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FUNGITOXIC ACTIVITY OF AQUEOUS AND ORGANIC SOLVENT EXTRACTS OF *TAGETES ERECTUS* ON PHYTOPATHOGENIC FUNGUS-*ASCOCHYTA RABIEI*

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Abstract

The *In vitro* fungitoxic potential of *Tagetes erectus* L. was scrutinized against *Ascochyta rabiei*, the causal agent of chickpea blight disease. The pathogen was exposed to various concentrations (1, 2, 3 and 4% w/v) of aqueous and methanol extracts of flower and shoot of *T. erectus* using food poisoning technique. All the employed concentrations of both flower and shoot extracts significantly suppressed the growth of target fungal pathogen. There was 4-35% and 55-73% reduction in colony diameter of *A. rabiei* due to different concentrations of aqueous flower and shoot extracts of *T. erectus* and 12-50% and 4-42% due to different concentrations of methanolic flower and shoot extracts of *T. erectus*, respectively.

Introduction

One of the most important legume crops of Pakistan is chickpea (*Cicer arietinum* L.), which is a major source of protein. In Pakistan, it is grown under rainfed conditions and 0.6752 million tons are produced annually (Anon., 2004). Although chickpea production is affected by various factors but blight disease is a major limiting factor for its reduced production (Ilyas & 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 management strategies are commenced to avoid the yield losses due to fungal plant diseases among which natural plants derived compounds contribute a lot in fight against pathogens (Vyvyan, 2002; Neerman, 2003). Many plant species are known to have fungitoxic effects on pathogenic fungal species (Carpinella *et al*., 2003; Bajwa *et al*., 2006, 2008; Shafique & Shafique, 2008). Numerous plant extracts e.g., *Magnolia grandiflora* (Ahmed & Abdelgaleil, 2005), *Aloe vera* (Bajwa *et al*., 2007), *Oryza sativa* (Javaid *et al*., 2008) etc., have been scrutinized for their phytopathogenic activity with an intention of exploring environmentally safe and economic alternatives of plant disease control. *Tagetes erectus* (marigold), an ornamental plant, which is also a popular bedding plant, can be used as a cover crop. It produces a substance with a pungent odour called alpha-terthienyl that is suggested useful for inhibiting attacks from root-knot nematodes, vine weevils and other disease promoting organisms such as insects, fungi, bacteria and viruses (Khan *et al*., 1971; Hethelyi *et al*., 1986; Soule, 1993). Thus the present study was designed to assess the antifungal potential of aqueous and organic solvent extracts of *Tagetes erectus* 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 flower of *Tagetes erectus* 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 25±2°C. Then material was filtered through muslin cloth followed by filter paper Whattman No. 1. This stock extract was stored at 4ºC and used within four days.

Preparation of organic solvent extract: The method of Alkhail (2005) was followed for the preparation of shoot and flower 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 Whattman No. 1. 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. The stock extract was stored at 4°C and used within four days.

The lower concentrations of 1, 2, 3 and 4% of both aqueous and methanol extracts of shoot and flower 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.

Laboratory bioassays: Malt extract agar (MEA) medium was prepared and cooled to 50°C. 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 flower extracts in the medium. Control received the same quantity of distilled water. The 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°C for 7 days. Fungal growth was measured by averaging the 3 times diameters taken at right angles for each colony. Percentage growth inhibition of the fungal colonies was calculated by applying the following formula:

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

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

Results

Effect of flower extract of *Tagetes erectus* on fungal biomass: The results obtained from growth assays of the test species in different concentrations of aqueous and methanolic extracts of flower of *T. erectus* are presented in Fig. 1A. A variable effect of various concentrations was recorded for the test species. The antifungal effect of all the concentrations of aqueous extracts was significant against the test fungal species except 1% extract that proved ineffective in suppressing the fungal growth. Maximum inhibition in fungal growth was evidenced by 2% aqueous extract whereas 3 and 4% concentrations exhibited slight and insignificant increase in fungal growth but revealed the significant antifungal potential. In case of methanol extract of flower of *T. erectus* there was a gradual decrease in growth of the test fungal species as the concentration of extract was increased from 1–4%. Relatively more toxicity of methanol extract was recorded as compared to aqueous extract. The 4% methanol extract concentration revealed the greatest inhibitory effect as it caused a significant decrease (50%) in fungal growth (Fig. 2A).

Effect of shoot extract of *Tagetes erectus* on fungal biomass: The *In vitro* antifungal potential of *T. erectus* shoot extracted by aqueous and methanol solvent is presented in Fig. 1B. In general, all concentrations of aqueous extract were found more inhibitory to the growth of test fungal species than methanol extract. All the concentrations significantly reduced the fungal growth in terms of colony diameter. Amongst these 4% concentration was the most effective in suppressing the growth up to 73% (Fig. 2B). Methanol extract also caused a significant reduction in growth of *A. rabiei* as compared to control but it proved to be comparatively less toxic than other extract examined. However in contrast to aqueous extract, 1% concentration of Methanol extract proved ineffective against suppressing the fungal growth as it depicted an increase (12.34%) in growth as compared to control. The highest antifungal activity of methanol extract was recorded at 4% concentration with maximum suppression of fungal growth up to 42% followed by 3 and 2% concentrations exhibiting 27 and 4.5% reduction, respectively (Fig. 2B).

Discussion

Different extracts used exhibited variable antimycotic activity against *In vitro* growth of *A. rabiei*. Among the two solvents, shoot aqueous extracts were found more inhibitory to test fungal growth than other extracts. Aqueous extracts of shoot of *T. erectus* reduced the fungal growth by 55-73%. Conversely, maximum reduction of 12-50% was exhibited by methanolic extract of flower. 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 solvents that resulted in variable activity of the extracts of same part of the plant in different solvents. There are many examples in literature which support these findings. Alkhail (2005) studied the effect of aqueous and ethanolic extracts of *Allium sativum, Carum carvi, Azidirachta indica* and *Eugenia caryophyllus* against *Fusarium oxysporum, Botrytis cinerea* and *Rhizoctonia solani* and found that aqueous extracts were more inhibitory to test fungal species growth than ethanolic extract. 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 viz., *Alternaria alternata, A. citri* and *A. tenuissima*. They reported that the inhibitory effect was found to be variable with the applied concentrations and caused a significant inhibition in biomass production of the three test fungi.

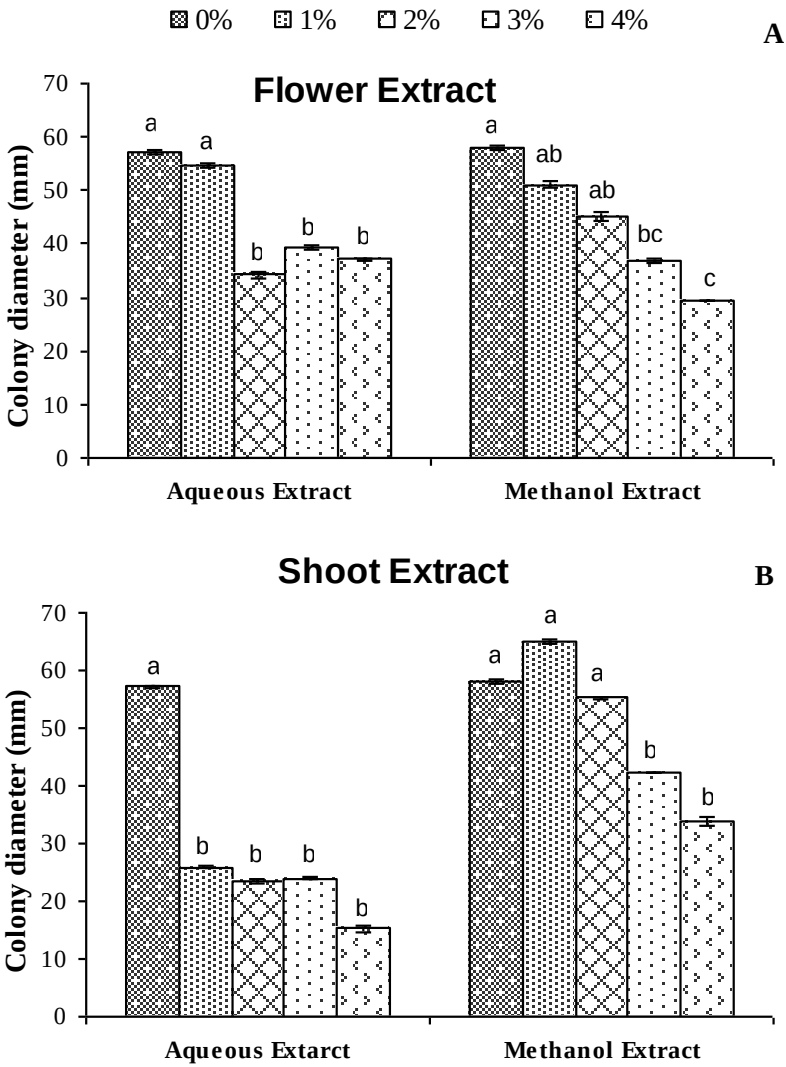

Fig. 1. Effect of aqueous and organic solvent extracts of *Tagetes erectus* on *In vitro* growth of *Ascochyta rabiei* after 7 days of incubation.
Vertical bars show standard error of means of three replicates. Values with different letters show significant difference as determined by DMR Test.

The extracts of different parts of *T. erectus* employed in the present study exhibited variable antifungal activity against the target pathogenic fungal species *A. rabiei.* This variability in antifungal potential of extracts may be attributed to the presence of different types of antifungal compounds in different parts of the test plant species. These findings are in line with the work conducted by Zafar *et al.*, (2002) who reported that chloroform extract of leaves of *M. azedarach* was active against *Fusarium chlamydosporium* 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.* Likewise, Carpinella *et al*., (2003) reported that hexanic and ethanolic extracts from fruit, seed kernels and senescent leaves exhibited fungistatic activity against *Aspergillus flavus*, *Diaporthe phaseolorum* var. *meridionales*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium verticillioides*, and *Sclerotinia sclerotiorum.* The antifungal activity of *M. azedarach* may be attributed to the presence of antifungal compounds viz., hydroxycoumarin scopoletin, vanillin, 4-hydroxy-3-methoxycinnamaldehyde and (±) pinoresinol (Carpinella *et al*., 2003, 2005).

The present study confirm that aqueous and organic solvent extracts of various parts (flower and shoot) of *T. erectus* contain ingredients that have antifungal potential which are highly effective against one of the most destructive pathogens of chickpea.

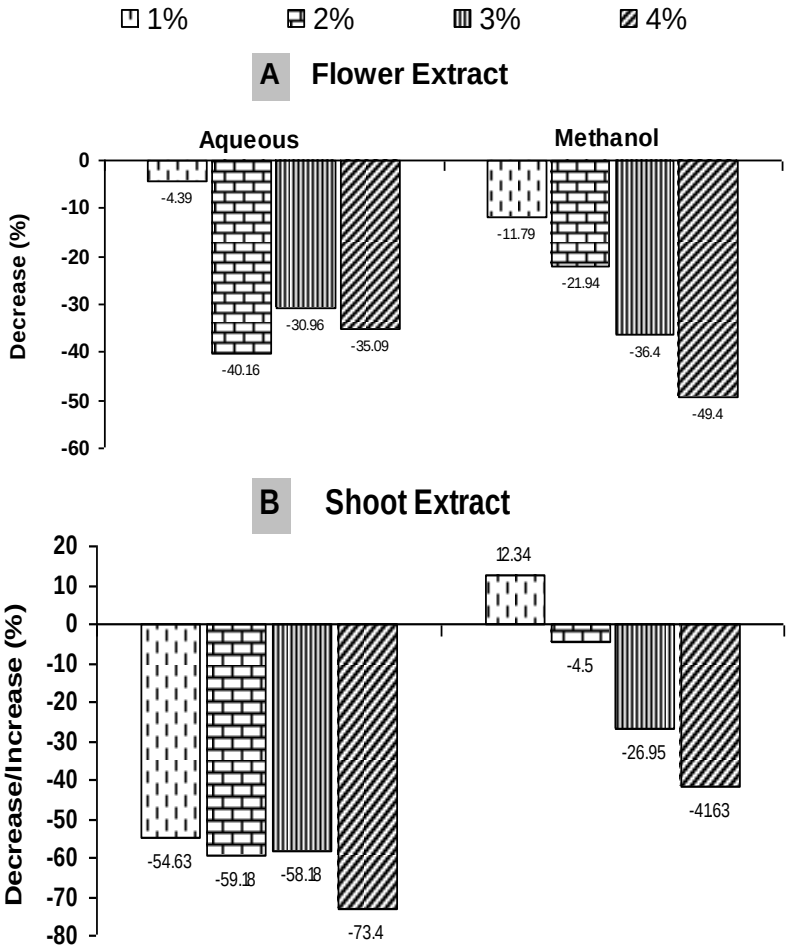

Fig. 2. Percentage increase/decrease in colony diameter of *Ascochyta rabiei* due to different concentrations of aqueous and organic solvent extracts of flower and shoot of *Tagetes erectus*.

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