Mycopath (2013) 11(1)**:** 9-13 **Research Article**

Antifungal activity of *Melia azedarach* L. fruit extract against *Sclerotium rolfsii*, the cause of collar rot disease of chickpea

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Abstract

Laboratory bioassays were carried out to evaluate the antifungal potential of methanolic fruit extract of *Melia azedarach* L. and its various fractions against *Sclerotium rolfsii,* the cause of collar rot of chickpea (*Cicer arietinum* L.). Different concentrations of methanolic fruit extract (0.5, 1.0, …, 3.5%) were prepared and their effect against the fungal pathogen was tested using malt extract broth in 100-mL conical flasks. All the tested concentrations of the methanolic extract significantly reduced fungal biomass by 81–84%. Methanolic extract was partitioned using *n*-hexane, ethyl acetate and *n*-butanol. The effect of different isolated fractions was tested against the pathogen in 10-mL volume test tubes. A concentration of 3.125 mg mL^{-1} of different fractions significantly decreased fungal biomass by 41–65%. The present study concludes that methanolic fruit extract of *M. azedarach* and its various fractions possess antifungal activity and can be used for management of *S. rolfsii.*

Keywords: Chickpea, collar rot disease, fruit extract, *Melia azedarach, Sclerotium rolfsii.*

Introduction

Chickpea (*Cicer arietinum* L.), a cool season crop of family Fabaceae, is grown worldwide as a major source of food (Doyle and Luckow, 2003). It is an important legume of tropics and dry land areas all over the world (Agarwal *et al.*, 2012). Seed is the main consumable part of the plant (Hossain et al., 2010). More than 50 countries are producing chickpea. South-Eastern Asia is producing 79% chickpea globally. Pakistan, Australia, Iran, USA, Canada, Turkey, Mexico, Ethiopia and Myanmar are the other most important chickpea producing countries. Worldwide total area under chickpea cultivation was 12.0 million ha, with 10.9 million MT productions and the average yield was 913 kg ha^{-1}. Widely growth of chickpea is in South Asia and the Mediterranean area of the world (Sheehy and Sharma, 2012). In spite of active breeding programs, the average yield of chickpea did not increase considerably over the years. During the previous era (1993–2011), worldwide annual production rate of chickpea has been low (0.007%) and average yield was nearly constant (Charrondiere *et al.,* 2011).

Sclerotium rolfsii is a soil-borne fungal pathogen that causes diseases in a wide range of horticultural and agricultural crop plants. It has over 500 species hosts in 100 plant families (Hegde *et al.*, 2010). Diseases caused by *S. rolfsii* are often assigned as southern blight. The fungus forms sclerotia that can remain in the soil for several years and function as overwintering structures as well as primary inoculum for the disease. Collar rot disease caused by *Sclerotium rolfsii* Sacc., is among the main biotic factors responsible for low chickpea production under conducive conditions (Hussain *et al.,* 2006; Maurya *et al.,* 2008). This disease can cause about 55-95% chickpea seedlings mortality (Gurha and Dubey, 1982). Good soil moisture, low organic matter and high soil temperature of 25-30 °C favour the disease (Mathur and Sinha, 1968). Use of chemical fungicides is an effective mean to control *S. rolfsii* (Conway *et al.,* 1996; Khattabi *et al.,* 2001). However, use of these chemical pesticides has a number of disadvantages because of their adverse effect on biotic and abiotic environment. These chemicals have broad spectrum activity and thus may destroy non-target organisms too (Haas *et al.,* 2000). Due to ill effects of synthetic agro-chemicals, scientists are now in search of alternatives from natural environment for management of plant diseases. Among these alternatives, use of crude and purified plant products for controlling plant pathogens are gaining much importance (Jabeen *et al.,* 2011; Kanwal *et al.,* 2011; Javaid *et al.,* 2012). The present study was conducted to investigate the antifungal activity of methanolic fruit extract of *Melia azedarach* for the management of *S. rolfsii.*

Materials and Methods

Bioassays with methanolic extract

Dried fruits of *M. azedarach* were crushed mechanically and 2 kg of this material was soaked in 6 L methanol at room temperature for two weeks. After filtration, methanol was evaporated under vacuum in a rotary evaporator to obtain 190 g of crude extract (Iqbal and Javaid, 2012). Crude extract (11.2 g) was dissolved in 6 mL dimethyl sulphoxide (DMSO) followed by addition of sterilized distilled water to prepare 14 mL of stock solution. In a similar way, 6 mL DMSO was dissolved in 8 mL sterilized distilled water to prepare control solution. Malt extract broth (76.5 mL) was autoclaved in 250 mL conical flasks and cooled at room temperature. Seven concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 g 100 mL^{-1}) were prepared by adding appropriate quantities of stock and control solutions to each flask to make the total volume of the medium 80 mL that was divided into four equal parts. Control treatment received 3.5 mL of control solution in 76.5 mL of malt extract broth to have the same quantity of DMSO in control and experimental treatments. Mycelial discs of *S. rolfsii* (5 mm diameter) were transferred to each 100-mL conical flask containing 20 mL of the medium. Each treatment was replicated four times. Flasks were incubated at 25±2 °C for 14 days followed by filtration of fungal biomass on pre-weighed filter papers (Javaid *et al.*, 2012).

Bioassay with different fractions of methanolic extracts

Methanolic fruit extract was subjected to fractionation using *n*-hexane (3 × 500 mL), ethyl acetate (400 mL) and *n*-butanol (400 mL). Solvents were evaporated on a rotary evaporator and *n*-hexane, ethyl acetate, *n*-butanol and aqueous fractions were obtained. The isolated fractions were tested *in vitro* against *S. rolfsii*. An amount of 1.2 g of each fraction was dissolved in 1 mL DMSO and 5 mL of malt extract broth was added in each beaker to get a concentration of 200 mg mL^{-1}. Half the amount of this medium was used for bioassays and the other half was serially double diluted by addition of malt extract broth to prepare 100, 50, 25, 12.5, 6.25, and 3.125 mg mL^{-1} concentrations. A series of control treatment was prepared without adding extracts but having the same amount of DMSO as was present in different extract treatments. Bioassays were conducted in 10 mL volume glass test tubes each containing 1 mL of medium. Test tubes were inoculated with 5 µL of *S. rolfsii* spore suspension aseptically. Each treatment was replicated thrice. Test tubes were incubated in an incubator at 25±2 °C for 14 days. Thereafter, fungal biomass in each test tube was filtered on pre-weighed filter papers, dried to constant weight in an electric oven at 60 °C and weighed (Javaid and Samad, 2012).

All the data were analyzed by analysis of variance followed by Tukey's HSD test at 5% level of probability.

Results and Discussion

Antifungal activity of methanolic fruit extract

Data regarding the effect of different concentrations of methanolic fruit extract on biomass of *S. rolfsii* is presented in Fig. 1. The adverse effect of all the concentrations of the extract was significant over control. There was 81-84% reduction in fungal biomass due to various concentrations of methanolic fruit extract.

Antifungal activity of different fractions of methanolic fruit Extracts

DMSO was used to dissolve different fractions of methanolic fruit extract of *M. azedarach.* This compound itself adversely affected the growth of the target fungal pathogen (Fig. 2-5). In general, there was gradual reduction in fungal biomass due to rise in DMSO concentration. In the present study, a series of control treatments were designed to avoid the effect of DMSO on the results of experiment. For each concentration of different fractions of methanolic fruit extract, there was a corresponding control treatment for comparison having the same amount of DMSO as was present in extract treatment. DMSO is also reported to reduced growth of *Macrophomina phaseolina, Alternaria alternata* and *Ascochyta rabiei* in some previous studies (Javaid and Munir, 2012; Javaid and Samad, 2012; Naqvi *et al.,* 2012).

The lowest concentration (3.125 mg mL^{-1}) of each fraction of methanolic extract was the most effective and significantly reduced fungal biomass by 41-65%. The effect of higher concentrations was generally less pronounced as compared to the lower most concentration (Fig. 2-5). This unusual antifungal pattern of higher concentrations of the methanolic extract fractions might be due to antifungal effect of DMSO. In case of higher extract concentrations, there were also higher concentrations of DMSO which adversely affected the fungal growth and consequently the effect of higher extract concentrations was not much pronounced as

compared to corresponding control treatment. Earlier, Jabeen *et al.* (2008) also reported antifungal activity of organic solvents and aqueous extracts of *M. azedarach* fruit against *Ascochyta rabiei*, the cause of destructive chickpea blight. Limonoids are the major compounds of *M. azedarach* fruits (Roy and Saraf, 2006), which have antifungal properties (Carpinella *et al.*, 2005), and could be responsible for antifungal activity of the fruit extract against *S. rolfsii*. The present study concludes that methanolic fruit extract of *M. azedarach* and its various fractions possess antifungal potential against *S. rolfsii*. Further studies are required to identify the effective antifungal constituents.

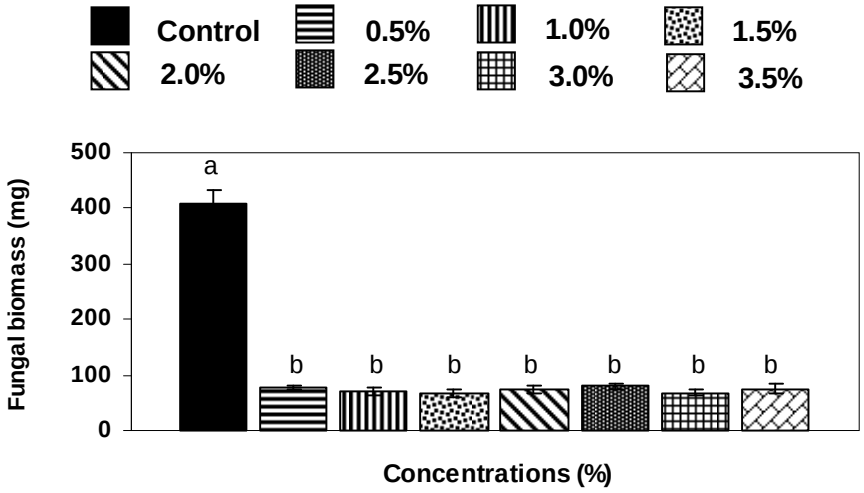

Fig. 1: Effect of different concentrations of methanol fruit extract of *Melia azedarach* on biomass of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey HSD Test.

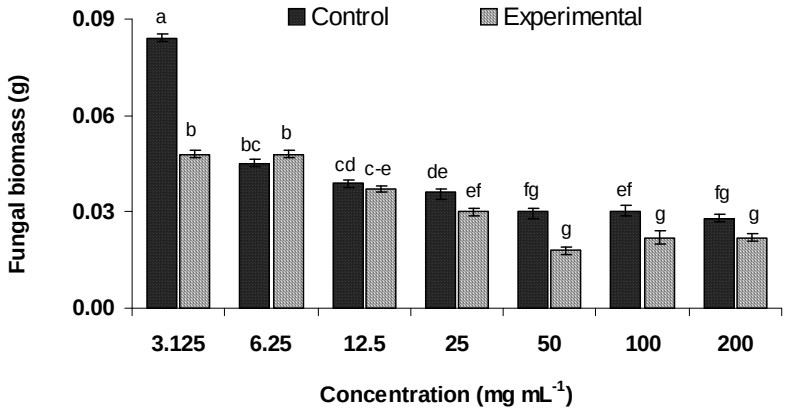

Fig. 2: Effect of different concentrations of *n*-hexane fraction of methanolic fruit extract of *Melia azedarach.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey HSD Test.

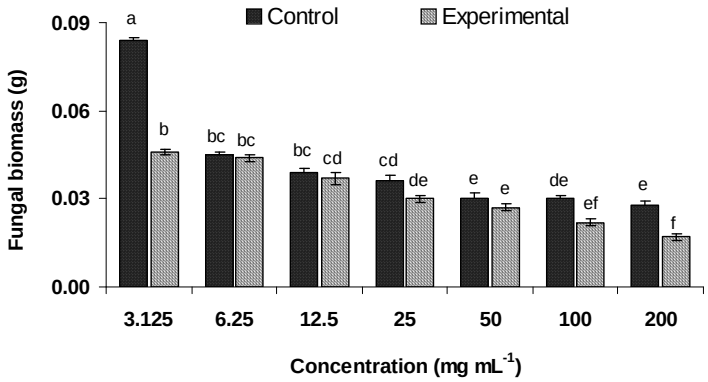

Fig. 3: Effect of different concentrations of ethyl acetate fraction of methanolic fruit extract of *Melia azedarach.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey HSD Test.

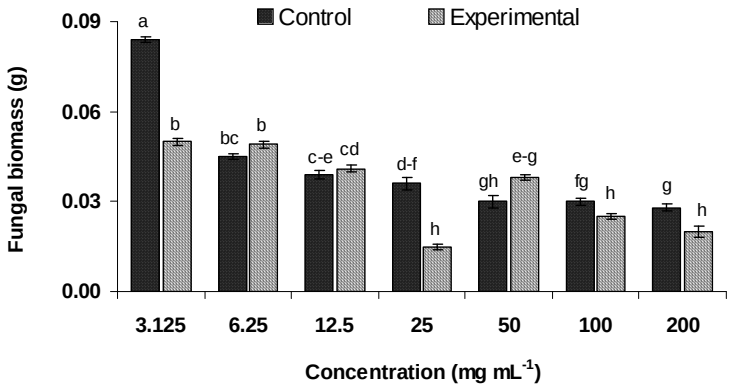

Fig. 4: Effect of different concentrations of *n*-butanol fraction of methanolic fruit extract of *Melia azedarach.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey HSD Test.

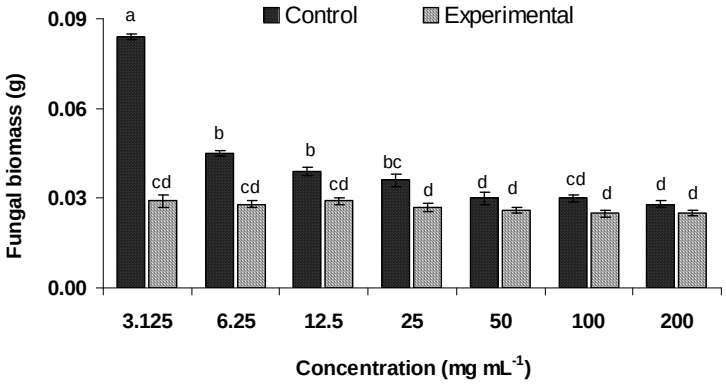

Fig. 5: Effect of different concentrations of aqueous fraction of methanolic fruit extract of *Melia azedarach.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey HSD Test.

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