Mycopath (2012) 10(2)**:** 51-55 **Research Article**

Prospects of using fruit and bark extracts of *Eucalyptus citriodora* for control of *Ascochyta rabiei,* the causal organism of chickpea blight

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Abstract

Ascochyta blight is a major disease of chickpea (*Cicer arietinum* L.) that is caused by *Ascochyta rabiei* (Pass.) Lab. Generally, fungicides are used to control this pathogen, which cause environmental pollution. In search of natural alternatives to these fungicides, efficacy of methanolic fruit, root-bark and stem-bark of *Eucalyptus citriodora* Hook. was investigated as antifungal agents against *A. rabiei.* Two hundred grams of dried and crushed materials of each plant part were extracted in methanol for two weeks. After evaporation of methanol on a rotary evaporator under reduced pressure, different concentrations (0.5, 1.0, 1.5, …, 4.0%) of crude methanolic extracts were prepared in malt extract broth. Fungal biomass was significantly reduced by all the concentrations of the extract. Antifungal efficacy of the extracts varied with concentrations and plant part assayed. In generally, fungal biomass production was inversely proportional to the extract concentration. Among the three plant parts, root-bark extract exhibited the highest antifungal activity followed by fruit and stem-bark extracts, respectively. There was 72–89%, 54–75% and 47–61% reduction in fungal biomass due to various concentrations of root-bark, fruit and stem-bark extracts, respectively, over negative control treatment. The present study concludes that methanolic root extract of *E. citriodora* possesses substantial antifungal potential to control *in vitro* growth of *A. rabiei.*
Keywords: *Ascochyta rabiei*, chickpea blight, *Eucalyptus citriodora*, methanolic extracts.

Introduction

In terms of area under cultivation, worldwide chickpea is the second most important legume (FAOSTAT, 2009). It is among the major sources of proteins in developing countries. It contains 20–23% protein in grains, 40% carbohydrates, 3–6% oil, and minerals including Zn, Fe, P, K, Mn and Mg (Gil *et al.,* 1996; Ibrikci *et al.,* 2003). Chickpea blight caused by *A. rabiei* is a highly damaging fungal pathogen globally that causes significant yield losses in the crop (Ali *et al.,* 2012). Under conditions suitable for pathogen to develop disease, 100% losses may occur (Alwawi *et al.,* 2009). Cultivation of blight resistant chickpea varieties is the most economic way of controlling this disease (Ilyas *et al*., 2007). However, resistance does not last long because of production of new races in the pathogen (Jamil *et al.,* 2010). Alternatively, farmers use fungicides to combat the menace. Successful management of the disease can be achieved by efficient and timely use of chemical fungicides. A range of fungicides including chlorothalonil, azoxystrobin, pyraclostrobin, boscalid, prothioconazole and mencozeb have been proved effective in controlling Ascochyta blight on chickpea (Davidson and Kim, 2007; Banniza *et al.,* 2011). However, use of fungicides pollutes the environment (Chang *et al.,* 2007). Researchers are in search of environmental friendly alternatives to these fungicides. Among these alternatives, use of natural plant products either in crude form or as purified compounds is gaining importance nowadays (Kanwal *et al.,* 2011; Javaid and Shoaib, 2012). Recently, Jabeen *et al.* (2011) identified a natural compound β-amyrin from leaves of *Melia azedarach* L. with potential antifungal activity against *A. rabiei.* The present study was carried out to investigate the antifungal activity of methanolic extracts of different parts of *E. citriodora* against *A. rabiei.*

Materials and Methods

Preparation of methanolic extracts

Stem-bark, root-bark and leaves of *E. citriodora* were collected from a mature tree, thoroughly washed under tap water and dried in sunlight. Weighed quantity (200 g) of different plant parts were crushed and soaked in 1.0 L of

methanol at room temperature for 14 days. Thereafter, soaked plant parts were passed first through muslin cloth and then through filter papers so that plant debris can be completely separated from the methanolic extracts. Filtrates were evaporated on a rotary evaporator and the crude extracts were stored for further experimentation.

Laboratory bioassays

In order to prepare solutions of different concentrations, stock solutions of methanolic extracts of different parts of *E. citriodora* were prepared by dissolving 14.4 g methanolic extract of different plant parts in 6 mL dimethyl sulphoxide (DMSO) and volume was raised to 18 mL by adding sterilized distilled water. Likewise, 6 mL DMSO was added to 12 mL distilled water to prepare a control solution. Seventy six millilitres of malt extract broth was autoclaved in 250-mL conical flasks and cooled the flasks at room temperature. Different quantities of stock solution (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 mL) and control solution (3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, 0 mL), respectively, were mixed in each flask and volume was raised to 80 mL. To positive control, only 4 mL of control solution was added. Similarly, in negative control treatment 4 mL of sterilized distilled water was added. Medium was divided into four parts in 100-mL flasks each containing 20 mL medium. Flasks were inoculated with fungal plugs of 5-mm diameter and incubated at 26 °C. Experiment was conducted in a completely randomized design with four replications. Fungal biomass from each flask was filtered on already weighed filter papers 10 days after inoculation, dried at 60 °C and weighed.

Statistical analysis

Analysis of variance followed by LSD Test was applied to analyze the data regarding fungal biomass at 5% level of significance using computer software Statistix 8.1.

Results and Discussion

Analysis of variance revealed that the effect of different parts of the plant (P) as well as concentration of the extract (C) was significant ($P \leq 0.001$) for biomass of *A. rabiei.* In a similar way, interactive effect of P × C was also significant for this studied parameter (Table 1).

Data regarding the effect of different concentrations of methanolic fruit extract of *E. citriodora* on biomass of *A. rabiei* is presented in Fig. 1A & 2. The effect of DMSO on fungal biomass was insignificant as there was not any pronounced difference in fungal biomass between negative and positive control treatments. Earlier studies have shown variable effects of DMSO on growth of other fungal species namely *Sclerotium rolfsii, Alternaria alternata* and *Macrophomina phaseolina* (Iqbal and Javaid, 2012; Javaid and Samad, 2012; Naqvi *et al.,* 2012). The effect of DMSO generally varies with the fungal species and concentrations of DMSO in the medium (Amin and Javaid, 2012). In the present study, all the concentrations of methanolic fruit extract significantly reduced fungal biomass by 54–75% and 53–74% over negative and positive control treatments, respectively (Fig. 1A & 2). Similarly, Jabeen and Javaid (2008) found that aqueous, ethanolic and *n*-hexane extracts of *E. citriodora* fruits markedly reduced growth of *A. rabiei.*

Data concerning the effect of various concentrations of methanolic stem-bark extract of *E. citriodora* on biomass of target fungal pathogen is illustrated in Fig. 1B & 2. Stem-bark extract generally the effect similar to that of different concentrations of fruit extract. Biomass of *A. rabiei* was significantly reduced by all the concentrations of stem-bark extract over control. There was 47–61% and 46–60% decline in fungal biomass due to different concentrations of the extract as compared to negative and positive control treatments, respectively. Data about the effect of different concentrations of methanolic root-bark extract of *E. citriodora* on biomass of the test fungal species is demonstrated in Fig. 1C & 2. Root-bark extract exhibited the more pronounced effect on fungal biomass than fruit and stem-bark extracts. Different concentrations of root-bark extract significantly reduced fungal biomass by 72–89% and 71–88% over negative and positive control treatments, respectively. Earlier, Fiori *et al*. (2000) demonstrated that crude *E. citriodora* extracts can effectively control the growth of fungus *Didymella bryoniae,* the cause of gummy stem blight in cucurbits. Antifungal activity of methanolic extracts of *E. citriodora* could possibly be due to eucalyptus oils (Fiori *et al.,* 2000). Ramezani *et al*. (2002) reported that volatile oil of *E. citriodora* controlled the growth of many plant pathogenic fungi namely *Fusarium oxysporum, Colletotrichum lindemuthianum, Alternaria triticina, Helminthosporium oryzae*, *Alternaria solani* and *Rhizoctonia solani*.

This study concludes that extracts of all the studied parts of *E. citriodora* have antifungal potential against *A. rabiei.* However, root-bark extract is the best against growth of this fungal pathogen.

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Table 1: Analysis of variance (ANOVA) for the effect of different concentrations of methanolic leaf, stem-bark and root-bark extracts of *Eucalyptus citriodora* on biomass of *Ascochyta rabiei*.

Sources of variation	df	SS	MS	F values
Treatments	29	4.235	0.146	243*
Plant parts (P)	2	0.451	0.225	374*
Concentration (C)	9	3.653	0.406	674*
P × C	18	0.132	0.0073	12*
Error	90	0.054	0.0006	
Total	119	4.289		

*, Significant at $P \leq 0.001$.

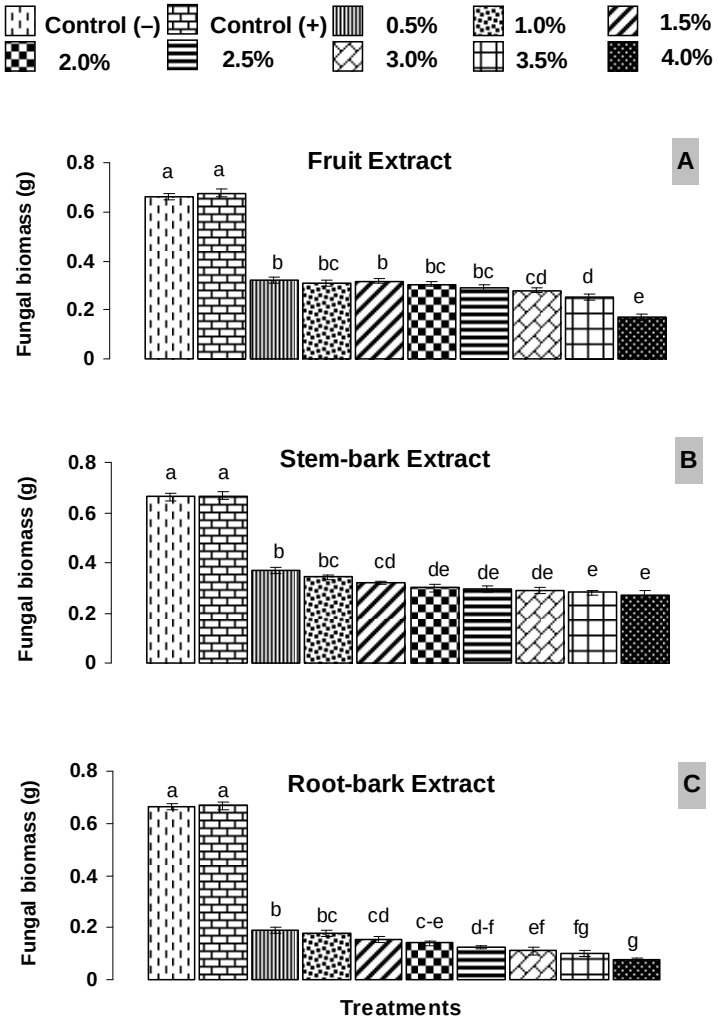

Fig. 1: Effect of different concentrations of methanolic leaf, stem-bark and root-bark extracts of *Eucalyptus citriodora* on growth of *Ascochyta rabiei*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

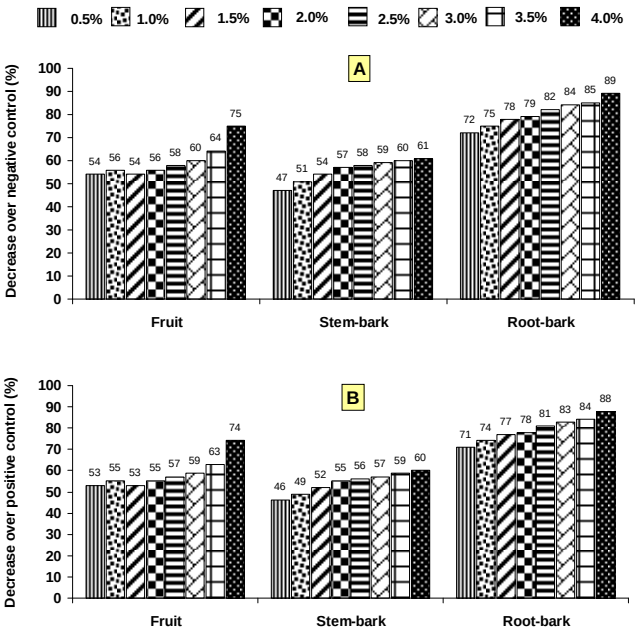

Fig. 2: Percentage decrease in biomass of *Ascochyta rabiei* due to different concentrations of methanolic leaf, stem-bark and root-bark extracts of *Eucalyptus citriodora* over negative and positive control treatments.

References

Ali H, Alam SS, Attanayake RN, Rahman M, Chen W, 2012. Population structure and mating type distribution of the chickpea blight pathogen *Ascochyta rabiei* from Pakistan and the United States. *J. Plant Pathol.,* **94**: 99-108.

Alwawi H, Moulla M, Choumane W, 2009. Genotype-environment interaction and genetics parameters in chickpea (*Cicer arietinum* L.). *Middle East J. Scientific Res.,* **4**: 231-236.

Amin M, Javaid A, 2012. Evaluation of methanolic leaf and bark extracts of *Syzygium cumini* against *Ascochyta rabiei. Mycopath*, **10**: 31-36.

Chang KF, Ahmad HU, Hwang BD, Gossen SE, 2007. Sensitivity of field popuations of *Ascochyta rabiei* to chlorothalonil, mancozeb and pyraclostrobin fungicides and effects of strobilurin fungicides on the progress of Ascochyta blight of chickpea. *Can. J. Plant Sci.,* **87**: 937-944.

FAOSTAT, 2009. Database http://faostat.fao.org/site/339/default.aspx

Gil J, Nadal S, Luna D, Moreno MT, 1996. Variability of some physico-chemical characters in Desi and Kabuli chickpea types. *J. Food Sci. Agric.,* **71**: 179-184.

Ibrikci H, Knewtson S, Grusak MA, 2003. Chickpea leaves as a vegetatble green for humans: Evaluation of mineral composition. *J. Food Sci. Agric.,* **85**: 945-950.

Ilyas MB, Chaudhry MA, Javed N, Ghazanfar MU, Khan MA, 2007. Sources of resistance in chickpea germplasm against Ascochyta blight. *Pak. J. Bot.,* **39**: 1843-1847.

Iqbal D, Javaid A, 2012. Bioassays guided fractionation of *Coronopus didymus* for its antifungal activity against *Sclerotium rolfsii. Nat. Prod. Res.,* **26**: 1638-1644.

Jabeen K, Javaid A, 2008. Antifungal activity of aqueous and organic solvent extracts of allelopathic trees against *Ascochyta rabiei. Allelopathy J.,* **22**: 231-238.

Jabeen K, Javaid A, Ahmad E, Athar M, 2011. Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei*-the cause of chickpea blight. *Nat. Prod. Res.,* **25**: 264-276.

Jamil FF, Sarwar M, Sarwar N, Khan JA, Zaid HM, Yousaf S, Imran HM, Haq I, 2010. Genotyping with RAPD markers resolves pathotype diversity in the Ascochyta blight and Fusarium wilt pathogens of chickpea in Pakistan. *Pak. J. Bot.,* **42**: 1369-1378.

Javaid A, Samad S, 2012. Screening of allelopathic trees for their antifungal potential against *Alternaria alternata* strains isolated from dying back *Eucalyptus* spp. *Nat. Prod. Res.,* **26**: 1697-1702.

Javaid A, Shoaib A, 2012. Chapter 12. Allelopathy for the Management of Phytopathogens. In: *Allelopathy: Current Trends and Future Applications*. Cheema ZA, Farooq M, Wahid A (Eds.). Springer Publishers. ISBN 978-3-642-30594-8. pp. 299-319.

Kanwal Q, Hussain I, Siddiqui HL, Javaid A, 2011. Antimicrobial activity screening of isolated flavonoids from *Azadirachta indica* leaves. *J. Serb. Chem. Soc.,* **76***:* 375-384.

Naqvi SF, Javaid A, Shoaib A, 2012. Evaluation of antifungal activity of methanolic extracts of *Dicanthium annulatum* for the management of *Macrophomina phaseolina. Afr. J. Microbiol. Res.,* **6**: 5882-5886.

Ramezani H, Singh HP, Batish DR, Kohli RK, 2002. Antifungal activity of the volatile oil of *Eucalyptus citriodora. Fitoterapia,* **73**: 261-262.