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Fungitoxicity of aqueous and organic solvent extracts of *Datura metel* against *Ascochyta rabiei*

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Abstract

In vitro efficacy of aqueous and methanolic extracts of *Datura metel* was evaluated against *Ascochyta rabiei*, (the causal agent of chickpea blight) and the sensitivity of colony growth was studied in terms of inhibition zone. The inhibitory potential of all the extracts was greatest at lower concentration. The aqueous and methanol extracts of shoot of *Datura metel* caused 21-34% and 20-40% reduction in growth of *Ascochyta rabiei* whereas the root extracts proved less effective as they caused 15-25% and 11-29% reduction in growth of *A. rabiei*, respectively.

Keywords: *Ascochyta rabiei*, chickpea, fungitoxicity, *Datura metel, aqueous and organic extracts.*

Introduction

One of the most important legume crops of Pakistan is Chickpea (*Cicer arietinum* L.), which is a major source of protein. It is grown over 0.963 m ha rainfed conditions with annual production of 0.6752 million tons with an average yield of 701 kg ha^{-1} (Anonymous, 2004), which is much lower than its potential. Blight disease is a major limiting factor for its reduced production (Ilyas and Bashir, 1983). *Ascochyta* blight is a major disease of chickpea in most growing areas of the world (Porta-Pugilia *et al*., 1997) that causes 20-25% yield loss in chickpea annually (Iqbal *et al.*, 2005).

Varieties of control measures are undertaken to avoid the implications of yield losses due to plant diseases. In this regard, the biological inhibitions by different natural substances, such as essential oils and plant extracts have been investigated widely against fungal activities. In last two decades, much work has been done on plant-derived compounds as environmentally safe alternatives to chemicals (Duke *et al*., 2000; 2002; Singh *et al*., 2004). In recent times Alkhail (2005) showed that aqueous extracts of plants viz. *Allium sativum, Cymbopogon proxims, Carum carvi, Azadirachta indica* and *Eugenia caryophyllus* had strong antifungal activity against fungi namely *Fusarium oxysporum, Botrytis cinerea* and *Rhizoctonia solani.* Similar effects of *Magnolia grandiflora* L. extracts against *Alternaria alternata, Helminthosporium* spp., *Fusarium oxysprum, F. culmorium* and *Rhizoctonia solani* have also been reported by Ahmad and Abdulgaleil (2005). More recently Bajwa *et al.* (2007) evaluated antifungal potential of aqueous and organic extracts of *Aloe vera* and reported that shoot aqueous and n-hexane extracts caused significant inhibition in growth and biomass production of the three tested fungi viz., *Alternaria alternata*, *A. citri* and *A. tenuissima*. Thus the present study was undertaken to evaluate the antifungal potential of aqueous and organic solvent extracts of *Datura metel* on *in vitro* growth of *Ascochyta rabiei*

Materials and Methods

Procurement and maintenance of target fungal species

Culture of target fungal species of *A. rabiei* was obtained from First Fungal Culture Bank of Pakistan, (FCBP) University of the Punjab, Quaid-e-Azam Campus Lahore and maintained on malt extract agar (MEA) medium.

Collection of plant materials

Fresh samples of shoot and root of *Datura metel* were collected from University of the Punjab, Quaid-e-Azam Campus Lahore and washed thoroughly under tap water, dried with blotting paper and cut into small pieces. The soluble ingredients of the plant material were then extracted by solubilization in water and methanol as different solvents.

Preparation of aqueous extract

Aqueous extract of water soluble ingredients of plant material was prepared

according to Bajwa *et al*,. (2007). A 20% w/v stock solution of plant extract was attained by soaking the crushed plant materials in sterilized distilled water for 48 h at 30±2 °C. Then material was filtered through muslin cloth followed by filter paper. This stock extract was stored at 4 ºC and used within four days.

Preparation of organic solvent extract

The method of Alkhail (2005) was followed for the preparation of shoot and root extract in methanol. The test plant was crushed and extracted by macerating 20 g of plant material in 100 mL of methanol for 48 h. Materials were filtered through muslin cloth followed by filter paper. Organic solvent extract was evaporated under vacuum until its volume was reduced to 2 ml and then diluted by adding appropriate quantity of sterilized distilled water to make final volume of 100 ml. These stock extracts were stored at 4 ºC and used within four days.

The lower concentrations of 1, 2, 3 and 4% of both aqueous and methanol extracts of shoot and root were prepared by adding appropriate quantity of sterilized distilled water. To make methanol control, 2 ml of methanol was added to sterilized distilled water to make final volume 100 ml.

Antifungal bioassays with extracts

Malt extract agar (MEA) medium was prepared and cooled to 50 ºC. Appropriate quantities of stock solution and distilled water were added to MEA medium to get 1, 2, 3 and 4% (v/v) concentrations of shoot and root extracts in the medium. Control received the same quantity of distilled water. The plant extracts were thoroughly mixed with the medium. Twenty ml of each medium was poured in each 9 cm diameter sterilized Petri plate. Mycelial discs of 5 mm diameter were taken with a pre-sterilized cork borer from 5-7 days old culture of *A. rabiei* and were placed in the centre of each Petri plate after solidification of the MEA medium. Each treatment was replicated thrice. Plates were incubated in an incubator at 25±2 ºC for 7 days. Fungal growth was measured by averaging the three diameters taken at right angles for each colony. Percentage growth inhibition of the fungal colonies was calculated by applying the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control - Growth in treatment}}{\text{Growth in control}} \times 100$$

Statistical analysis

All the data were analyzed by analysis of variance followed by Duncan's Multiple Range Test (Steel and Torrie, 1980) using computer software SPSS and COSTAT, respectively.

Results and Discussion

Effect of aqueous extract of *D. metel* on growth rate of *A. rabiei*

The results obtained from periodic growth assays of *A. rabiei* in various concentrations of shoot and root aqueous extract of *D. metel* showed significant antifungal activity in all the concentrations in comparison to control (Fig. 1). The results revealed that shoot extract was significantly the most effective in suppressing the colony growth as compared to root extract. The assessment of concentration effect revealed an increase in growth rate with increased incubation period. At initial growth stages all the concentrations invariably and insignificantly depressed the fungal growth while after 7 days incubation all extract concentrations depicted a considerable depression in growth rate. The maximum antimycotic activity was observed by 1 & 3% shoot extract. In contrast 2 & 4% concentrations depicted less toxicity against *A. rabiei*. In case of root extract a variable pattern of antimycotic activity was observed as 1 & 4% concentration of root extract was the most effective in suppressing the growth of *A. rabiei* whereas 2 & 3% concentrations were less depressive (Fig. 3A). Shoot extract caused about 21-33% depression in growth rate of the test organism while 15-25% reduction was noticed by root extract. The 1% concentration of both shoot and root aqueous extracts showed maximum decrease in fungal growth which was 33% in shoot extract and 25% by root extract (Fig. 4A).

Effect of organic extract of *D. metel* on growth rate of *A. rabiei*

Methanolic fractions exhibited more promising results in suppressing the fungal growth than aqueous fractions. The periodic data regarding fungal growth, exposed to various concentrations of methanolic extracts of *Datura metel* are presented in Fig. 2. Differences in growth rate were exhibited with respect to the concentrations employed. The periodic assays revealed a significant reduction in fungal growth rate in all concentrations. The fractions of shoot and root organic extracts did not show any particular trend in response to inhibition. However, the lowest concentration 1% caused significant reduction in mycelial growth (Fig. 3B).

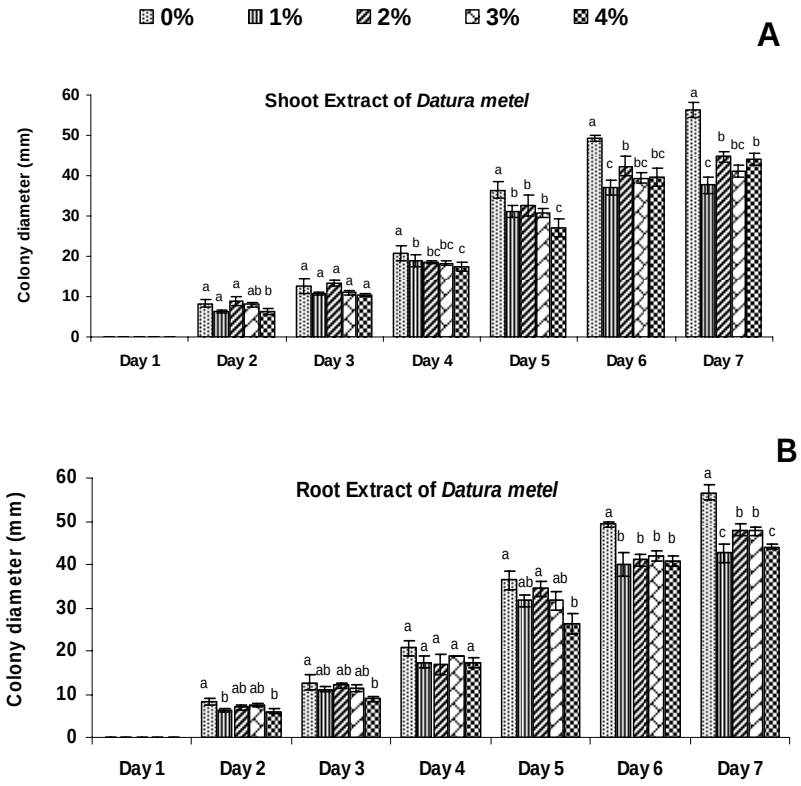

Fig. 1: Periodic effect of aqueous shoot and root extracts of *Datura metel* on fungal colony growth of *Ascochyta rabiei*.
Verticle bars show standard errors of means of three replicates.
For each day values with different letters show significant difference (P = 0.05) as determined by Duncan's Multiple Range Test.

The comparison of different concentrations of shoot and root organic extracts showed that the %age colony growth inhibition was significantly greater in shoot extract in contrast to root extract (Fig. 4B). There was 20-40% reduction in fungal growth due to various employed concentrations of shoot extract. The 1% shoot extract caused highest reduction of about 40% in fungal growth. Further increase in extract concentration exhibited significant difference as compared to 1% extract. Different concentrations of root extract caused 10-29% reduction in fungal growth. Maximum reduction of 29% was depicted by 1% root extract.

In the present study, two types of extracts of *D. metel* were used against *A. rabiei*. The results of this study clearly reflect that *D. metel* has the potential to induce toxic effects on mycelial growth and proliferation of fungi. The relative intensity of this effect, however, varies with the species involved, as well as the concentration of the extract employed. Earlier Shafique *et al.*, (2006) have reported that allelopathic trees viz., *Azadirachta indica*, *Mangifera indica*, *Melia azedarach* and *Syzygium cumini* significantly suppress the growth of *Alternaria alternata*. Similarly, Bajwa *et al.*, (2007) carried out the study on antifungal activity of aqueous and n-hexane shoot extracts of *Aloe vera* against few pathogenic species of *Alternaria alternata*, *A. citri* and *A. tenuissima*. They reported that the inhibitory effect was found to be variable with the applied concentration and caused a significant inhibition in biomass production of the three test fungi Likewise, Dabur *et al.*, (2004) reported that phytochemical extraction of *D. metel* showed antifungal (MIC 87.5 mg mL^{-1}) activity against *Aspergillus fumigatus*.

Extracts in different solvents exhibited variable antifungal activity against i*n vitro* growth of *A. rabiei.* Methanolic fractions exhibited more promising results in suppressing the fungal growth than aqueous fractions. Likewise Dwivedi and Dubey (1986) reported that volatile fractions of two medicinal plants,

Azadirachta indica and *Eucalyptus globules* were more effective against *Macrophomina phaseolina* than non-volatile fractions. The variation in antifungal activity of the extracts in different solvents may be attributed to the different chemical nature of the solvents. It is likely that different types of chemicals were dissolved in different solvent that resulted in variable activity of the extracts of same part of the plant in different solvents. There are many examples in the literature which support our findings. Zafar *et al.*, (2002) reported that chloroform extract of leaves of *M. azedarach* was active against *Fusarium chlamdosporum* while hexane, ethanol and water extracts were not. In a similar kind of work Bajwa *et al.*, (2006) reported the antimycotic activity of aqueous and dichloromethane fractions of *Cicer arietinum* against *Drechslera tetramera* and *D. hawaiiensis.*

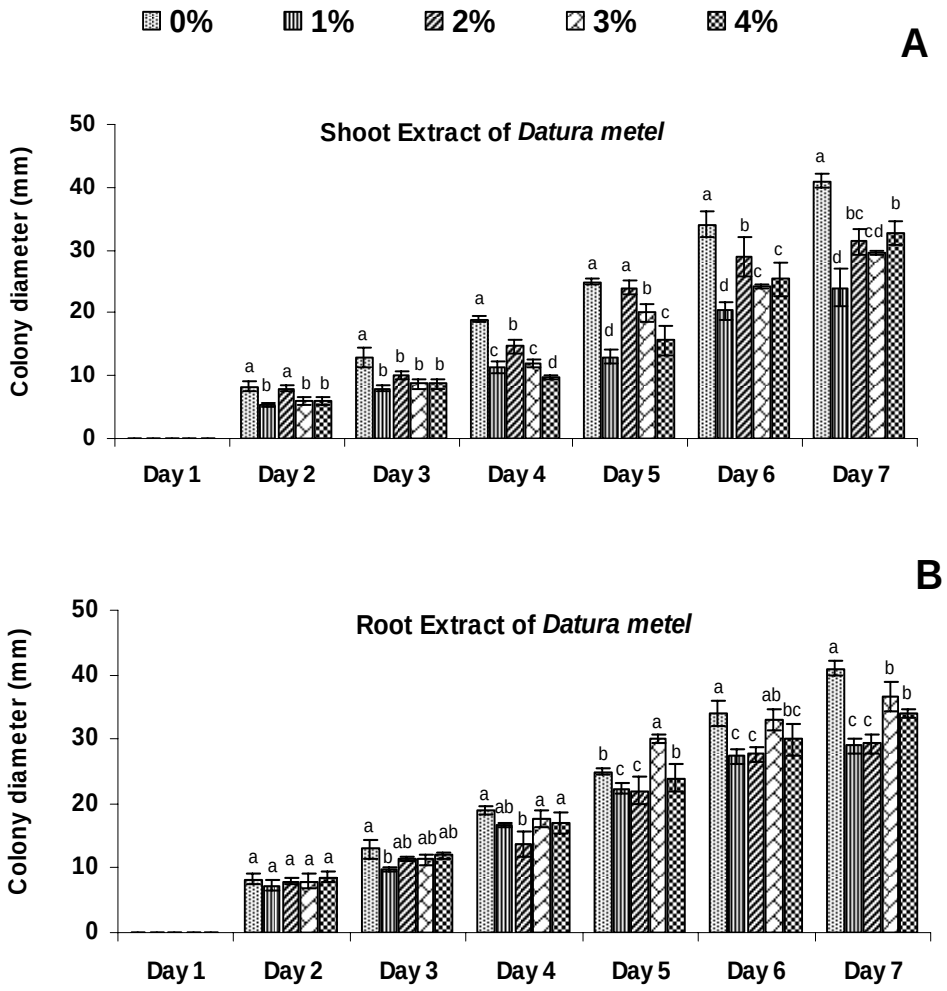

Fig. 2: Periodic effect of organic shoot and root extracts of *Datura metel* on fungal colony growth of *Ascochyta rabiei*.
Verticle bars show standard errors of means of three replicates.
For each day values with different letters show significant difference (P = 0.05) as determined by Duncan's Multiple Range Test.

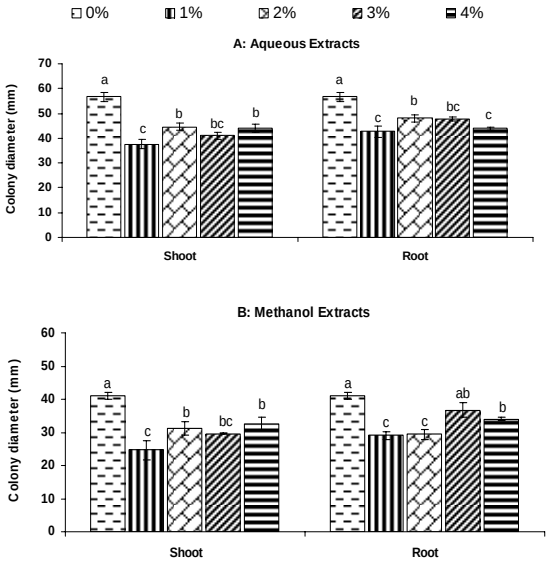

Fig. 3: Effect of aqueous and organic solvent extracts of *Datura metel* on in vitro growth of *Ascochyta rabiei* after 7 days of incubation.
Vertical bars show standard error of means of three replicates. Values with different letters show significant difference as determined by DMR Test.

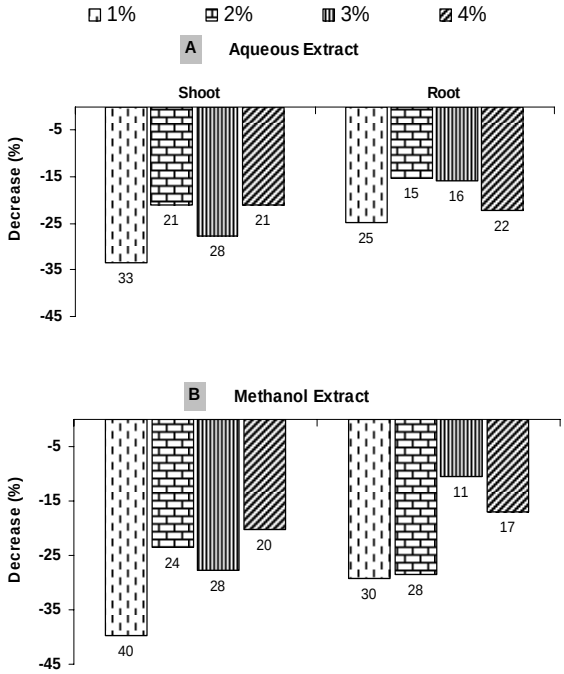

Fig. 4: Percentage decrease in colony diameter of *Ascochyta rabiei* due to different concentrations of aqueous and organic solvent extracts of shoot and root of *Datura metel*.

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