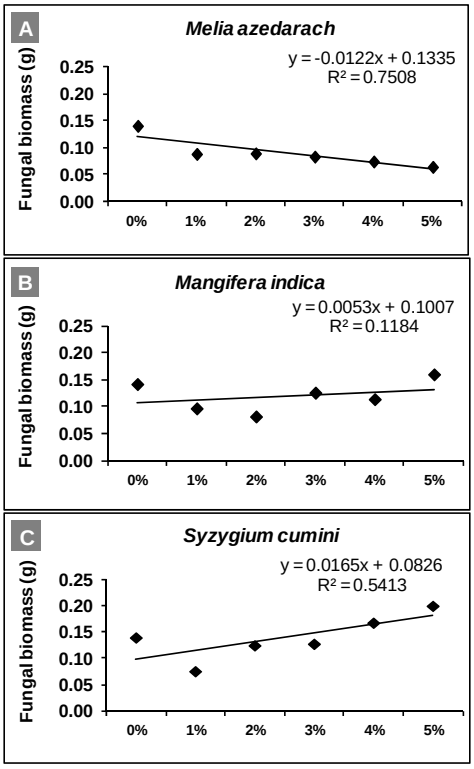

Fig. 3. Relationship between different concentrations of methanolic leaf extract of allelopathic trees and biomass of *Sclerotium rolfsii.*

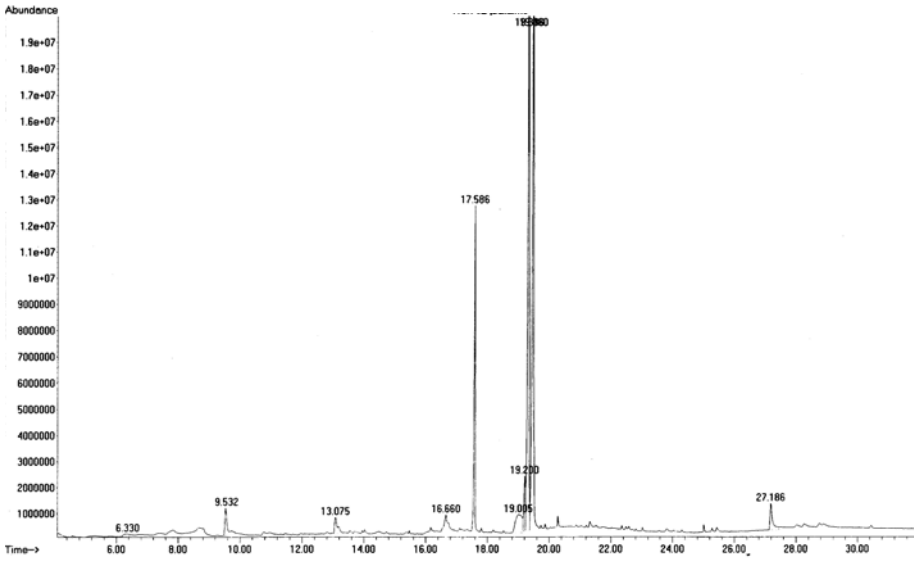

Fig. 4. GC-MS analysis of methanolic leaf extract of *Melia azedarach.*

Table 1. Compounds identified from methanolic leaf extract of *Melia azedarach* through GC-MS analysis.

Comp. No.	Names of compounds	Group	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	2-Heptanol	Alcohol	$C_7H_{16}O_2$	116	6.330	0.6
2	Hexanedioic acid, dimethyl ester	Fatty acid	$C_8H_{14}O_4$	174	9.532	2.3
3	1, 4-Benzenedicarboxylic acid	Ethyl methyl ester	$C_{10}H_{10}O_4$	194	13.075	1.3
4	7-Methyl-Z-tetradecen-1-ol acetate	Acetate compound	$C_{17}H_{32}O_2$	268	16.660	2.5
5	Hexadecanoic acid, methyl ester	Oxygenated diterpenes	$C_{17}H_{34}O_2$	270	17.586	12.3
6	Cyclopropaneoctanoic acid, 2-[[2-[(ethylcyclopropyl) methyl] cyclopropyl] methyl-, methyl ester	Fatty acid	$C_{22}H_{38}O_2$	334	19.200	2.8
7	9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	Linolenic acid	$C_{19}H_{32}O_2$	292	19.336	34.8
8	Phytol	Oxygenated diterpenes	$C_{20}H_{40}O$	296	19.480	36.0
9	Vitamin E	Vitamin compound	$C_{29}H_{50}O_2$	430	27.186	2.7

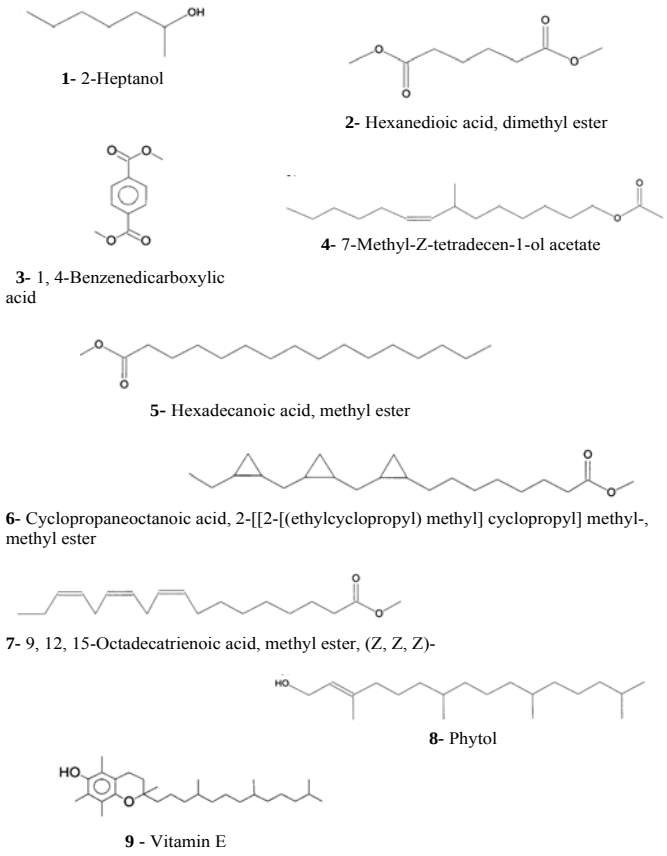

Fig. 5. Structures of compounds identified in methanolic leaf extract of *Melia azedarach* through GC-MS analysis.

Methanolic leaf extract of *M. azedarach* showed the best antifungal activity, therefore, this extract was subjected to GC-MS analysis. GC-MS chromatogram of this extract revealed the presence of nine compounds (Fig. 4). The active principles with their molecular formula, molecular weight, retention time and peak area are shown in Table 1. The identified constituents were phytol (36%); 9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z)- (34.8%); hexadecanoic acid, methyl ester (12.3%); cyclopropaneoctanoic acid, 2-[2-[(ethylcyclopropyl) methyl] cyclopropyl] methyl-, methyl ester (2.8%); vitamin E (2.7%); 7-methyl-Z-tetradecen-1-ol acetate (2.5%); hexanedioic acid, dimethyl ester (2.3%); and hexanedioic acid, dimethyl ester (1.3%); 2-heptanol (0.6%). Structures of the identified phytochemicals are presented in Fig. 5. Phytol is important member of branched chain unsaturated terpene and is well-known for its antifungal activity (Omoruyi *et al*. 2014). The antimicrobial activity of phytol is attributed to its antioxidant and antiradical potential (Pejin *et al*. 2014). Literature is scanty about the antimicrobial activity of 9, 12, 15-octadecatrienoic acid, methyl ester, (Z, Z, Z) and hexadecanoic acid, methyl ester, so far their presence has been detected in many medicinal plants including *Melia* sp. (Gopalakrishnan and Udayakumar 2014, Jahirhussain *et al*. 2015). Vitamin is powerful, fat-soluble antioxidant comprised of four different tocopherols and four different tocotrienols with antifungal as well as antiviral potential (Narayanamoorthi *et al.* 2015). Cyclopropaneoctanoic acid, 2-[[2-[(ethylcyclopropyl) methyl] cyclopropyl] methyl-, methyl ester is fatty acid and 7-methyl-Z-tetradecen-1-ol acetate is acetate compound, and their antifungal activity is not known (Gnanavel and Saral 2013, Omoruyi *et al*. 2014). The presence of hexanedioic acid, dimethyl ester (Omoruyi *et al.* 2014), 1, 4-benzenedicarboxylic acid (Ezhilan and Neelamegam, 2012) and 2-Heptanol (Swamy *et al*. 2015) has been confirmed in plants of antimicrobial nature.

It was concluded from the current investigation that the methanolic leaf extract of *M. azedarach* possess antifungal activity against *S. rolfsii.*

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