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GC-MS ANALYSIS AND ANTIFUNGAL ACTIVITY OF METHANOLIC ROOT EXTRACT OF *Chenopodium album* AGAINST *Sclerotium rolfsii*

*Análise por Cromatografia Gasosa com Espectrômetro de Massa (GC-MS) e Atividade Antifúngica de Extrato Metanólico de Raiz de **Chenopodium album** Diante de **Sclerotium rolfsii***

ABSTRACT - *Sclerotium rolfsii* is a soil-borne fungal plant pathogen that causes diseases in more than 500 plant species. Chemical fungicides used to control this disease cause environmental pollution, therefore, plant derived compounds can be used as alternative to synthetic fungicides to reduce environmental pollution. *Chenopodium album* is a weed of family Chenopodiaceae that is used as food and also has medicinal importance. In the present study, antifungal activity of methanolic root extract of *C. album* was evaluated against *S. rolfsii* using six concentrations viz. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g 100 mL⁻¹ amended in malt extract as growth medium. All the root extract concentrations significantly reduced fungal biomass by 15-58% over control. Gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic root extract of *C. album* was performed. Six compounds were identified in methanolic root extract through GC-MS analysis. The most abundant compound was 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (58.56%) followed by 9-octadecenoic acid (Z)-, methyl ester (12.75%) and 9-octadecenoic acid (Z)-, methyl ester (10.27%), which might be responsible for antifungal activity of methanolic root extract of *C. album*.

Keywords: biological control, natural compounds, reduction use of agrochemical.

RESUMO - *Sclerotium rolfsii* é um patógeno vegetal fungoso do solo que causa doenças em mais de 500 espécies de plantas. Os fungicidas químicos utilizados para controlar essa doença causam poluição ambiental. Portanto, compostos derivados de plantas podem ser usados como alternativa aos fungicidas sintéticos para reduzir a poluição ambiental. *Chenopodium album* é uma planta daninha da família Chenopodiaceae que é utilizada como alimento e também tem importância medicinal. No presente estudo, a atividade antifúngica do extrato metanólico de raiz *C. album* foi avaliada diante de *S. rolfsii* utilizando seis concentrações, ou seja, 0,5, 1,0, 1,5, 2,0, 2,5 e 3,0 g 100 mL⁻¹, alteradas em extrato de malte como meio de crescimento. Todas as concentrações de extrato de raiz reduziram significativamente a biomassa fúngica em 15-58% em relação ao controle. Foi feita a análise por cromatografia gasosa com espectrômetro de massa (GC-MS) do extrato metanólico de raiz de *C. album*. Seis compostos foram identificados em extrato metanólico de raiz por meio de análise por GC-MS. O composto mais abundante foi o ácido 1,2-benzenodicarboxílico, mono (2-etil-hexil) éster (58,56%), seguido de ácido 9-octadecenoico (Z)-, éster metílico (12,75%), e ácido

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9-octadecenoico (Z)-, éster metílico (10,27%), o que pode ser responsável pela atividade antifúngica de extrato metanólico de raiz de C. album.

Palavras-chave: fungicida natural, controle biológico, redução do uso de agroquímico.

INTRODUCTION

Sclerotium rolfsii Sacc., is one of the oldest known plant pathogenic fungus that was reported for the first time in 1892 on tomatoes in Florida (Aycock, 1966). Its occurrence in warm temperate and subtropical regions of the world has increased its host range (Hameeda et al., 2010). Therefore, over 500 plant species belonging to different groups are being attacked by this fungus. The extensive host range, abundant growth, and ability to produce persistent sclerotia make it a robust fungus that has been responsible for causing massive economic losses and included it in the list of fungi that are difficult to manage through physical and cultural practices (Farooq et al., 2011; Eslami et al., 2014). In chickpea (*Cicer arietinum*), the collar rot disease caused by *S. rolfsii* is responsible for 55-95% mortality of seedlings (Gurha and Dubey, 1982). Fungicides are normally utilized to minimize the losses (Azhar et al., 2006; Lakpale et al., 2007).

Increasing awareness of public concern regarding the deteriorating impact of the indiscriminate use of chemicals on the environment has led the researchers to seek for alternative disease management strategies for sustainability in agriculture (Rauf and Javaid, 2013). The use of natural compounds from plants could fulfill the criteria of a sustainable agriculture. Many recent studies have shown that plant crude extracts and purified compounds have great potential to be used as antifungal agents. Crude extracts, pure compounds or soil amendment with dry biomass of mango (*Mangifera indica*), *Datura metel*, *Coronopus didymus*, *Imperata cylindrica*, *Withania somnifera* and radish (*Raphanus sativus*) have been proved very useful in the management of many soil-borne plant pathogenic fungi namely *Macrophomina phaseolina*, *S. rolfsii* and *Fusarium oxysporum* (Kanwal et al., 2010; Javaid and Saddique, 2011; Iqbal and Javaid, 2012; Javaid and Akhtar, 2015; Javaid and Bashir, 2015; Javaid et al., 2015).

Chenopodium album is a summer annual, fast-growing weed and is commonly used for food and it also possesses medicinal values (Singh et al., 2011). The tender shoots are eaten raw in salads or with curd, cooked as vegetables and are also used as fodder (Karwani and Sisodia, 2015). It has been found to have an antipruritic, antinociceptic (Kumar et al., 2007), and sperm immobilizing activity (Pande and Anupam, 2010). Its leaves are useful in the cure of influenza, anorexia, cough, dysentery, pneumonia, diarrhea, piles and typhoid (Cheryl et al., 2006; Singh et al., 2011). Besides, the antifungal activity of *C. album* has been documented against some plant pathogenic fungi. Singh's (2005) results have revealed a strong toxic effect of leaves of *C. album* against *S. rolfsii*. Javaid and Amin (2009) have found a considerable reduction of up to 96% in growth of *M. phaseolina* due to leaf, stem, root and inflorescence extracts of *C. album*. Jabeen et al. (2014) have found a significant antifungal activity of leaf extract of *C. album* against *Ascochyta rabiei*, responsible for chickpea blight. Recently, Javaid and Rauf (2015) findings have explored the potential of soil amendment with dry leaf biomass of *C. album* against the management of *Fusarium oxysporum* f. sp. *cepae*. However, studies regarding antifungal activity of *C. album* root extract against *S. rolfsii* are lacking. Therefore, the aim of the present study was to investigate the antifungal potential of methanolic root extract of *C. album* against *S. rolfsii*.

MATERIALS AND METHODS

Isolation of pathogen

Chickpea (*Cicer arietinum*) plants infected with collar rot disease were collected from the field of district Sahiwal of province Punjab, Pakistan, during 2014. Infected portions of a basal plant part were surface sterilized with a solution of sodium hypochlorite (1%) for 1 min and then rinsed several times with sterilized distilled water. These pieces were plated on 2% malt extract agar (MEA) and incubated at 24 °C for 7 days. After that, culture purification was done by

sub-culturing of an isolated pathogenic fungus using the single spore transfer technique. The isolated fungal species was identified as *S. rolfsii* on the basis of morphological characters (Watanabe, 2002). In order to check the pathogenicity of the isolated fungus, its inoculum was mixed in the pot soil and chickpea seeds were sown in these pots. The symptoms of collar rot appeared on chickpea seedling, confirming the pathogenicity of the isolated fungal species. Pure culture of the fungus was preserved at 4 °C for further use.

Preparations of methanolic extracts

Roots of *C. album* were collected from different vicinities of Lahore, Pakistan. The collected material was thoroughly washed under tap water and then dried at 45 °C using an electric oven. The dried material was ground to a fine powder. Powdered roots (200 g) were soaked in methanol (2 L) in a plastic jar for 7 days at room temperature. Later on, the soaked material was filtered and a rotary evaporator was used to evaporate the filtered extract at 45 °C under vacuum.

Antifungal assays

Bioassay experiments were done using methanolic extract of *C. album* root *in vitro*. Nine grams of methanolic root extract were dissolved in 5 mL dimethyl sulfoxide (DMSO) followed by the addition of sterilized distilled water to prepare 15 mL of stock solution. In a similar way, 5 mL DMSO were mixed in 10 mL distilled water to prepare a control solution. Malt extract broth medium (55 mL) was prepared in 250 mL conical flasks and then autoclaved at 121 °C for 30 min. After autoclaving, an antibacterial capsule (chloromycetin at 250 mg L⁻¹) was added in each media flask to avoid bacterial contamination. Six concentrations viz. 0, 10, 20, 30, 40, and 50 mg mL⁻¹ were prepared by adding 0, 1, 2, 3, 4, and 5 mL stock solution and 5, 4, 3, 2, and 1 mL control solution, respectively, to 55 mL growth medium to make the total volume of the medium 60 mL in each flask. Four replicates were made by allocating equal parts of growth medium in each replicate, i.e., 15 mL. A mycelial disc (5 mm) of *S. rolfsii* was transferred in each flask by a sterile borer and then incubated at 27 °C for 10 days. Afterwards, filtration of fungal mass was done using pre-weighed filter papers (Whatman No.1). Finally, the filtered material was dried in an electric oven at 70 °C to obtain a fungal dry biomass (Rauf and Javaid, 2013).

GC-MS analysis

GC-MS (gas chromatography-mass spectroscopy) analysis was done on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO 6859, USA), consisting of a 30 m length and 0.25 mm diameter capillary tube consisting of 95% dimethyl polysiloxane and a second service provider gas called helium also used with flow amount of 0.5 mL min⁻¹. The obtained peak areas were measured by Turbo-Mass-OCPTVS-Demo SPL software.

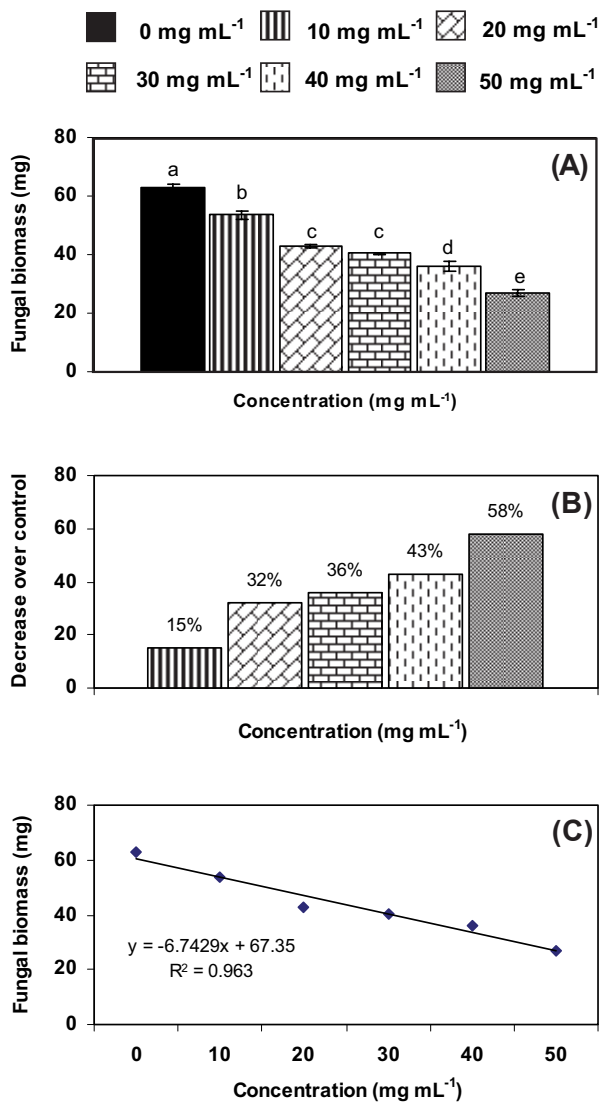
Statistical analysis

All the data were subjected to one-way ANOVA. Means were compared by applying Tukey's HSD Test at 5% level of significance using computer software Statistics 8.1. Relationship between fungal biomass and extract concentration was computed using MS Excel.

RESULTS AND DISCUSSION

Data about the effect of methanolic root extract on biomass of *S. rolfsii* is presented in Figure 1A-C. Different concentrations of methanolic root extract of *C. album* (10 to 50 mg mL⁻¹) significantly reduced fungal biomass by 15-58% over the negative control treatment. A linear correlation between extract concentration and biomass of *S. rolfsii* was recorded with R² = 0.963.

Six compounds belonging to fatty acid methyl esters, aromatic dicarboxylic ester and ketone group were identified in methanolic root extract of *C. album* (Figure 2, Table 1 and 2). These compounds were 2(3H)-furanone, dihydro-4,4-dimethyl (12.75%); 6-methylenebicyclo(3.2.0)hept-3-en-2-one (7.21%); hexadecanoic acid, methyl ester (5.44%); 9,12-octadecadienoic acid (Z,Z)-,



Values with different letters at their top show a significant difference ($P \leq 0.05$), as determined by Tukey's HSD Test. Vertical bars show standard errors of means of three replicates.

Figure 1 - (A) effect of different concentrations of methanol root extract of *Chenopodium album* on biomass of *Sclerotium rolfsii*, (B) percentage reduction in fungal biomass due to different concentrations of methanolic extract over control, C: relationship between extract concentration and fungal biomass.

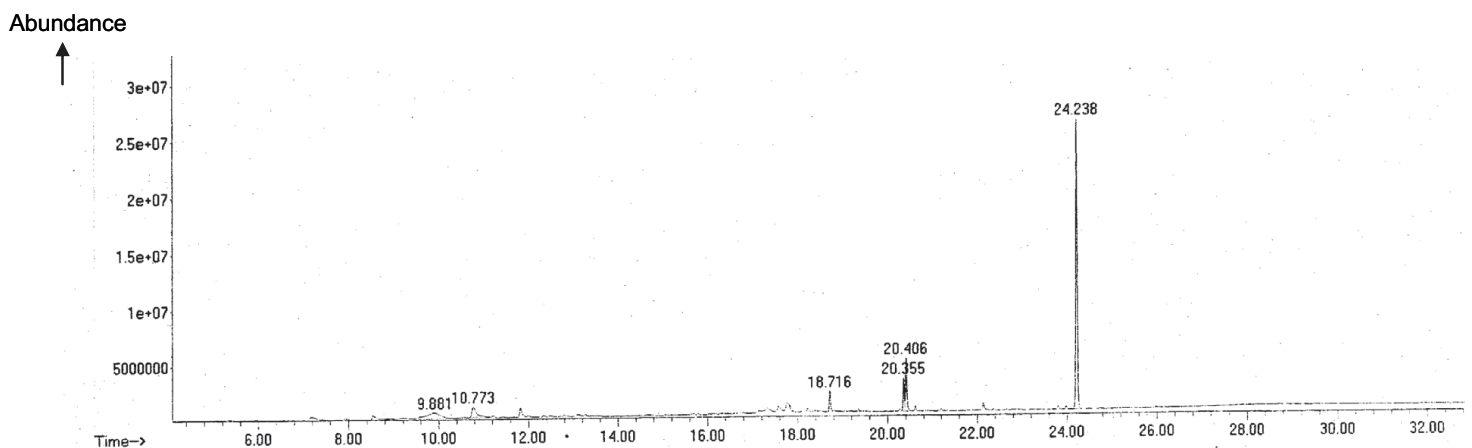


Figure 2 - GC-MS chromatogram of methanolic root extract of *Chenopodium album*.

Table 1 - Compounds identified from methanolic root extract of *Chenopodium album* through GC-MS analysis

Comp. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	2(3H)-Furanone, dihydro-4,4-dimethyl	C ₆ H ₁₀ O ₂	114	9.881	12.75
2	6-Methylenebicyclo(3.2.0)hept-3-en-2-one	C ₈ H ₈ O	120	10.773	7.21
3	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	18.716	5.44
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294	20.355	5.81
5	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296	20.406	10.27
6	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C ₁₆ H ₂₂ O ₄	278	24.238	58.50

Table 2 - Nature and properties of compounds identified from methanolic root extract of *Chenopodium album* through GC-MS analysis

Comp. No.	Names of compounds	Nature	Property	Reference
1	2(3H)-Furanone, dihydro-4,4-dimethyl	Ketone	No activity reported	
2	6-Methylenebicyclo(3.2.0)hept-3-en-2-one	ketone	Bacteriostatic, fungistatic, antiparasitic	Hussein et al. (2016)
3	Hexadecanoic acid, methyl ester	Palmitic acid methyl ester (fatty acid methyl ester)	Antifungal, Antioxidant, Antibacterial	Agoramoorthy et al. (2007)
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid methyl ester (fatty acid methyl ester)	Antibacterial	Lima et al. (2011)
5	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid methyl ester (fatty acid methyl ester)	Antimicrobial, Nematicidal	Chandrasekharan et al. (2008); Lima et al. (2011)
6	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	Aromatic dicarboxylic ester	Antimicrobial, Cytotoxicity, Antioxidant, Anti-inflammatory, Antiviral	Govindappa et al. (2014); Krishnan et al. (2014)

methyl ester (5.81%); 9-octadecenoic acid (Z)-, methyl ester (10.27%); and 1,8-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (58.50%). Names of these compounds along with their molecular formulas, molecular weights, retention time, and peak areas are presented in Table 1. Structures of these compounds are given in Figure 3. Three out of the six identified compounds (21.52%) belonged to the fatty acid methyl ester group, namely linoleic acid methyl ester, oleic acid methyl ester, and palmitic acid methyl ester (Tables 1 and 2). Fatty acid methyl esters are known to possess antifungal properties (Agoramoorthy et al., 2007). Chandrasekharan et al. (2008) have evaluated the antimicrobial activity of four halophytes of the Chenopodiaceae family and found that fatty acid methyl esters extract of *Salicornia brachiata* possessed the highest antifungal activity. Lima et al. (2011) have reported that fatty acid methyl esters of seeds of *Annona cornifolia*, mainly oleic acid methyl ester (51.5%), linoleic acid methyl ester (19.1%), and palmitic acid methyl ester (16.9%) as in the present study, have inhibited the growth of 12 strains of a clinical pathogenic fungus *Paracoccidioides brasiliensis*. According to Agoramoorthy et al. (2007), fatty acid methyl ester extract of *Excoecaria agallocha*, rich in palmitic acid methyl ester (56.02%), suppressed growth of four species of *Candida* namely *C. albicans*, *C. krusei*, *C. tropicalis* and *C. parapsilosis*. Similarly, 6-methylenebicyclo (3.2.0)hept-3-en-2-one is also known to exhibit a fungistatic activity (Hussein et al., 2016).

The most abundant compound 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester has been previously identified from marine *Streptomyces* sp., *Alternaria* sp. and metabolites of a

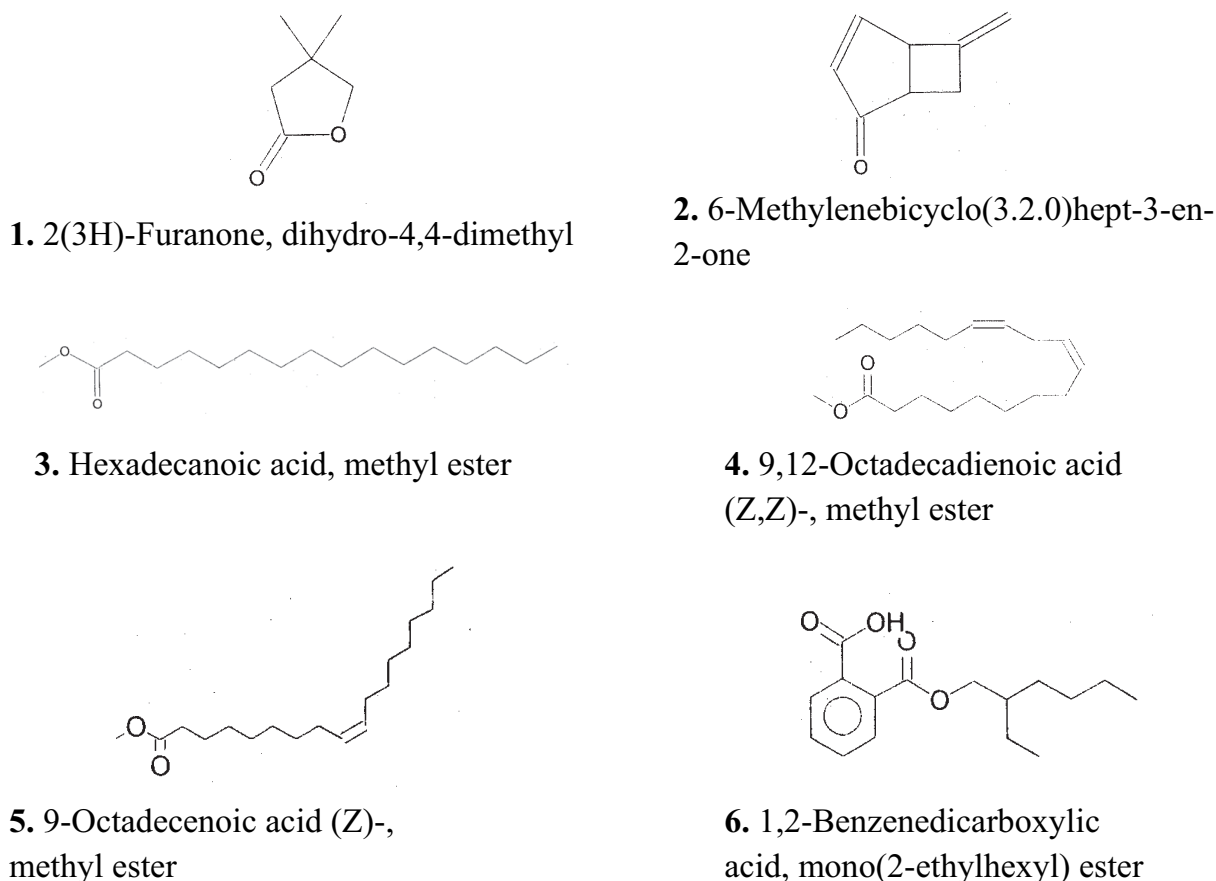


Figure 3 - Structures of compounds identified in methanolic root extract of *Chenopodium album* through GC-MS analysis.

cyanobacterium *Oscillatoria terebriformis*. This compound is known to have a number of biological properties, including cytotoxicity, antioxidant, anti-inflammatory, antimicrobial, and antiviral activity (Govindappa et al., 2014; Krishnan et al., 2014; Mukund et al., 2014). Since it is a biologically active compound and also found as the major compound in the present study (58.05%), therefore, there is possibility that it might have had a role in the antifungal activity of methanolic root extract of *C. album* in the present study.

The present study concludes that *S. rolfisii* can be managed by the methanolic root extract of *C. album*. The antifungal activity of this extract is possibly because of various fatty acid methyl esters and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester.

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