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ANTIFUNGAL ACTIVITY OF *MELIA AZEDARACH* L. FRUIT EXTRACT AGAINST *ASCOCHYTA RABIEI* (PASS.) LAB.

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A B S T R A C T

Ascochyta rabiei (Pass.) Lab. causes blight that is a major constraint in chickpea (*Cicer arietinum* L.) production worldwide including Pakistan. Disease is generally controlled by repeated applications of foliar fungicides that cause environmental pollution and also responsible for health hazards. The present study was carried out to investigate the antifungal potential of different organic solvent fractions of methanolic fruit extract of *Melia azedarach* L. against *A. rabiei* (Pass.) Lab. Methanolic fruit extract of this tree was partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. After evaporation of solvents in a rotary evaporator, different concentrations of the crud organic fraction were prepared and evaluated against the target fungal pathogen. All the concentrations of organic solvent fractions significantly reduced the fungal biomass over corresponding control treatments. There was 43-87%, 75-94%, 69-95%, 38-89% and 63-94% reduction in fungal biomass due to different concentrations of *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fraction of methanolic fruit extract of *M. azedarach,* respectively. The present study concludes that all these fractions of methanolic fruit extract of *M. azedarach* possess substantial antifungal potential against *A. rabiei.* These fractions and their purified compounds may be used as alternatives to synthetic fungicides for the management of *A. rabiei.*

Keywords: *Ascochyta rabiei*, chickpea, *Melia azedarach*, natural fungicides.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an edible legume that is cultivated extensively for its nutrient rich seeds, containing sufficient amount of protein (20-23%), carbohydrates (40%), vitamins, minerals (Mg, K, P, Fe, Zn, and Mn) and dietary fibers (Gil *et al.,* 1996; Ibrikci *et al*., 2003; Kimurto *et al.,* 2013). Among food legumes, it ranks third in production in the world cultivated on an area of over 11.5 million hectares in over 40 counties with annual production of 10.5 million tonnes (FAOSTAT, 2011). It also plays an important role in sustainability of legume-cereal cropping systems where it increases soil nitrogen through biological nitrogen fixation and also breaks disease cycles of cereal pathogens (Pande *et al*., 2011). This valuable crop is attacked by *Ascochyta rabiei* (Pass.) Lab. [telomorph: *Didymella rabiei* (Kovachevski) Arx.] that causes blight disease and is among the major constraints to yield improvement in chickpea (Taran *et al.,* 2013). Under suitable environmental conditions for disease development, almost 100% yield losses have been reported (Chongo and Gossen, 2001). The most important solution for long-term management of a disease is the development of resistant varieties. Chickpea cultivars having better levels of resistance to *A. rabiei* have also been developed and commercialized (Taran *et al.,* 2011). However, improved varieties are only moderately resistant to the pathogen due to availability of only partial resistance among the cultivated chickpea germplasm (Taran *et al.,* 2013). Consequently, mostly foliar and some systemic fungicides are used to combat the menace (Shtienberg *et al.,* 2005, 2006; Ahmed *et al.,* 2008; Banniza *et al.,* 2011). This practice increases the production cost due to multiple applications of the fungicides that is a typical requirement of this disease (Acikgoz *et al.,* 1994). In addition, indiscriminate use of synthetic agro-chemicals

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cause environmental pollution and health hazards (Oruc, 2010). Due to these reasons, scientists are in search of less harmful and eco-friendly alternatives to these fungicides from plants (Jabeen *et al.,* 2011; Javaid and Samad, 2012; Rauf and Javaid, 2013). The present study was therefore carried out to investigate the fungicidal potential of different organic solvent fractions of methanolic fruit extract of *M. azedarach* against *A. rabiei.*

MATERIALS AND METHODS

Fully grown fruits of *M. azedarach* were collected when they were still green and dried in sunlight. Four kilograms of dried fruits were crushed and soaked in 7.0 L of methanol at room temperature for two weeks. Thereafter materials were filtered through a muslin cloth. Residues were again soaked in methanol for one week and filtered. Following filtration, the combined methanolic extract was evaporated under vacuum in a rotary evaporator to get crude fruit extract.

The crude methanolic extract was mixed in 500 mL distilled water and the mixture was extracted with 500 mL of *n*-hexane in a separating funnel. The process was repeated many times until all the *n*-hexane soluble materials were removed from the mixture indicated by a clear *n*-hexane layer in the separating funnel. The *n*-hexane phase was collected and evaporated under vacuum at 45 ˚C until dryness affording the 10 g of *n*-hexane fraction. Then the remaining extract was subjected to further fractionation by successive solvent extractions with chloroform, ethyl acetate, and *n*-butanol, to yield 16 g of chloroform fraction, 7 g of ethyl acetate fraction and 6 g of *n*-butanol fraction. Finally, the remaining aqueous extract was evaporated to dryness under reduced pressure at 55 ˚C to give 100 g of aqueous fraction (Rauf and Javaid, 2013).

The various fractions from methanolic fruit extract of *M. azedarach* were tested *in vitro* against the target fungal pathogen. Weighed amount (1.6 g) of each of the five fractions were dissolved in 1 mL Dimethyl sulfoxide (DMSO) and added to 7 mL of malt extract broth separately. A volume of 4 mL of these stock solutions (200 mg mL^{-1}) were used for bioassays and to rest of the solutions, 4 mL malt extract broth was added. In this way, the solutions were serially double diluted by adding malt extract broth to prepare lower concentrations of 100, 50, 25, 12.5, 6.25, 3.125 and 1.562 mg mL^{-1}. For control, 1 mL of DMSO was dissolved in 7 mL malt extract broth and serially double diluted to prepare control treatments corresponding to various extract concentrations. Bioassays were conducted in 10 mL glass test tubes each containing 1 mL of the medium. Inoculum of *A. rabiei* was prepared by adding 5 mL of sterilized distilled water on 8 days old colony of the fungus, scratched the surface of the fungal colony gently and passed the materials through a sterilized muslin cloth. Filtrate was collected and used as inoculum. One drop of inoculum was added to each test tube. Each treatment was replicated four times. Test tubes were incubated at room temperature. After 7 days, fungal biomass in each test tube was filtered on pre-weighed filter papers. Filter papers were dried at 70 ˚C and weighed. The weight of fungal biomass was obtained by subtracting the weight of filter papers from the combined weight of filter paper plus fungal biomass.

Statistical analysis: Analysis of variance followed by Duncan's Multiple Range Test (Steel *et al.,* 1997) was applied to analyze the data regarding fungal biomass at 5% level of significance using computer software COSTAT.

RESULTS AND DISCUSSION

Various fractions of methanolic fruit extract of *M. azedarach* in different solvents showed pronounced antifungal activity. In general, all the concentration of *n*-hexane fraction significantly reduced the fungal biomass. However, a marked variation in antifungal activity among the various concentrations was evident. The highest concentration of 200 mg mL^{-1} and 100 mg mL^{-1} showed the greatest antifungal activity resulting in 87% and 89% reduction in fungal biomass over control. However, the rest of the concentrations reduced fungal biomass by 43–82%. There was a gradual increase in fungal biomass as the extract concentration decreased from 50 mg mL^{-1} to 1.562 mg mL^{-1} (Figure 1A & 2). Likewise various concentration of chloroform fraction significantly reduced the fungal biomass by 75–95% (Figure 1B & 2).

There was a significant adverse effect of all the concentrations of ethyl acetate fraction against the growth of *A. rabiei.* Various concentrations of this fraction reduced the fungal biomass by 69–94% over control (Figure 1C & 2). *n*-butanol fraction exhibited comparatively lower antifungal activity than rest of the organic solvent fractions. Antifungal activity of this fraction gradually decreased with the decrease of extract concentration. In general, all the concentrations significantly reduced the fungal biomass over corresponding control treatments (Figure 1D).

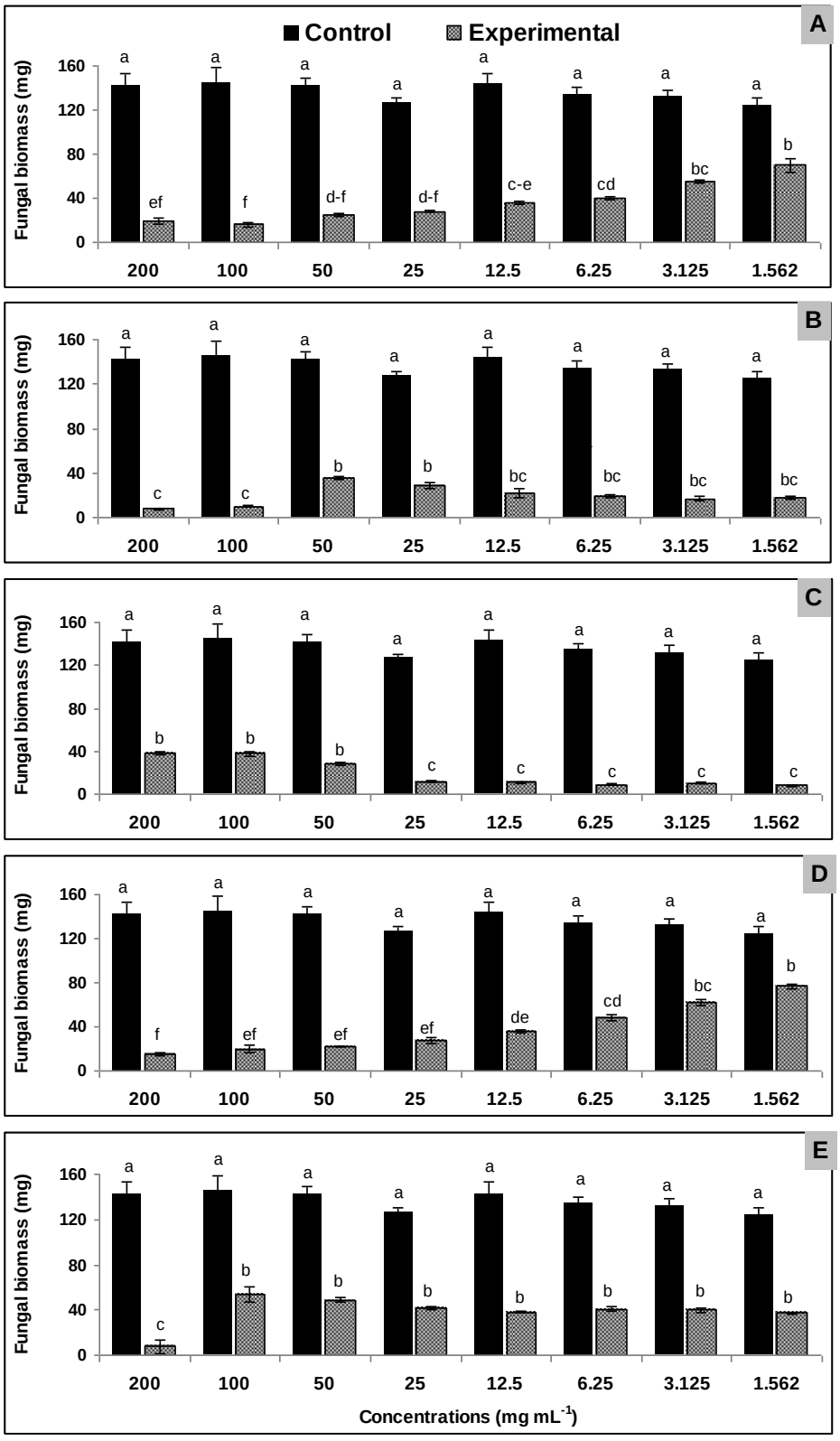

Figure 1 (A-E). Effect of different concentrations of *n*-hexane (A), chloroform (B), ethyl acetate (C), *n*-butanol (D) and aqueous fraction (E) of methanolic fruit extract of *Melia azedarach* on *in vitro* growth of *Ascochyta rabiei*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

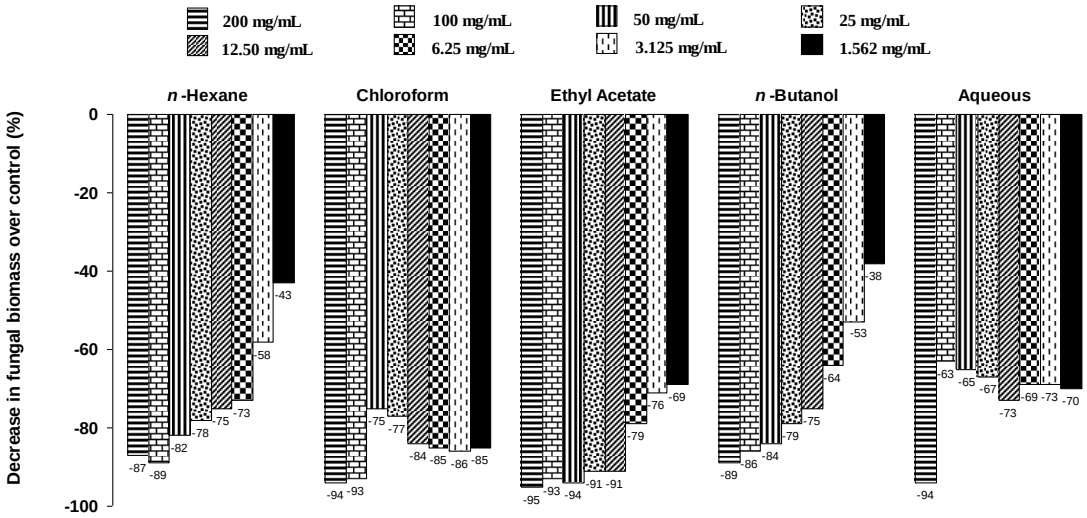

Figure 2. Percentage decrease in biomass of *Ascochyta rabiei* due to different concentrations of *n*-hexane, chloroform, ethyl acetate, *n*-butanol and Aqueous fractions of methanolic fruit extract of *Melia azedarach* over control.

There was 38–89% reduction in fungal biomass due to different concentrations of *n*-butanol fraction (Figure 2). All the concentrations of aqueous fraction showed significant antifungal effect against the target fungal pathogen. The effect of highest concentration of 200 mg mL^{-1} was much more pronounced as compared to all the lower concentrations. The highest concentration reduced the fungal biomass by 94% as compared to 63–73% reduction due to lower concentrations of 1.562–100 mg mL^{-1} (Figure 1E & 2). Although all the fractions of methanolic fruit extract of *M. azedarach* significantly reduced fungal biomass, however, there was a marked variation in antifungal potential of among the fractions. This could be attributed to presence of different types of compounds in different fractions. The four solvents used in this study for partitioning of methanolic extract had different polarity, *n*-hexane is non-polar and *n*-butanol is highly polar. Consequently, different compounds were dissolved in these fractions depending upon their polarities. Similar variation in antifungal activity of different fractions of methanolic extracts of other plant species have also been reported against fungal plant pathogens such as *Macrophomina phaseolina, Alternaria alternata, Sclerotium rolfsii* etc. (Iqbal and Javaid, 2012; Javaid and Saddique, 2012; Javaid and Samad, 2012). The chief group of biologically active constituents present in *M. azedarach* fruits are limonoids, a group of lipophilic substances (Roy and Saraf, 2006). These compounds might be responsible for the antifungal activity of the extracts against *A. rabiei* (Carpinella *et al.,* 2005). The present study concludes that methanolic fruit extract and its various organic solvent fractions have substantial antifungal potential against *A. rabiei.* Further studies are needed to identify the effective antifungal constituents from these fractions.

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