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# USE OF METHANOLIC EXTRACTS OF AN ASTERACEOUS WEED *Eclipta alba* FOR CONTROL OF *Macrophomina phaseolina*

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**ABSTRACT**

*Macrophomina phaseolina (Tassi) Goid is a destructive fungal phytopathogen causing diseases in hundreds of plant species. No registered fungicide is available so far to control this pathogen. This study was carried out to explore the antifungal activity of methanolic extracts of different parts of Eclipta alba (L.) Hassk, a weed of family Asteraceae, against M. phaseolina. Different concentrations (0, 1, 2, 3, 4 and 5%) of methanolic extracts of leaf, stem, root and inflorescence of this weed were evaluated against the target fungal pathogen using malt extract broth as growth medium. In general, extracts of all the plant parts exhibited variable antifungal activity. There was a linear and inverse relationship between fungal biomass and extract concentrations. Leaf and stem extracts showed the best antifungal activities resulting in up to 64% and 61% decrease in biomass of M. phaseolina, respectively. Methanolic extracts of root and inflorescence were proved comparatively less effective than extracts of other plant parts and reduced fungal biomass up to 32% and 19%, respectively.*

**Key words:** Antifungal activity, asteraceous weed, *Eclipta alba, Macrophomina phaseolina,* methanolic extracts.

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## INTRODUCTION

*Macrophomina phaseolina* causes diseases on a wide range of plants (more than 500 plant species) in about 100 angiosperm families in many regions of the world (Ijaz *et al*., 2013). Most common diseases caused by this fungal pathogen are charcoal rot, dry rot, leaf blight, ashy stem blight and damping off. Hosts of this pathogen include grasses [*Zea mays* L., *Avena sativa* L., *Sorghum bicolor* (L.)

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Moench], legumes [*Glycine max* (L.) Merr.*, Arachis hypogaea* L.*, Cicer arietinum* L.*, Vigna unguiculata* (L.) Walp.], woody plants and many other important agricultural crops such as sunflower (Ali and Dennis 1992; Rayatpanah *et al.,* 2012; Yildiz and Benlioğlu, 2014). Although it generally causes diseases in plants, however, it is also a human opportunistic pathogen (Srinivasan *et al.,* 2009). It is also a seed-borne pathogen present on seed coat and cotyledons (Reuveni *et al.,* 1983). When infected seeds are sown, the fungus infects the seedling and later on transmits to fruits through peduncle where it infects the seeds (Ijaz *et al.,* 2013). The diseases due to *M. phaseolina* are most common in areas with low precipitation and high temperatures (Mirza and Beg, 1983).

Although some chemical fungicides such as 5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide and tetramethylthiuram disulfide are effective against *in vitro* growth of *M. phaseolina* (Shahda *et al.,* 1991)*,* however, there is not any registered fungicide against this pathogen so far. Moreover, synthetic agrochemicals also cause environmental pollution and ill effects on health (Oruc, 2010). Therefore, some alternative environmental friendly measures are needed to combat the menace. In recent years, scientists have explored large number of natural resources against plant pathogens and very encouraging results have been obtained (Javaid and Shoaib, 2012; Javaid *et al.,* 2014). Many recent studies showed that crude extracts of various plant species such as *Chenopodium album* L.*, Chenopodium murale* L.*, Syzygium cumini* (L.) Skeels*, Coronopus didymus* (L.) Sm and *Datura metel* L. were very effective in the management of phytopathogens namely *M. phaseolina, Ascochyta rabiei* (Pass.) Lab. and *Sclerotium rolfsii* Sacc. (Javaid and Amin, 2009; Jabeen and Javaid, 2010; Iqbal and Javaid, 2012; Jabeen *et al.,* 2014). Researchers have also isolated many potential antifungal compounds from plants such as β-amyrin from *Melia azedarach* L. (Jabeen *et al.,* 2011), 7-*O*--glucoside and (-)-epi-catechin from *Azadirachta indica* A. Juss. (Kanwal *et al.,* 2011), and 6-nonadecynoic acid from *Sommera sabiceoides* K. Schum. (Li *et al.,* 2008). Likewise, Kanwal *et al.* (2010) isolated two antifungal compounds namely (-)-epicatechin-3-O-β-glucopyranoside and 6-(*p*-hydroxybenzyl) taxifolin-7-O-β-D-glucoside (tricuspid) from mango (*Mangifera indica* L.), which were very effective against *M. phaseolina.*

*E. alba* commonly grows as a weed in moist places in warm temperate to tropical areas globally. It has a long history of medicinal uses. It has been used as antihaemorrhagic, antimycotoxic, antioxidant, antihepatotoxic, antihyperglycemic, antibacterial, analgesic and immunomodulatory agent (Chokotia *et al.,* 2013). Previously, most of the studies on this plant have been carried out

regarding its pharmaceutical properties (Nahar, 1993; Sawant *et al.,* 2004; Kumar *et al.,* 2005; Kima *et al.,* 2008), and little is known about its antifungal activities especially against fungal plant pathogens. The present study was, therefore, carried out to assess the antifungal activity of methanolic extracts of different parts of this plant against *M. phaseolina.*

## MATERIALS AND METHODS

### Preparation of methanolic extracts

For preparation of methanolic extracts of *E. alba,* 200 g of dried stems, leaves, roots and inflorescence were separately soaked in 1.0 L methanol in closed jars for 15 days. Materials were filtered through double layer muslin cloth and filtrates were again filtered through filter paper. Filtrates were evaporated under reduced pressure on rotary evaporator at 45 °C to remove methanol. Materials obtained were placed in pre-weighed beakers and further dried in an oven at 45 °C to completely evaporate methanol. Finally, 16.8 g stem extract, 12 g leaf extract, 10.75 g root extract and 14.6 g inflorescence extract were obtained.

### Bioassays with methanolic extracts

*In vitro* bioassays were carried out with methanolic extracts of *E. alba.* Crude methanolic extract (9.0 g) of each part of *E. alba* was dissolved in 5 mL dimethyl sulphoxide (DMSO) followed by addition of autoclaved distilled water to prepare 15 mL of stock solution. In a similar way, 5 mL DMSO was added in 10 mL autoclaved distilled water to prepare control solution. Fifty five milliliters malt extract (ME) broth was autoclaved at 121 ºC for half an hour in 250 mL conical flasks and cooled at room temperature. Chloromycetin at 50 mg 100 $mL^{-1}$ of the medium was added to avoid bacterial contamination. Five concentrations viz. 0, 1, 2, 3, 4, 5 g 100 $mL^{-1}$ were prepared by adding appropriate quantities of stock solution and control solution to each 250 mL flask to make total volume of the medium 60 mL. These 60 mL were divided into four 15 mL portions in 100 mL conical flasks. Experiment was carried out using completely randomized design. Flasks were inoculated with mycelial plugs (5 mm diameter) from 7 days old colony of *M. phaseolina* and incubated for one week. Thereafter, fungal biomass was filtered, dried at 70 ºC in an oven and weighed (Javaid and Iqbal, 2014).

### Statistical analysis

All the data were analyzed by analysis of variance. Means were separated by Tukey's HSD Test 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 presented in Table-1 shows that the effect of plant parts (P), extract concentrations (C) and P×C was significant (P≤0.001) for biomass of *M. phaseolina.* Among the four types of methanolic extracts, leaf extract showed the best antifungal activity. All the concentrations of this extract significantly declined biomass of *M. phaseolina* by 10-64% (Fig. 1A & 2). There was a linear and inverse relationship between extract concentration and fungal biomass with $R^2$ = 0.9699 (Fig. 3 A). Methanolic stem extract was also found highly effective against the target fungus. All the concentrations of stem extract except 1% significantly suppressed biomass of the fungus. There was 3-61% decrease in biomass of *M. phaseolina* due to 1-5% concentrations of the stem extract (Fig. 1B & 2). There was a parallel reduction in fungal biomass with an increase of extract concentration. Relationship between extract concentration and fungal biomass was linear with $R^2$ = 0.9732 (Fig. 3 B). Earlier, Bakht *et al.* (2011) reported that methanolic extract of *E. alba* exhibited strong antifungal activity against *Candida albicans* (Robin) Berkhout*.* Peraman *et al.* (2011) found that ethanolic extract of *E. alba* showed moderate antifungal activity against *Aspergillus niger* van Tieghem and *C. albicans.* Methanolic leaf extract of *E. alba* also showed antifungal activity against *A. niger, Penicillium citrinum* Thom and *Aspergillus flavus* Link (Prabu *et al.,* 2011). *E. alba* contains a large number of bioactive substances including alkaloids, glycosides, polyacetylenes, coumestans, triterpenoids and flavonoids (Chokotia *et al.,* 2013). Leaves of *E. alba* possess wedelolactone, a-terthienylmethanol, stigmasterol, demethylwedelolactone-7-glucoside and dimethyl-wedelolactone (Wagner *et al.,* 1986), which may be responsible for antifungal activity. Venkatesan and Ravi (2004) suggested an alkaloid from shoot extract of *E. alba* with strong antifungal activity against *Candida tropicalis* Berkhout*, Rhodotorula glutinis* and *C. albicans*.

Methanolic extract of *E. alba* roots was relatively less antifungal than leaf and stem extracts. All concentrations of this extract except the lowermost one (1%) significantly reduced biomass of *M. phaseolina.* Different concentrations of the root extract decreased biomass of the fungus by 2-32% over control (Fig. 1C & 2). Methanolic extract of inflorescence exhibited the least antifungal activity where only 3% and higher concentrations had a significant effect on fungal biomass. There was only 1-19% reduction in fungal biomass due to various concentrations of this extract (Fig. 1D & 2). There were also linear and inverse relationships between concentrations and fungal biomass for methanolic root and inflorescence extracts (Fig. 3C & D).

## CONCLUSION

The present study concludes that methanolic leaf and stem extracts are very effective against *in vitro* growth of *M. phaseolina.* More studies are needed to isolate and identify the effective antifungal constituents from these extracts.

**Table-1.** Analysis of variance (ANOVA) for the effect of different concentrations of methanolic leaf, stem, root and inflorescence extracts of *Eclipta alba* on biomass of *Macrophomina phaseolina*.

| Sources of variation | df | SS | MS | F values |
|---|---|---|---|---|
| Plant parts (P) | 3 | 21844 | 7281 | 1209* |
| Concentration (C) | 5 | 56936 | 11387 | 1890* |
| P × C | 15 | 12865 | 858 | 142* |
| Error | 72 | 434 | 6 | |
| Total | 95 | 92078 | | |

* Significant at P≤0.001.

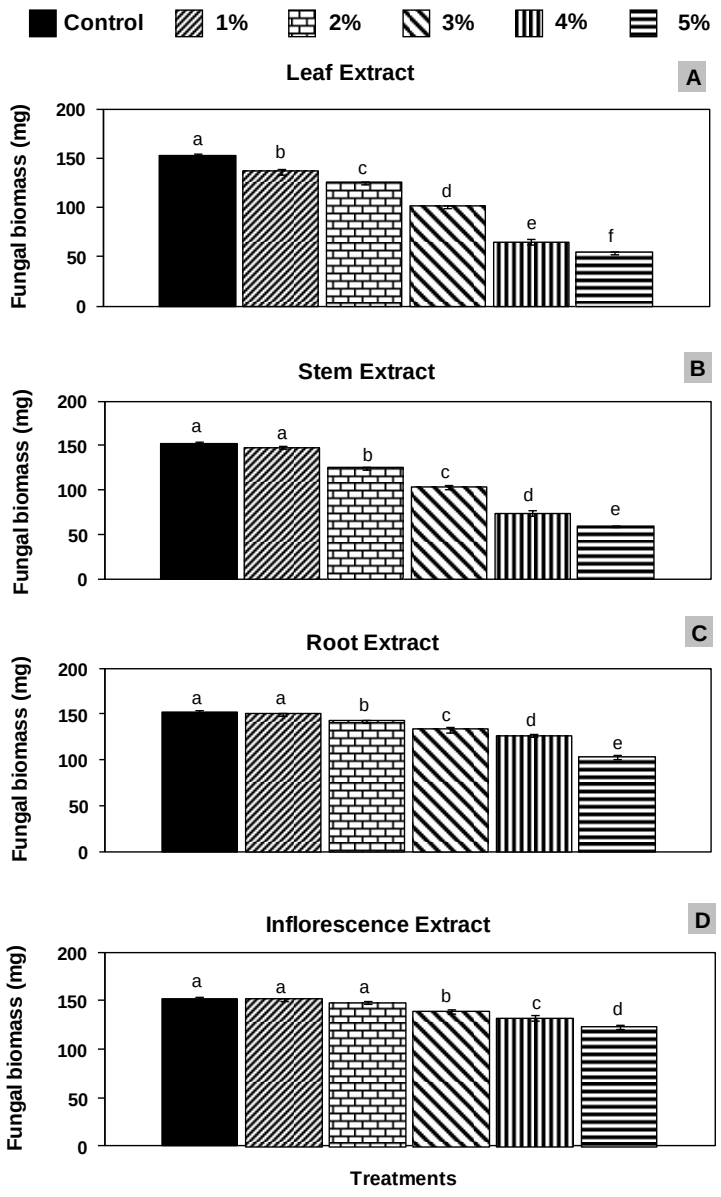


**Figure 1.** Effect of different concentrations of methanol extracts of *Eclipta alba* on biomass of *Macrophomina phaseolina.* Vertical bars show standard errors of means of three replicates. In the figures, the values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

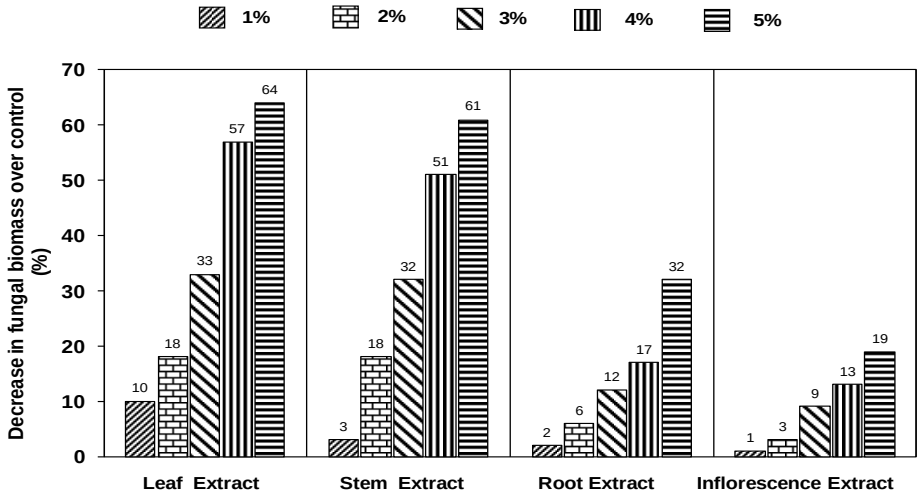


**Figure 2.** Percentage decrease in biomass of *Macrophomina phaseolina* due to different concentrations of methanolic leaf, stem, root and inflorescence extracts of *Eclipta alba* over control.

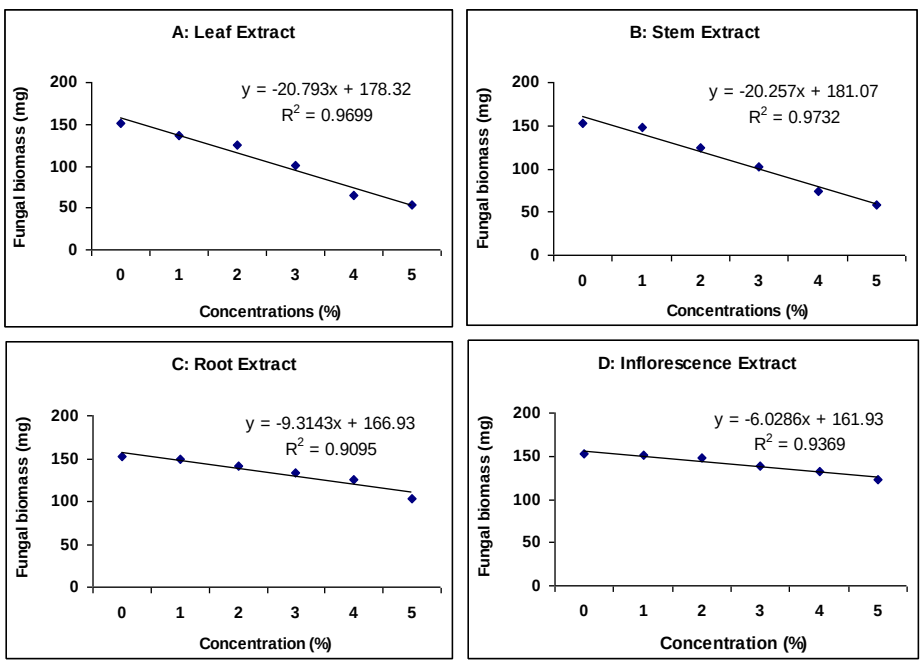


**Figure 3.** Regression analysis for the effect of different concentrations of methanolic leaf, stem, root and inflorescence extracts of *E. alba* on biomass of *M. phaseolina*.

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