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Antifungal activity of *n*-hexane extracts of *Datura metel* against *Ascochyta rabiei*

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Abstract

The antimycotic potential of *Datura metel* (syn. *Datura alba* Nees.) was investigated *in vitro* against *Ascochyta rabiei*, the cause of chickpea blight disease. The pathogen was exposed to various n-hexane concentrations (1, 2, 3 and 4% w/v) of shoot and root extracts of *D. metel* using poisoning food technique. All the employed concentrations of both root and shoot extracts significantly suppressed the growth of the target fungal pathogen. There was 28-34% and 23-41% reduction in colony diameter of *A. rabiei* due to different concentrations of n-hexane shoot and root extracts of *D. metel.*

Keywords: *Ascochyta rabiei*, chickpea, n-hexane extract, *Datura metel.*

Introduction

Chickpea (*Cicer arietinum* L.) is an important legume crop. It is grown under wide agro-ecological conditions and rank first among food legumes in Indian Subcontinent and Mediterranean basin (Anonymous, 1994). In Pakistan, it is grown under rainfed conditions and 0.6752 million tons are produced annually (Anonymous, 2004). Although chickpea production is affected by various factors but blight disease caused by *Ascochyta rabiei* is the major limiting factor (Ilyas and Bashir, 1983) which cause about 20-25% annual loss in chickpea yield and total crop failure under favourable epidemic conditions (Iqbal *et al.*, 2005).

The exploitation of resistant varieties has been supported as the best control of disease (Iqbal *et al.*, 2002), however, cultivars with resistance against *Ascochyta* blight have not exhibited the desired results due to lack of stability in tolerance because of appearance of new pathotypes of the pathogen (Iqbal *et al.*, 2002, 2005). Chemical control has been proved efficient and economical in controlling blight disease. However, increasing public concern on environmental issues desires that alternative management systems be evolved either to reduce pesticide dependant or naturally occurring compounds be explored to constrain the pathogen attack (Singh *et al.*, 2003; Cuthbertson and Murchie, 2005). Natural plants derived compounds contribute a lot in fight against pathogens (Vyvyan, 2002). Several plant families like Acanthaceae, Amranthceae, Apiaceae and Magnoliaceae have antifungal and cytotoxic properties (Mansilla and Palenzuela, 1999; Neerman, 2003). Numerous studies conducted in Pakistan revealed a wide spectrum prospects of using extracts of plants for biological control of pathogenic fungi (Bajwa *et al*., 2001; Bajwa *et al*., 2002; Ahmad and Abdelgaliel, 2005; Braga *et al.*, 2007). Various plant extracts e.g., *Cicer arietinum* (Bajwa *et al.*, 2006), *Parthenium hysterophorus* (Bajwa *et al.*, 2004) and *Magnolia grandiflora* (Ahmed and Abdelgaleil, 2005) etc. have also been examined for their antifungal activity with the objective of exploring environmentally safe alternatives of plant disease control. Thus with the objective to contribute to these studies, the antifungal activity of n-hexane extracts of *Datura metel* was investigated against *Ascochyta rabiei.*

Materials and Methods

Culture of *A. rabiei* FCBP-702 was obtained from First Fungal Culture Bank of Pakistan, University of the Punjab, Quaid-e-Azam Campus Lahore and maintained on malt extract agar (MEA) medium.

Shoots and roots of mature *Datura metel* plants were collected from University of the Punjab, Quaid-e-Azam Campus Lahore. After thorough washing under tap water, the materials were surface sterilized with 1% sodium hypochlorite solution followed by thorough washing with sterilized water. Plant materials were crushed and soaked in organic solvent n-hexane @ 20 g/100 ml for 48 hours. The extracts were filtered through muslin cloth followed by filter paper. Organic solvent was evaporated under

vacuum to 2 ml and the remaining extract was diluted by adding appropriate quantity of sterilized distilled water to make 20% extract. These stock extracts were stored at 4 ºC and used within four days. To make n-hexane control, 2 ml of organic solvent was added to sterilize distilled water to make final volume 100 ml.

Two percent MEA medium was prepared by autoclaving at 121 ºC and cooled to 50 ºC. Afterwards, appropriate quantities of stock solution and distilled water were added to MEA medium to get 1, 2, 3 and 4% (w/v) concentrations of shoot and root extracts in the medium. The plant extracts were thoroughly mixed with the medium. Control treatments received only n-hexane control mixture. Twenty ml of each medium was poured in each 9 cm diameter sterilized Petri plates. Mycelial discs of 5 mm diameter were taken with a sterilized cork borer from 7 days old cultures of *A. rabiei* and were placed in the centre of each Petri plate after solidification of the medium. Each treatment was replicated thrice. Plates were incubated 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:

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

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

Results and Discussion

The sensitivity of colony growth of the causal organism of blight disease was evaluated using inhibition zone technique at various concentrations. The data regarding the colony growth revealed that the growth rate was different in all the concentrations employed (Fig. 1A & B). Generally, root extract was more toxic than shoot extract to fungal growth. The periodic growth assays showed that the colony growth increased from first day to seventh day in all the treatments, however a nominal depression was observed after two and three days of incubation but at 7 days period growth rate was found to be significantly depressed as compared to control. The variation in antifungal activity of the shoot and root extracts may be attributed to the different chemical nature of the compounds present in these parts (Afsharypuor *et al*., 1995). Generally all the concentrations significantly suppressed fungal growth. No particular trend was observed in response to fractions of shoot and root extracts. The maximum antimycotic activity was observed under 1% shoot extract. In contrast, the higher concentrations (from 2-4%) depicted less toxicity against *A. rabiei*. In case of root extract inverse pattern of antimycotic activity was observed as 4% concentration of root organic extract was found to be the most effective in suppressing the growth of *A. rabiei* (Fig. 2). *Datura metel* is well known for tropane alkaloids and with asteroids, yielded several sphingosine derivatives, one of which was characterised as (4E,8Z)-1-O-(beta-D-glucopyranosyl)-N-(2'-hydroxyhexadecanoyl)-sphinga-4,8-dienine from comprehensive spectral analysis of its hexaacetate and known to have antifungal properties (Sahai *et al*., 1999). Dabur *et al*. (2004) isolated a new pyrrole derivative 1 from the leaves of *D. metel* which was characterised as 2-beta-(3, 4-dimethyl-2, 5-dihydro-1H-pyrrol-2-yl)-1'-methylethyl pentanoate on the basis of spectral data analyses and chemical reactions which was endowed with antifungal activity. Earlier Carpinella *et al.* (2003) have reported that hexanic and ethanolic extracts from fruit, seed kernels and senescent leaves of *Melia azedarach* exhibited fungistatic activity against *Aspergillus flavus*, *Diaporthe phaseolorum* var. *meridionales*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium verticillioides*, and *Sclerotinia sclerotiorum.* Similarly, Kagale *et al.* (2004) found variable antimicrobial activity of *D. metel in vitro* against *Rhizoctonia solani* and *Xanthomonas oryzae.*

The comparison of means in case of different concentrations of shoot and root extracts showed that the percentage colony growth inhibition was significantly greater in root extract in contrast to shoot extract (Fig. 3). There was 22-35% reduction in fungal growth due to various employed concentrations of shoot extract. The 1% shoot extract caused the highest reduction of about 35% in fungal growth. Further increase in extract concentration exhibited significant difference in antimycotic activity as compared to 1% extract. Different concentrations of root extract caused 22-41% reduction in fungal growth. Maximum reduction of 41% was depicted by 4% root extract. Earlier, Dabur *et al.* (2004) reported that phytochemical extraction of *D. metel* showed antifungal (MIC 87.5 mg/ml) activity against *Aspergillus fumigatus*.

In conclusion, this study demonstrates that n-hexane extracts of *D. metel* exhibit an effective antifungal potential against the test organism, *A. rabiei*. Finally, current research offers the possibility of developing strategies for controlling plant pathogens with natural extracts or bioactive metabolites of medicinal plants.

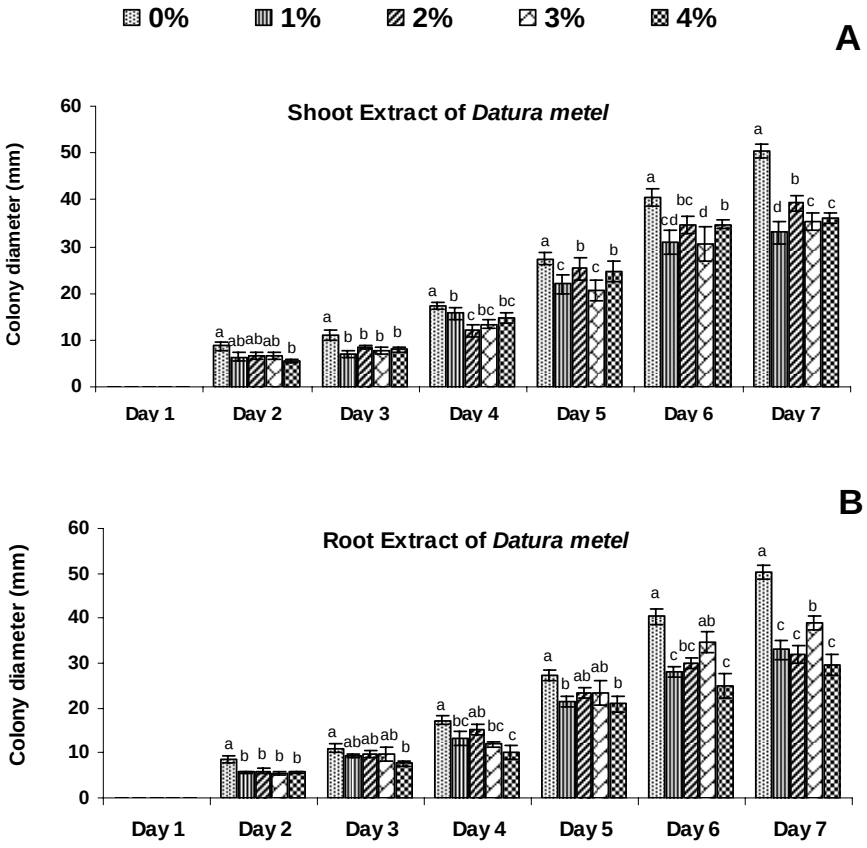

Fig. 1: Periodic effect of shoot and root extracts of *Datura metel* on fungal colony growth of *Ascochyta rabiei*.
Verticle bars show standard errors of means of three replicates.
For each day values with different letters show significant difference (P = 0.05) as determined by Duncan's Multiple Range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

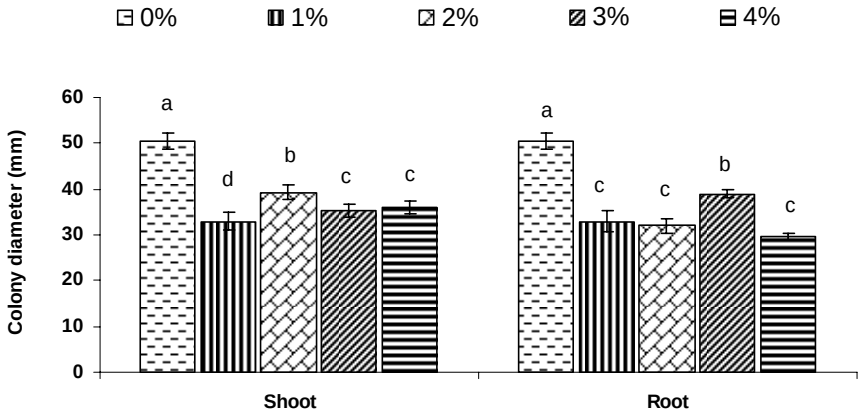

Fig. 2: Effect of n-hexane extracts on *in vitro* growth of *Ascochyta rabiei* after 7 days of incubation.
Vertical bars show standard error of means of three replicates. Values with different letters show significant difference as determined by DMR Test.

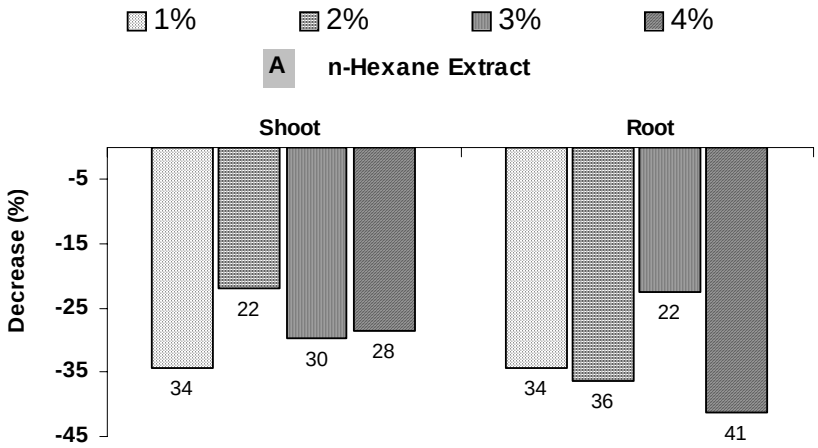

Fig. 3: Percentage decrease in colony diameter of *Ascochyta rabiei* due to different concentrations of n-hexane shoot and root extract of *Datura metel*.

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