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BIOEFFICACY OF EXTRACT OF *AGERATUM CONYZOIDES* AGAINST *DRECHSLERA AUSTRALIENSIS* AND *DRECHSLERA HOLMII*

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A B S T R A C T

Ageratum conyzoides L. a medicinal plant of family Asteraceae, possesses analgesic, anti-inflammatory and antimicrobial activities. The current study was intended for appraisal of the effect of antifungal activity of root, shoot and leaf of *A. conyzoides* against two pathogenic *Drechslera* species namely *D. australiensis* and *D. holmii*,which causes leaf blight, brown spot, crown rot, and root rot in important crops. In order to achieve this goal, 1-4% concentrations of aqueous extracts, dichloromethane (DCM) fraction and essential oils were assessed for *in vitro* antimycotic potential. Essential oils were found the most inhibitory against the test fungal growth by exhibiting 100% growth reduction than aqueous and DCM fraction of plant extracts. Among the remaining, dichloromethane fraction was proved more effective than aqueous extracts. Dichloromethane fraction (4%) exhibited maximal depression in biomass that was 92% in *Drechslera australiensis* and 91% in *Drechslera holmii.* These findings suggest the usage of aqueous extracts as well as DCM fractions and oils of *A. conyzoides* for control of fungal pathogens.

Keywords: *Ageratum conyzoides,* antifungal activity, aqueous extracts, DCM fractions, *Drechslera* species, essential oils.

INTRODUCTION

The plant derived metabolites are referred to as allelochemicals which are responsible for resistance against plant antagonism, microbial spell or insect/animal predation. Thousands of plant-derived allelochemicals have already been identified, with activity against weeds (Javaid *et al*., 2008), fungal pathogens (Shafique *et al*., 2011), nematodes and insects (Khan *et al.*, 1971). Allelopathic compounds in aqueous extract of many plants are known to parade antimycotic assets, which diminish the sprouting and mycelium proliferation of fungi. These by products are ecofriendly as compared to artificial compounds (Singh *et al*., 2003). Extracts and essential oils from many plants and herbs have been known to possess antimicrobial activity (Wilson *et al*., 1997; Bajwa *et al*., 2006). Biologically active essential oils of plants also signify a potent substitute that is globally more adequate composites for disease management. The antifungal potential of essential oils is well recognized (Guynot *et al*., 2005; Dikbas *et al*., 2008; Linde *et al*., 2010). The allelopathic potential of volatile allelochemicals from *A. conyzoides* has been reported by many workers (Kong *et al.,* 2002; 2004). Thus the purpose of underlying research was to estimate the fungitoxic potential of extracts and oils of *Ageratum conyzoides,* against two plant pathogenic *Drechslera* spp. and to determine the type and concentration which inhibits the growth and development of these pytopathogenic fungi.

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MATERIALS AND METHODS

Assortment of test plant materials: Fresh samples of roots, shoots and leaves of *Ageratum conyzoides* L. were collected from University of the Punjab, Lahore, and washed thoroughly under tap water followed by 1% sodium hypochlorite solution and three to four successive washings with distilled water. Then plant material was dried at 40 °C in an electrical oven for about overnight and grinded to fine powder in a fine electrical grinder.

Procurement and culturing of target fungal species: Cultures of pathogenic fungi, *Drechslera holmii* (Luttr.) Subram. & B. L. Jain and *Drechslera autsrliensis* Bugnic. ex M.B. Ellis were attained from First Fungal Culture

Bank of Pakistan, (FCBP) University of the Punjab, Lahore and preserved on malt extract agar (MEA) in sterilized Petri plates and refrigerated at 4 ºC.

Preparation of aqueous extract: The powdered plant material (20 g) of samples (root, shoot and leaves) was separately soaked in 100 mL autoclaved distilled water for 24 hrs at 30±2 °C to prepare 20% w/v extracts. Then the matter was subjected to filtration using muslin cloth tracked by Wattman No.1 filter paper and stored at 4 °C and used within two days.

Preparation of organic solvent extract: Aqueous plant extract was prepared @ 20 g in 100 mL (w/v) sterilized distilled water and partitioned with 100 mL of dichloromethane which was removed using rotary evaporator. The concentrate was remixed in 100 mL of autoclaved distilled water to make 20% stock solution. Then dilutions were primed by adding appropriate quantity of water.

Anti-fungal activity assay: Antifungal extract bioassays were carried out in 2% liquid malt extract medium. To avoid bacterial contamination, antibacterial Chloromycetin capsules were used. In 250 mL flasks, calculated measures of respective stock solution and distilled water were poured for preparing 1-4% concentrations (100 mL in each flask). Control contained same quantity of distilled water. Fresh inoculum discs of 5 mm width of *D. australensis* and *D. holmii,* were inoculated in all treatment flasks aseptically in triplicates and incubated at 25±2 °C. The fungal mass from every set was gathered on pre-weighed filter paper after 7 days and dry weight was calculated after 24 h oven drying at 60 °C (Bajwa *et al*., 2006).

Extraction of essential oil: Fresh sterilized leaves were cut about into 2 cm pieces. One hundred grams of the sample was introduced in a round bottom flask and 500 mL of distilled water was poured. Hydrodistillation was done up to 14 h in a modified Clevenger apparatus. Anhydrous sodium sulphate was employed to dehydrate oils and refrigerated at 4 °C.

Oil antifungal bioassays: Antifungal oil bioassay was also carried out in 2% liquid malt extract medium. In each test tube, 4 mL of ME and 1 mL of each of 1-4% stock solution of oil of *A. conyzoides* was added. Control received the same quantity of water. Inoculum suspension was prepared from one week mature actively emergent cultures of *D. australensis* and *D. holmii,* and 0.2 mL of conidial suspension, containing 7 × 10^6 conidia mL^{-1}, was inoculated in each test tube aseptically. Each conduct was in triplicate and incubated at 25±2 °C. For the assessment of fungal biomass yield, harvest was taken after 7-days. From all sets, mycelial biomass was poised on pre-weighed filter papers and dry weight was calculated after 24 h oven drying at 60 °C (Bajwa *et al.,* 2006).

Statistical analysis: This experimentation was directed using a completely randomized design. Standard errors of averages of all the five replicates were figured using computer software Microsoft Excel. Percentage suppression/increment of biomass after employed all concentrations of the treatment against control was deliberated by following formula:

$$\text{Biomass reduction/increment (\%)} = \frac{\text{Biomas in control} - \text{Biomass in treatment}}{\text{Biomass in contol}} \text{X100}$$

The data were analyzed by applying Duncan's Multiple Range Test (Steel and Torrie, 1980) using computer software COSTAT.

RESULTS

Effect of aqueous shoot extract of *A. conyzoides* on fungal biomass production: The data from the biomass assays of two test species of *Drechslera* exposed to employed concentrations of aqueous shoot extract of *A. conyzoides* are presented in Figure 1. In general aqueous extract of shoot was found to be inhibitory to fungal growth of both the target species. The antimycotic potency of all the concentrations of aqueous shoot extract was significant against test fungi except 4% extract concentration as it promoted the growth of *D. australiensis* up to 5%. In general, there was 12-23% reduction and 22-42% reduction in biomass production of *D. holmii* and *D. australiensis,* respectively, by employing different concentrations of aqueous shoot extract. In case of *D. holmii* all the concentrations significantly reduced the fungal biomass production. Among this 1% concentration was the most effective in suppressing the biomass production. The highest antifungal activity was recorded against *D. australiensis* where maximum suppression of fungal growth was recorded in 2% concentration.

Effect of aqueous leaf extract of *A. conyzoides* on fungal biomass: The relative intensity of antifungal effect was found to be varying with the species involved, as well as the concentration of the leaf extract employed. A variable effect of various concentrations was recorded for both test species. In case of *D. holmii* the highest antifungal activity was recorded in 4%

followed by 1% and 3% concentrations except 2% concentrations which caused least reduction in fungal biomass production (Figure 2). In case of *D. australiensis* 1% and 2% concentrations were the most effective in suppressing the biomass production, while 3% and 4% concentrations rather promoted the fungal biomass production. No particular drift was instituted in inhibition of fungal biomass production of both species but the lower concentrations were more effective against *D. australiensis* while the higher concentrations not significantly reduced the fungal growth rather 3% and 4% concentrations enhanced the fungal growth. About 42% reduction in biomass was recorded for D*. holmii* and 32% reduction in case of *D. australiensis* at 4% and 2% concentrations, respectively.

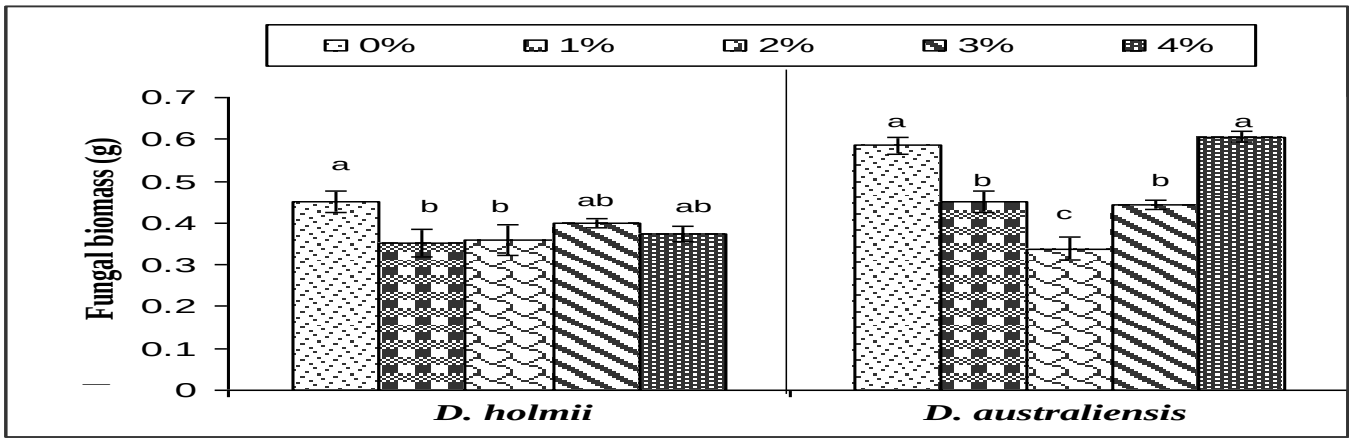

Figure 1. Effect of different concentrations of aqueous shoot extract of *Ageratum conyzoides* on biomass of *D. holmii* and *D. australiensis* after 7 days of incubation.Vertical bars show standard errors of means of three replicates. Values with different letters show significant differences (P = 0.05) as determined by DMR Test.

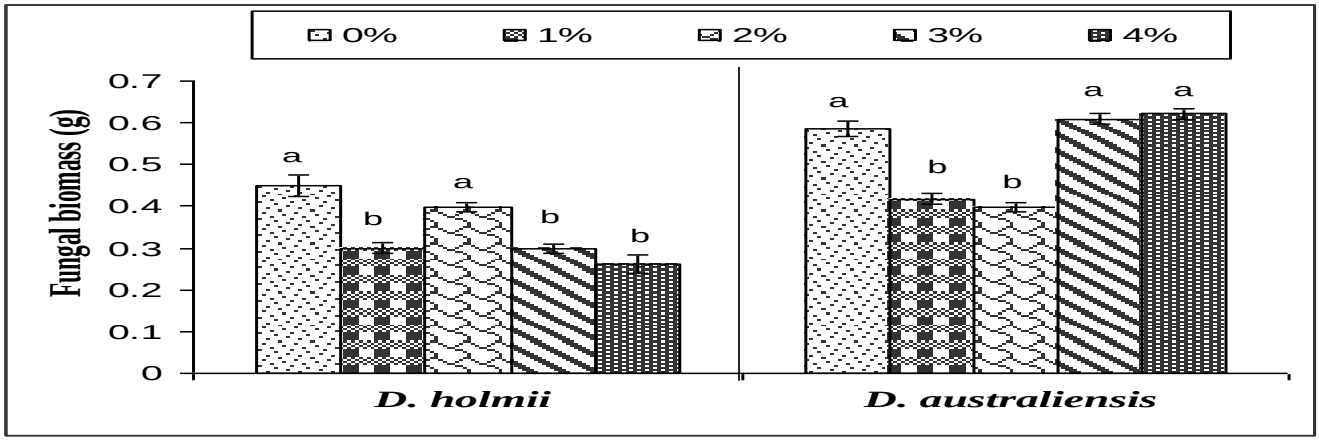

Figure 2. Effect of different concentrations of aqueous leaf extract of *Ageratum conyzoides* on biomass of *D. holmii* and *D. australiensis.* Vertical bars show standard errors of means of three replicates. Values with different letters show significant differences (P = 0.05) as determined by DMR Test.

Effect of aqueous root extract of *A. conyzoides* on fungal biomass production: In case of aqueous root extract of *A. conyzoides,* the mycelial yield of the test fungus *D. holmii*, was found to be significantly suppressed in all experimental treatments as compared to control. Maximum reduction in biomass production was evident at 3% concentration where the phytotoxic stress was very obvious in terms of dry biomass reduction (Figure 3). The 4% concentration depicted less deleterious effect on biomass inhibition but it was significantly lower than control. There was 22, 18, 32 and 11% reduction in biomass of *D. holmii* due to various concentrations (1-4%) of aqueous root extract of *A. conyzoides.* The antimycotic effect of all the concentrations of the aqueous root extract was found to be inhibitory to *D. australiensis* except 4% concentration. The antifungal effect of all the concentrations was significant and 2% concentration exhibited a persistent

negative effect on fungal biomass production. In contrast to lower concentrations, where the allelopathic stress was obvious in terms of dry biomass reduction, 4% concentration caused a significant increase in fungal dry biomass production up to 7%. There was 12, 26 and 23% reduction in biomass production of *D. australiensis* due to aqueous root extract concentrations of 1-3% of *A. conyzoide*, respectively.

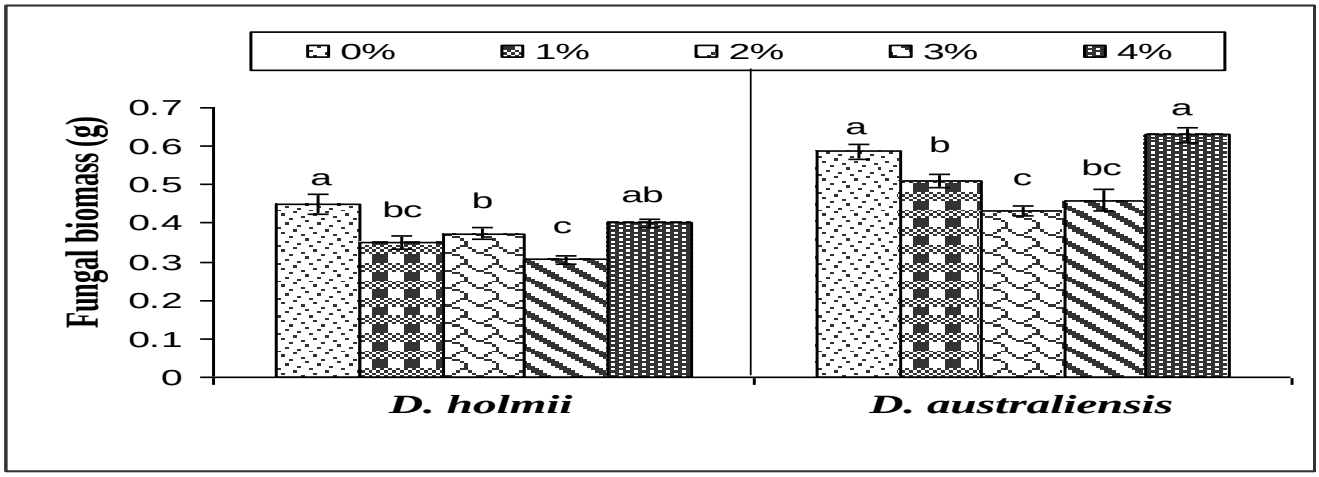

Figure 3. Effect of different concentrations of aqueous root extract of *Ageratum conyzoides* on biomass of *D. holmii* and *D. australiensis* after 7 days of incubation. Vertical bars show standard errors of means of three replicates. Values with different letters show significant differences (P = 0.05) as determined by DMR Test.

Effect of Dichloromethane fraction of shoot extract of *A. conyzoides* on fungal biomass production: DCM fractions exhibited more promising results in suppressing the fungal growth than aqueous fractions. The differences in growth rate were found to be varied with respect to variability in concentrations employed. The Biomass assays revealed that all the applied concentrations of DCM fraction of *A. conyzoides* decreased the fungal biomass in both test fungi. There was a gradual decrease in biomass of the two fungal species as the concentration of extract was increased from 1-4% (Figure 4). The 1% DCM shoot extract concentration caused the highest reduction of about 31% in growth of *D. holmii* and similarly 31% reduction in growth of *D. australiensis* and further increase in concentration exhibited significant decrease in growth rate as compared to control. Relatively more toxicity of the extract was recorded against *D. australiensis*. There was 35-91% and 42-92% drop of growth by a number of laboring concentrations of DCM fraction of shoot extract, in *D. holmii* and *D. australiensis,* respectively.

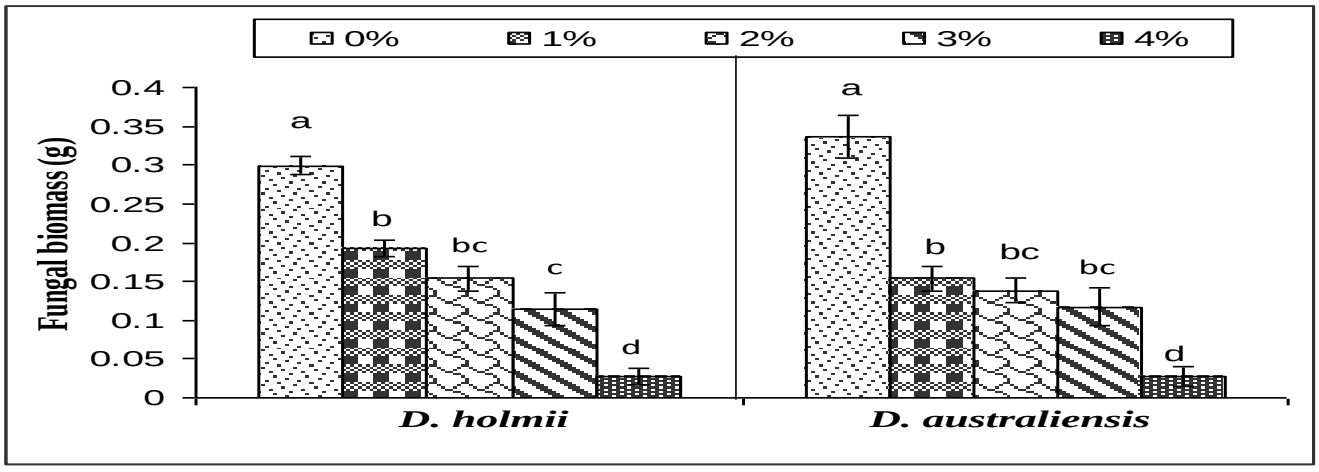

Figure 4. Effect of different concentrations of DCM fraction of shoot extract of *Ageratum conyzoides* on biomass of *D. holmii* and *D. australiensis* after 7 days of incubation. Vertical bars show standard errors of means of three replicates. Values with different letters show significant differences (P= 0.05) as determined by DMR Test.

Effect of Dichloromethane fraction of leaf extract of *A. conyzoides* on fungal biomass production: In general DCM fraction of leaf extract of *A. comyzoides* was found to be more inhibitory to test fungal growth than aqueous extracts. The antifungal effect of all the concentrations of DCM fraction was significant against both the test fungal species. It is apparent from the results that the growth reduction was found to be parallel with the increase in fraction concentration. In general, *D. australiensis* was proved to be more susceptible to the DCM fractions employed. The maximum antifungal activity was observed at 4% concentration in both test fungal species. There was 36-69% and 47-79% decline in growth because of different working concentrations of DCM fraction of leaf extract, in *D. holmii* and *D. australiensis,* respectively (Figure 5).

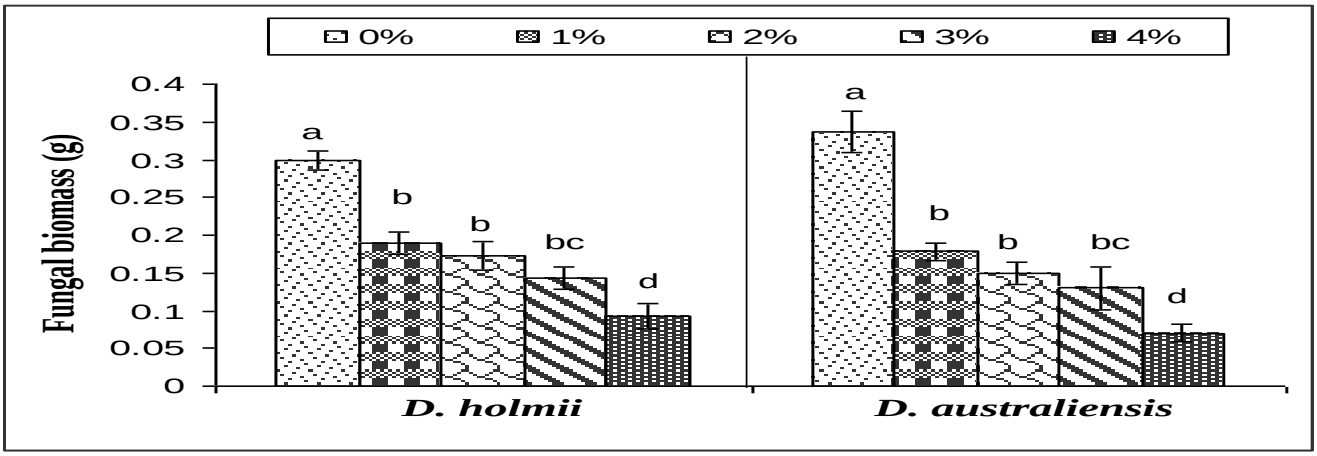

Figure 5. Effect of different concentrations of DCM fraction of leaf extract of *Ageratum conyzoides* on biomass of *D. holmii* and *D. australiensis* after 7 days of incubation. Vertical bars show standard errors of means of three replicates. Values with different letters show significant differences (P= 0.05) as determined by DMR Test.

Effect of Dichloromethane fraction of root extract of *A. conyzoides* on fungal biomass production: DCM fraction of root extract also presented the same trend as was exhibited by shoot and leaf extract fractions. All the concentrations (1-4%) diminished *in vitro* biomass that was found proportionate with increase in fraction concentration (Figure 6). The highest and statistically significant allelopathic suppression was induced by the highest concentration (4%) causing a decline of 51 and 79% in biomass production of *D. holmii* and *D. australiensis,* repectively. The fractions (1-4%) depressed the mycelial yield up to 31%-51% of *D. holmii.* In case of *D. australiensi* the decrease in biomass production was recorded as 31-79%.

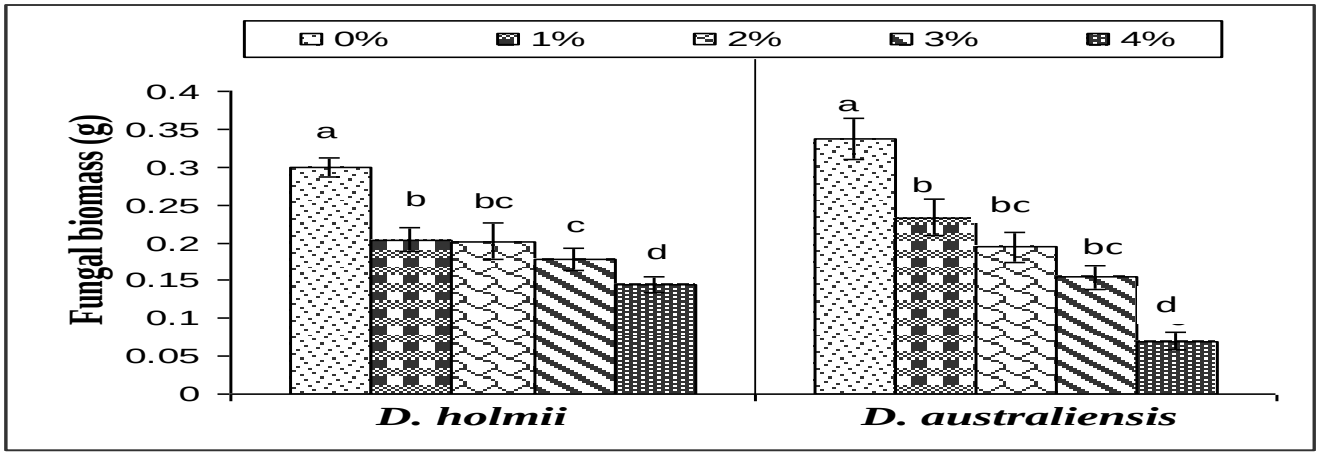

Figure 6. Effect of different concentrations of DCM fraction of root extract of *Ageratum conyzoides* on biomass of *D. holmii* and *D. australiensis* after 7 days of incubation.

Vertical bars show standard errors of means of three replicates. Values with different letters show significant differences (P= 0.05) as determined by DMR Test.

Effect of different concentrations of essential oil of *A. conyzoides* on fungal biomass production: The essential oil was found superior in reducing the biomass production of target fungal species. All the concentrations (1-4%) showed no mycelial growth (Figure 7). It may be due to the aromatic volatile compounds present in essential oil. The decrease in fungal biomass production was recorded as 100% for both test fungi.

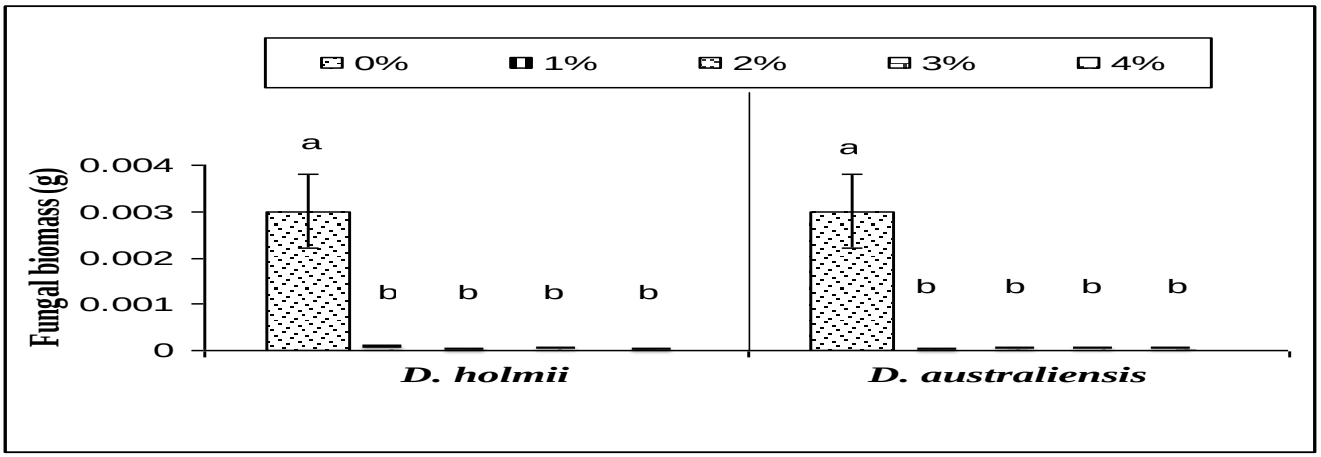

Figure 7. Effect of different concentrations of essential oil of *Ageratum conyzoides* on biomass of *D. holmii* and *D. australiensis* after 7 days of incubation. Vertical bars show standard errors of means of three replicates Values with different letters show significant differences (P = 0.05) as determined by DMR Test.

DISCUSSION

Exploiting antifungal activity of natural plant products for controlling fungal pathogens is becoming a popular area of research these days (Mughal *et al*., 1996; Khan *et al*., 1998; Bajwa *et al*., 2003). Presently the employed extract types of *A. conyzoides* significantly suppressed the growth of two pathogenic test species belonging to genus *Drechslera*. The effect of plant extracts were varied with the test plant parts, test fungal species, concentration of the aqueous and organic extracts (Zafar *et al*., 2002; Bajwa *et al.,* 2006).

Presently, two types of plant extracts were used to examine the antifungal activity of the *A. conyzoides* against the pathogenic fungi. The organic plant extracts showed more phytotoxic potential than the aqueous extracts. Previously, Zafar *et al*. (2002) demonstrated the maximum inhibitory potential of chloroform leaf extract of *M. azedarach* against *Fusarium chlamdosporum* whereas aqueous and other organic extracts were not.

In the present study, generally higher concentrations of organic extracts were more effective in controlling growth of the test fungi however higher concentrations of aqueous extracts of all plant parts failed to suppress the growth of *D. australiensis* at 7 days growth stage, while lower concentrations caused significant reduction in biomass production. In case of aqueous extracts more arrest in growth was observed at lower concentrations whereas higher concentrations supported the mycelial growth rate. The proliferation in growth might be owing to reclaiming aptitude of fungi to allelochemicals or due to utility of these chemicals as nutrients by fungi (Sicker, 1998). Less pH value of medium may also favour growth production at higher concentrations. In the same way certain allelopathic compounds possess inconstant responses in varying concentrations that could be positive or negative (Parius *et al.,* 1985). In case of aqueous extracts the more antifungal effect was recorded in shoot and leaf extracts against the test fungal species and cause 42% inhibition in biomass production. In similar kind of study Bajwa *et al.* (2007) evaluated the antifungal activities of shoot and root extracts of *Parthenium hysterophorus* L. and *Ageratum conyzoides* against *Macrophomina phaseolina* (Tassi) Goid., the cause of charcoal rot disease of sunflower. A measured reduction in *M. phaseolina* biomass was observed due to aqueous extracts. The lowest concentration of 2% of both root and shoot extract of *P. hysterophorus* markedly suppressed the biomass. Whereas in case of *A. conyzoides* 4% of both root and shoot extract was proved the most effective.

The present study revealed that dichloromethane fraction from aqueous root extract showed strong antifungal potential that was parallel to increase in fraction concentration. DCM fraction (4%) depicted extreme suppression in biomass that was 92% in *D.*

australiensis and 91% in *D. holmii.* The DCM fraction from aqueous leaf extract decreased fungal growth up to 79% in *D. australiensis* and 69% in *D. holmii,* while in DCM fraction from aqueous shoot extract; minimum growth reduction of 51% for *D. holmii* was recorded. These results are supported by previous studies of Ahmad and Abdulgaleil (2005) who reported the antifungal activity of dichloromethane extracts of leaves and stem bark of *Magnolia gradiflora L.* against six pathogenic fungi, *Alternaria alternata*, *Helminthosporium* sp., *Nigrospora* sp., *Fusarium oxysporum*, *F. culmorium* and *Rhizoctonia solani*. They observed that dichloromethane extracts of leaves and stem bark exhibited an obvious antifungal activity against four of the six test fungi. In another research work Bajwa *et al.* (2006) have studied the *in vitro* antifungal activity of *Cicer arietinum* L. as natural alternatives of plant disease control against *Drechslera tetramera* and *Drechslera hawaiiensis*. They found that crude water extract exhibited the most significant antifungal activity even at lower concentration (5%) while in case of extraction in Dichloromethane fraction, the inhibitory effect was found to be proportional with the applied concentration. The essential oil was found superior in reducing the biomass production. All the concentrations showed no mycelial growth. This may be due to the compounds present in essential oils. This fact is supported by the work of Juliana *et al.* (2010) who have recently studied inhibitory effects of essential oil of *A. conyzoides* on the mycelial growth and aflatoxin B1 production by *Aspergillus flavus*. The essential oil inhibited fungal growth to different extents depending on the concentration and completely inhibited aflatoxin production at concentrations above 0.10 µg mL^{-1}.

CONCLUSION

The results obtained in this work indicate antifungal properties of *A. conyzoides* against *D. holmii* and *D. australiensis.* Thus, *A. conyzoides* aqueous and DCM fractions as well as essential oil may be attractive for the use of a natural product for control of fungi that attack industrial crops, avoiding chemical fungicides application.

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