USING EXTRACTS OF LAMBSQUARTERS WEED FOR CONTROL OF *Alternaria alternata*

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ABSTRACT

Antifungal potential of extracts of different parts of lambs quarters weed (Chenopodium album L.) against a plant pathogenic fungus Alternaria alternate assessed through laboratory bioassays. Different parts of the weed were soaked in methanol for two weeks to get the extracts. The solvent was evaporated under reduced pressure and different concentrations of the extracts of each plant part (1 to 5%) were prepared in malt extract broth. Leaf, root and fruit extracts exhibited pronounced antifungal activity resulting in 23–95%, 29–96% and 9–94% suppression in biomass of A. alternata. The effect of stem extract was insignificant. This study concluded that all parts of C. album except stem contain potent antifungal constituents to control A. alternata.

Keywords: *Alternaria alternata, Chenopodium album,* fungicidal, methanolic extract.

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INTRODUCTION

A number of plant pathogenic fungi produce toxins that damage the plant and plant products (Proctor *et al.*, 2018). Species of genus *Alternaria* are saprophytic in nature and produce various secondary metabolites with toxic effects on agricultural commodities (Lawrence *et al.*, 2016). *Alternaria alternata* is considered as an opportunistic, ubiquitous, cosmopolitan and seed-inhabiting plant pathogen responsible for economic yield losses (Wenderoth *et al.*, 2017). It produces enormous mycotoxins such as altenuene, altenuisol, altenusin, alternariol, tentoxin, altertoxin and altenin (Dang *et al*., 2015). It causes leaf spot, leaf blight, stem canker, rot, black spot, necrotic lesion and brown spot diseases in more than 380 host plant species (Al-Ghafri *et al*., 2019). Generally, the isolates of *A. alternata* pose a serious threat to crops by producing dark colored, multicellular, single or multiple chains of conidia attached with conidiophores (Guevara *et al.*, 2019). The fungus also survives in the form of mycelium for considerable time by overwintering in soil, plant debris and dead decayed plant materials (Gabriel *et al.*, 2016). Chemical control is considered as an effective control measure to reduce the pathogen inoculum. Many synthetic fungicides are available in market against this destructive pathogen but due to the development of new pathogenic fungal strains and their hazardous impact on environment and human beings, their usage has been restricted (Sondhia and Waseem, 2019). The difficulties encountered for the treatment of *A. alternata* have stimulated the search for plant-based products as an attractive, cheap and environment friendly alternate to the synthetic chemicals (Javaid and Samad, 2012; Li *et al.*, 2018).

Plant derived products are gaining importance worldwide because of their safe status, consumer acceptance and wide host range against the pathogens (Mishra *et al*., 2018). *Chenopodium album* is an annual fast-growing weed plant widely grown in Europe, America and Asia (Tanveer and Shah, 2017). It is a rich source of medicinally important bioactive constituents including antioxidant, antinociceptive, antifungal, anticancer, antibacterial and antimicrobial properties (Lone *et al*., 2017; Khomarlou *et al*., 2018). Its leaves are traditionally used for the cure of diarrhea, typhoid, pneumonia, blood purifier, appetizer, cough, anorexia, influenza, dysentery and piles (Khomarlou *et al*., 2017). *C. album* extracts have previously been found very effectual against *Sclerotium rolfsii*, *Ascochyta rabiei*, *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium oxysporum* (Ali *et al*., 2017; Alkooranee *et al*., 2020). The present investigation was undertaken to assess the antifungal potential of different parts of *C. album* methanolic extracts against *A. Alternata.*

MATERIALS AND METHODS

Plants of *C. album* were collected from waste places in Lahore Pakistan. Plants were carefully uprooted and after washing with tap water, their different parts were separated. After drying in shade, materials of different parts were thoroughly crushed and stored for further use. Each plant part (100 g) was soaked in 500 mL methanol for 14 days. The solvent was then separated from debris by filtration. The solvent was then evaporated at 45 ºC using a rotary evaporator. For evaporation of final traces of the solvent, the materials were poured in 100-mL beakers and put them in an oven at 45 ºC.

For laboratory bioassays, stock solutions of methanolic extract of each plant part *viz.* leaf, stem, root and inflorescence were prepared. Equal amount of extract of each part (0.30 g) was dissolved in 0.25 mL dimethyl sulphoxide (DMSO) and added autoclaved malt extract broth (MEB) to prepare 6 mL of 50 mg mL^{-1} concentration stock solutions. For maintaining same concentration of DMSO in experimental and control treatments, a control solution was also made by dissolving 0.25 mL DMSO in 5.75 mL MEB to have 6 mL of a control

solution. A portion (3 mL) of both types of solutions was double diluted serially by addition of MEB. Consequently, different concentrations (1.562, 3.125, 6.25, 12.50, 25 and 50 mg mL^{-1}) were prepared. Bioassays were carried out in triplicates in test tubes (10-mL). There was 1 mL of the medium in each test tube. All the tubes were inoculated with 20 µL conidial suspension of *A. alternata* and incubated for 7 days at room temperature. At the end of experiment, biomass of *A. alternata* was collected on pre-weighed filter papers, and weighed after drying at 70 ºC. Percentage decrease in fungal biomass due to different concentrations over corresponding control was calculated by the following formula:

$$\text{Decrease over control (\%)} = \frac{\text{Biomass in control - Biomass in an extract treatment}}{\text{Biomass in control}} \times 100$$

All the data were analyzed with ANOVA techniques followed by LSD test at $P \leq 0.05$ using software Statistix 8.1.

RESULTS AND DISCUSSION

A variety of growth responses of the fungus were observed when *A. alternata* was exposed to different concentrations of extracts of common lambsquarters (*C. album).* Leaf extract exhibited highly pronounced antifungal activity that was increased with increasing concentrations of the extract. There was 23–95% decline in biomass of *A. alternata* when the fungus was grown in 1.562–50 mg mL^{-1} concentrations of leaf extract (Fig. 1A and 2). Regression analysis revealed that there was a linear relationship between extract concentration and fungal biomass with R^2 = 0.930 (Fig. 3A). Previously, Singh (2005) found leaf extract of *C. album* highly toxic to growth of *Sclerotium rolfsii.* According to Javaid and Amin (2009), a 4% leaf extract of this weed suppressed biomass of *Macrophomina phaseolina* by up to 44%. Leaf extract of this weed was also highly antifungal against *Ascochyta rabiei* (Jabeen *et al.,* 2014). Alkooranee *et al.* (2020) reported that leaf extract of *C. album* inhibited growth of *Sclerotinia sclerotium, Fusarium solani, Pythium aphanidermatum* and *Rhizoctonia solani* probably due to presence of fatty acid methyl esters in the extracts. When *C. album* leaves were mixed in the soil, they controlled basal rot of onion (Javaid and Rauf, 2015). An antifungal compound mucondialdehyde has been identified previously from *C. album* leaves (Tahara *et al.,* 1994).

None of the concentration of stem extract proved inhibitory to fungal growth (Fig. 1B). By contrast, earlier studies showed that stem extract of *C. album* was highly toxic to growth of other fungal species. Stem extract of 1–4% concentrations reduced biomass of *M. phaseolina* by 81–94% (Javaid and Amin, 2009). Likewise, up to 53% reduction in growth of *F. oxysporum* has been reported due to a 3% stem extract of *C. album* (Rauf and Javaid, 2013)). These variable finding suggest that methanolic stem extract of this weed is specific regarding its antifungal activity against different fungal species.

Root extract was highly effective in arresting fungal growth. A concentration of 50 mg mL^{-1} of this extract almost completely controlled the growth of *A. alternata* by suppressing its biomass by 96% while lower concentrations reduced fungal growth by 29–89% (Fig. 1C and 2). Linear regression between extract concentration and fungal biomass is given in Fig. 3C with R^2 = 0.994. Earlier, Javaid and Amin (2009) found remarkable suppression in growth of *Macrophomina phaseolina* due to root extract of *C. album.* Root extract of *C. album* (0.5–3.0%) can reduce growth of *Sclerotium rolfsii* by 15– 58% (Ali *et al.,* 2017). They identified various fatty acid methyl esters in root extract including, 12-octadecadienoic acid (Z,Z)-, methyl ester and hexadecanoic acid, methyl ester which could be the cause of antifungal activity of this extract (Agoramoorthy *et al.,* 2007). Lavaud *et al.* (2000) identified various saponins from root extract of this weed including chikusetsusaponin IVa and calenduloside E, which are also likely to be responsible for control of *A. alternata.* Saponins isolated from *Polyscias fulva* are known to possess antifungal activity against a number of yeasts and dermatophytes including *Candida albicans*, *C. lucitaniae*, *C. guilliermondii*, *Trichophyton rubrum*,

T. equinum and *T. terrestre* (Njateng *et al.,* 2015). Saponins control fungal growth possibly by interfering with fungal sterols (Trda *et al.,* 2019).

Inflorescence extract was also inhibitory to the fungal pathogen. Its various concentrations reduced fungal biomass by 9–94% (Fig. 1D and 2). Relationship between extract concentration and biomass of the pathogen is shown in Fig. 3D. Methanolic extract of this weed was very effective against *M. phaseolina.* A 4% extract the weed decreased growth of this pathogen by 96% (Javaid and Amin, 2009). Likewise, this extract was also found the most effective when extracts of different parts of *C. album* were tried against *F. oxysporum* f. sp. *cepae.* Its different concentrations (0.5–3%) caused 24–80% reduction in the fungal growth (Rauf and Javaid, 2013).

It is concluded that methanolic leaf, root and inflorescence extracts are highly antifungal against growth of *A. alternata.*

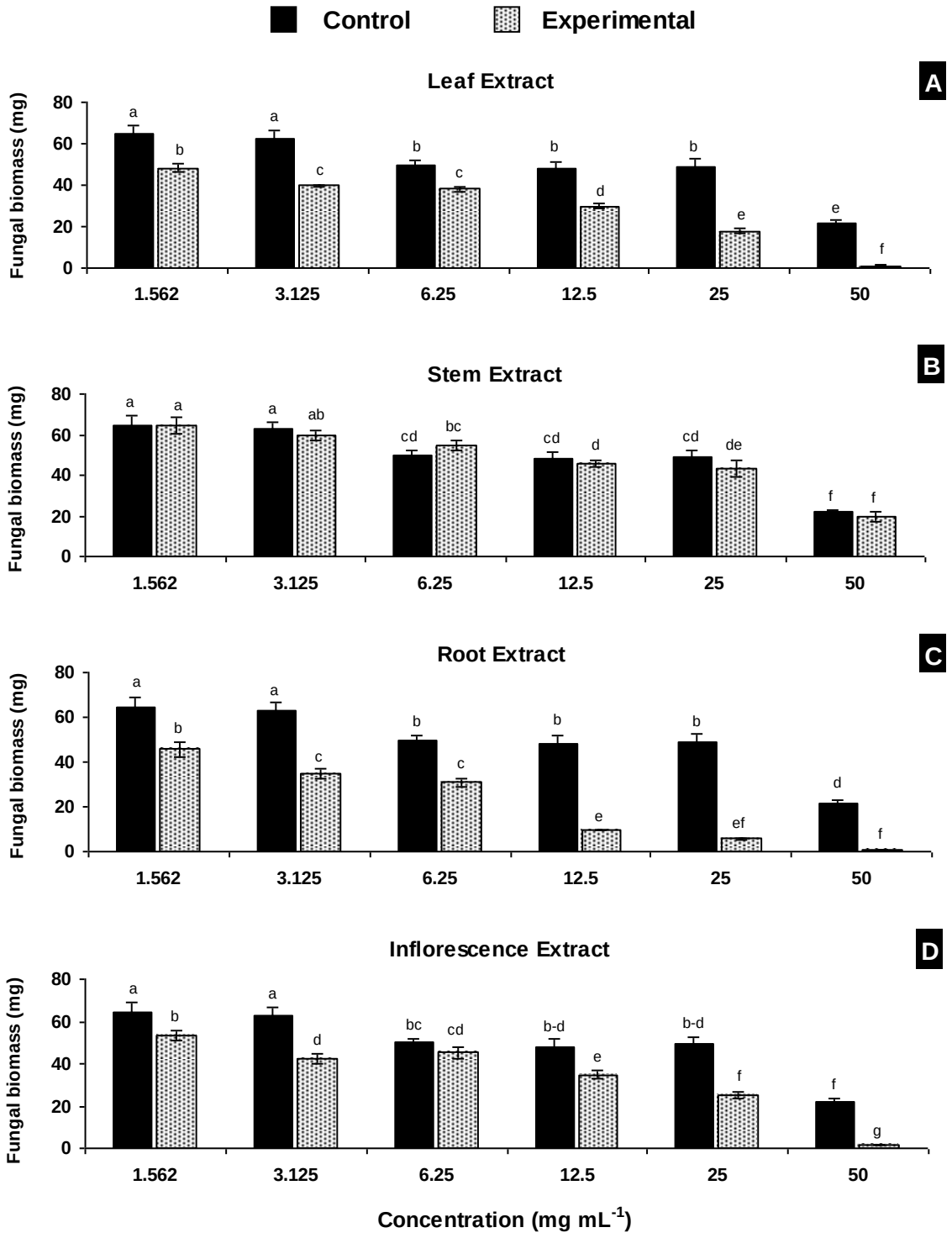

Fig. 1: Effect of different concentrations of methanolic extracts of *Chenopodium album* on biomass of *Alternaria alternata*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test

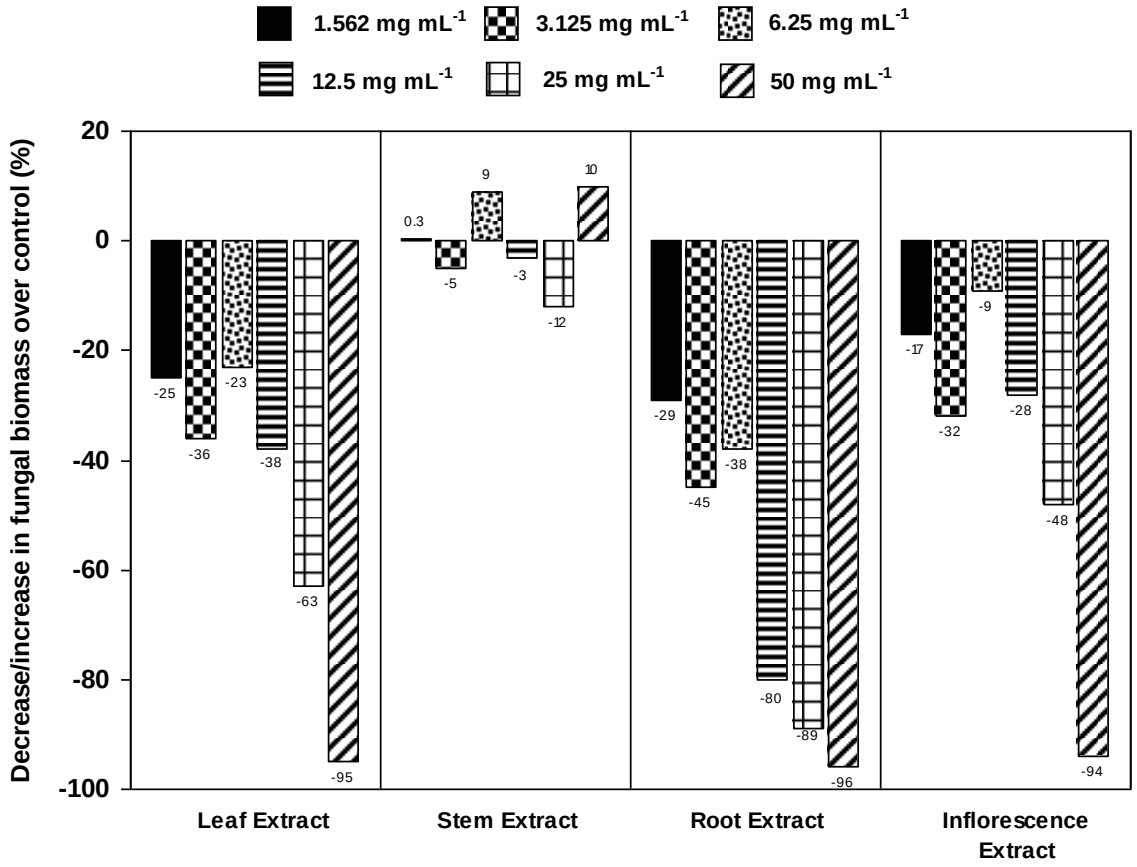

Fig. 2: Percentage decrease/increase in biomass of *Alternaria alternata* due to different concentrations of methanolic extracts of *Chenopodium album.*

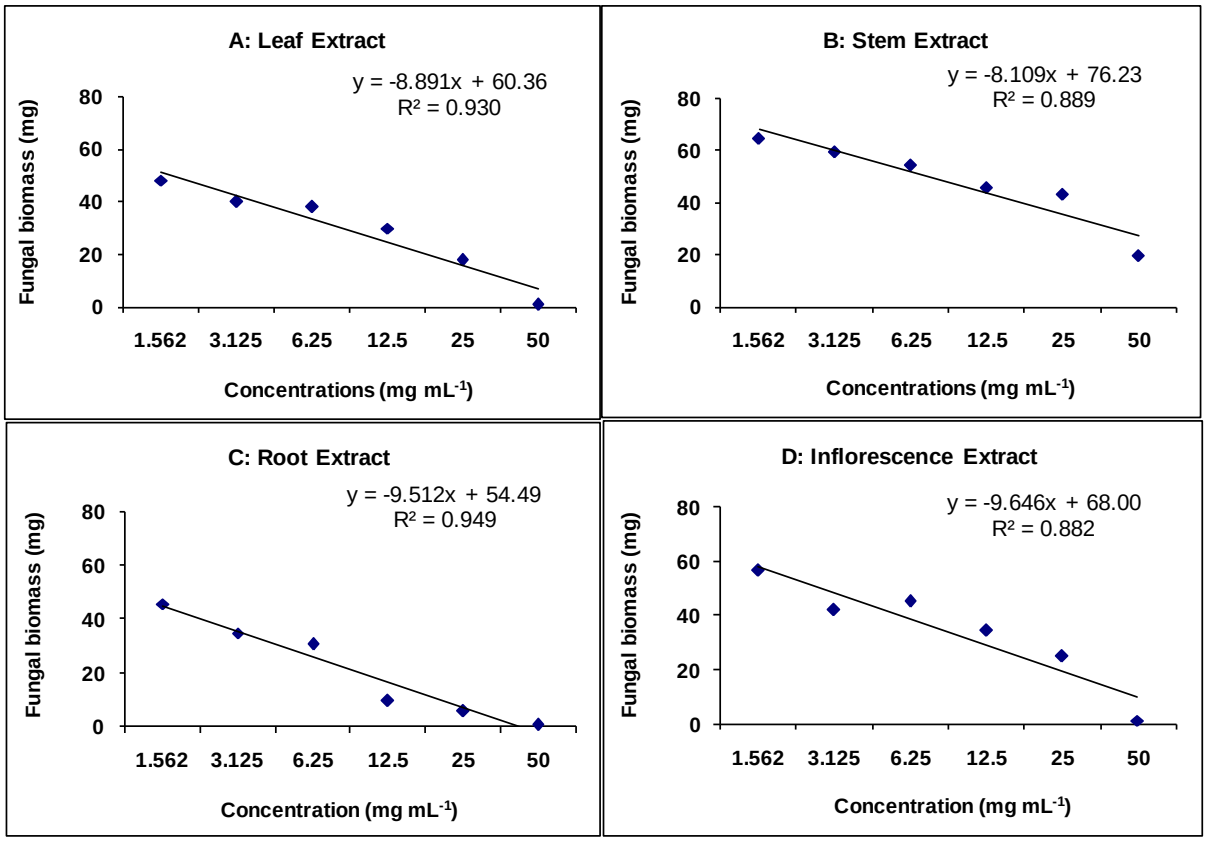

Fig. 3: Regression analysis for the effect of different concentrations of methanolic leaf, stem, root and inflorescence extracts of *Ageratum conyzoides* on biomass of *Alternaria alternata*.

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