Mycopath (2015) 13(1)**:** 7-11 **Research Article**

Management of *Macrophomina phaseolina* by extracts of *Launea nudicaulis*

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Abstract

Laboratory bioassays were carried out to assess antifungal activity of different parts of *Launea nudicaulis,* a weed of family Asteraceae against a highly problematic soil-borne fugal plant pathogen *Macrophomina phaseolina*. Dried and crushed different parts of the weed namely leaf, stem, root and inflorescence were extracted with methanol for two weeks. After evaporation of methanol on a rotary evaporator, the remaining materials were dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water to prepare different concentrations ranging from 10 to 50 mg mL^{-1}. In general, all the extracts showed variable antifungal activity. The highest antifungal activity was exhibited by methanolic leaf extract followed by stem, root and inflorescence extract resulting in 20–75%, 9–66%, 5–58% and 3–39% decline in fungal biomass as compared to control, respectively. A linear relationship was recorded between different concentrations of methanolic leaf, stem, root and inflorescence extract, and fungal biomass with R^2 = 0.9733, 0.9768 and 0.8854 and 0.9642, respectively. This study concludes that methanolic leaf and stem extracts of *L. nudicaulis* possess potent antifungal activity against *M. phaseolina.*
Keywords: Antifungal activity, asteraceous weed, *Launea nudicaulis, Macrophomina phaseolina*.

Introduction

Macrophomina phaseolina is an important soil- and seed-borne pathogen that causes diseases especially charcoal rot in a large number of plant species including many economically important crops such as maize, cotton, sunflower, soybean, and sesame (Mayek-Perez *et al.*, 2002; Beas-Fernandez *et al.*, 2006; Abdel-Kader *et al.*, 2010; Ijaz *et al*., 2013). Charcoal rot is of prime importance in reducing crop yield especially in arid regions of the world. It is an important disease during hot and dry weather or when unfavorable environmental conditions prevails (Dhingra *et al.*, 1977). Some chemical fungicides like benomyl, thiophanate-methyl, thiram, thiabendazole, triforine, and captan have been proved effective in laboratory tests against *M. phaseolina* (Ilyas *et al.*, 1975). However, synthetic agrochemical also cause environment pollution and ill effects on humans and animals health (Gurjar *et al*., 2012). Therefore, some alternative environmental friendly measures are needed to combat the hazard. In recent years, scientists have explored large number of natural resources against plant pathogens and very encouraging results have been obtained (Tripathi *et al*., 2008; Javaid and Iqbal, 2014; Javaid and Rauf, 2015). Many scientists used crude extracts as well as purified compounds of various plant species like *Chenopodium album* L., *Chenopodium murale* L., *Melia azedarach* L., *Coronopus didymus* (L.) Sm and *Datura metel* L. which found very effective in the management of plant pathogens namely *M. phaseolina, Ascochyta rabiei* (Pass.) Lab. and *Sclerotium rolfsii* Sacc. (Javaid and Amin, 2009; Jabeen *et al.*, 2011; Javaid and Iqbal, 2014; Jabeen *et al.*, 2014).

Launea nudicaulis (family Asteraceae) is a perennial naked stemmed herb containing about 2 cm wide yellow flowers with sweet and pleasant smell and is particularly and frequently used by local people in medicines (Alkhatalan *et al*., 2014). Its leaves are used to relieve fever in children, also used for treatment of skin itches, cuts, ulcers, swelling, bilious fever, eczema eruptions and rheumatism and roots are used for tooth ache (Rashid *et al*., 2000; Khan *et al.*, 2012). Due to its wide application in folk medicine and various potent biological activities including insecticidal, cytotoxic, antimicrobial, hypoglycemic and anti-inflammatory (Al-Mahrezi *et al.*, 2011), this plant has been extensively studied and reported to be rich source of various classes of compounds like; flavonoid, terpenoids, acetylene, sphingolipids, steroids and their glycosides (Daur, 2012; Alkhathalan *et al.*, 2014). However, studies regarding its antifungal activity especially against *M. phaseolina* are lacking. Therefore, the present study was carried out to investigate antifungal potential of methanolic extracts of various parts of this weed against *M. phaseolina.*

Materials and Methods

Plant materials and extract preparation

Plants of *L. nudicaulis* were collected during spring and summer of 2014 from different localities of Punjab University, Quaid-e-Azam Campus, Lahore, Pakistan. Different plant parts viz. leaves, stems, roots and inflorescence were separated, washed with water and then air dried at room temperature for three weeks. The dried plant parts were milled to a fine powder and stored in polythene bags at room temperature until used. Two hundred grams of the dried powdered sample of each part were soaked in 1.5 L methanol in covered containers and left for 2 weeks. Thereafter, the plant materials were filtered with cheese cloth and then filtered with filter paper. The filtrates were evaporated on a rotary evaporator at 45 °C. To completely evaporate methanol, the materials were taken in pre weighed beakers and evaporated in an electric oven at 45 °C. Finally, 15.5 g of stem extract, 17.4 g of leaf extract, 12.75 g of root extract and 9.75 g of inflorescence extract were obtained.

Determination of antifungal properties of the extracts

Plant extracts were tested for their efficiency against *M. phaseolina* by using protocol described by Javaid and Iqbal (2014). Stock solutions of methanolic extracts of each of the four plant parts were prepared by dissolving 9 g of each extract in 5 mL of dimethyl sulphoxide (DMSO) followed by addition of sterilized distilled water to make a total volume 15 mL. Likewise, a control solution was also prepared by dissolving 5 mL DMSO in 10 mL of sterilized distilled water. Purpose of this control solution to maintain equal amount of DMSO in different concentration of the extracts in the finally prepared medium. Different concentrations of the extracts viz. 1%, 2%, 3%, 4% and 5% were prepared in malt extract (ME) broth. For each concentration, 55 mL of ME broth were autoclaved at 121 °C, cooled at roomed temperature and added specified quantities of stock and control solutions to prepare 60 mL of the medium of a specific concentrations. For control treatment, only 5 mL of control solution was added to 55 mL autoclaved ME broth. Chloromycetein was added in each flask to avoid bacterial contamination. Sixty milliliters ME broth of each concentration was divided into 15 mL portions and poured in sterilized 100-mL volume flasks. Each flask was inoculated with mycelial disc of *M. phaseolina* of 4 mm diameter. Each treatment was replicated four times. Inoculated flasks were incubated at 25±2 °C for seven days. Thereafter, fungal biomass was filtered; oven dried at 70 ºC and weighed. Experiment was carried out using completely randomized design.

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Data analysis

Acquired experimental data were analyzed by analysis of variance. Means were separated by LSD method at 5% level of significance using computer software Statistix 8.1. Relationship between extract concentrations and fungal biomass was calculated by using MS Excel.

Results and Discussion

Analysis of variance revealed a significant effect of different parts of *L. nudicaulis* (P), concentration of methanolic extract (C) as well as effect of P×V for biomass production of *M. phaseolina* (Table 1). Variation in antifungal activities of different parts have also been reported in *Coronopus didymus*, *Datura metel* and *Chenopodium album* against *Sclerotium rolfsii*, *M. phaseolina* and *Fusarium oxysporum* f. sp. *cepae* (Iqbal and Javaid, 2012; Javaid and Saddique, 2012; Rauf and Javaid, 2013). Difference in antifungal activity of different parts of *L. nudicaulis* and other plant species may be attributed to different types of compounds or their quantities in different plant parts.

Out of four parts of *L. nudicaulis* used in bioassays, methanolic leaf extract exhibited the highest antifungal activity against *M. phaeolina.* Different concentrations of this extract significantly reduced fungal biomass by 20–75% over control (Fig. 1 & 2). Regression analysis showed that there was a linear relationship between extract concentrations and biomass of the fungal pathogen with R^2 = 0.9733 (Fig. 3A). Methanolic stem and root extract extracts also expressed pronounced antifungal activity against *M. phaseolina* resulting in 9–66% and 5–58% suppression in fungal biomass over control (Fig. 1 & 2). There was a linear relationship between stem and root extracts concentrations and fungal biomass with R^2 = 0.9768 and 0.8854, respectively (Fig. 2 B & C). Methanolic inflorescence extract showed the least antifungal activity. Although 20 mg mL^{-1} and higher concentrations had a significant adverse effect on fungal biomass, however, there was just 3–39% decline in fungal biomass due to different concentrations of the extract (Fig. 1 & 2). Relationship between extract concentration and fungal biomass was linear with R^2 = 0.9642 (Fig. 3 D). Methanolic extract of *L.*

nudicaulis also known to reduce growth of *Aspergillus* spp. (Rashid *et al.*, 2000). Recently, Nivas and Boominathan (2015) reported antimicrobial activity of methanolic extracts of aerial parts of *L. nudicaulis* against *Streptococcus pneumoniae.* They found alkloids, flavonoids and saponins in the extract. Zellagui *et al.* (2012) reported antimicrobial activity of *L. nudicaulis* and 19 compounds in essential oils of this plant, with dioctyl phthalate as the major constituent (39.84%) possibly responsible for antimicrobial activity.

The present study concludes that methanolic leaf and stem extracts contain potent antifungal constituents for the management of *M. phaseolina.*

Table 1: Analysis of variance (ANOVA) for the effect of methanolic extracts of different parts of *Launea nudicaulis* on biomass of *Macrophomina phaseolina*.

Sources of variation	df	SS	MS	F values
Plant parts (P)	3	22729	7576	800*
Concentration (C)	5	97132	19426	2051*
P × C	15	9033	602	64*
Error	72	682	9.5	
Total	95	129577		

*, Significant at P≤0.001.

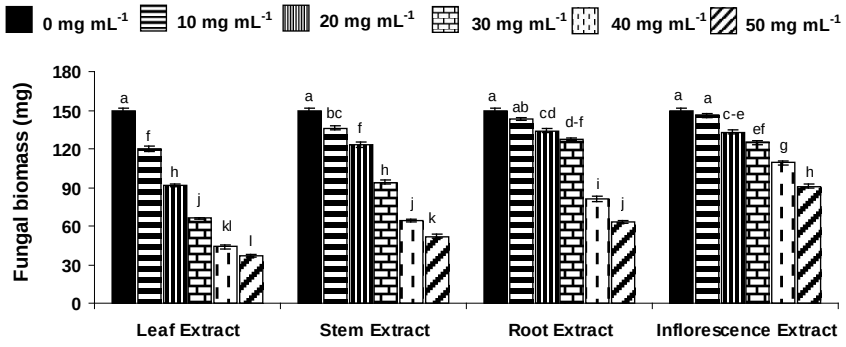

Fig. 1: Effect of methanol extracts of *Launea nudicaulis* on biomass of *Macrophomina phaseolina.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

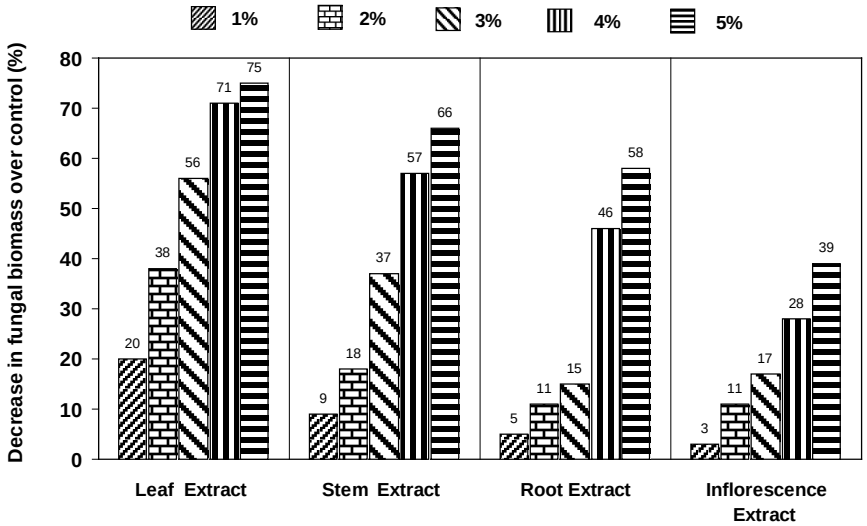

Fig. 2: Percentage reduction in biomass of *Macrophomina phaseolina* due to methanolic extracts of different parts of *Launea nudicaulis* over control.

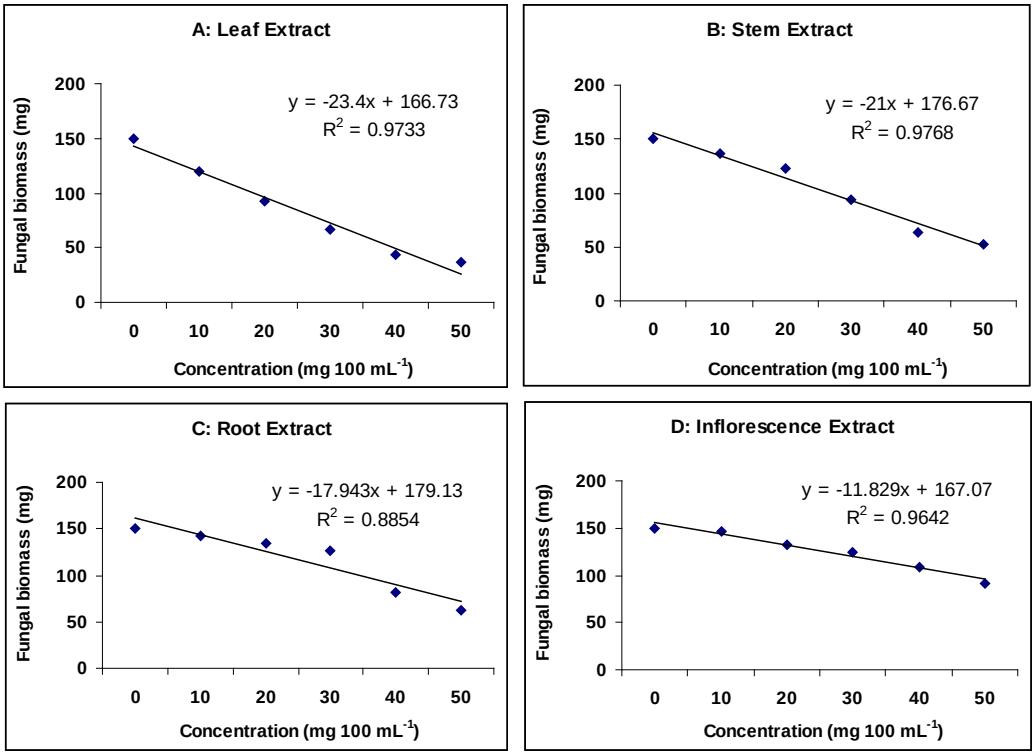

Fig. 3: Relationship between concentrations of methanolic leaf, stem, root and inflorescence extracts of *Launea nudicaulis* on biomass of *Macrophomina phaseolina*.

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