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Antifungal Activity of Allelopathic Plant Extracts IV: Growth Response of *Drechslera hawaiiensis*, *Alternaria alternata* and *Fusarium moniliforme* to Aqueous Extract of *Parthenium hysterophorus*

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ABSTRACT

Allelopathic potential of *Parthenium hysterophorus* L., against three pathogenic fungal species viz. *Drechslera hawaiiensis* (M.B.Ellis), *Alternaria alternata* (Fr.) Keissl and *Fusarium moniliforme* Sheld was studied. These species were subjected to various concentrations of aqueous extracts of aerial parts of *P. hysterophorus* in liquid malt extract medium. The dry biomass productivity assays were carried out periodically with 5 days intervals up to fifteen days. The growth of all the three test pathogenic species was generally inhibited by lower concentrations viz. 10, 20, 30 and 50% of the *Parthenium* extracts while aqueous extracts of higher concentrations (60 and 70%) stimulated biomass production of test fungal species.

Key Words: Allelopathy; *Parthenium hysterophorus; Drechslera hawaiiensis; Alternaria alternata; Fusarium moniliforme*

INTRODUCTION

Parthenium hysterophorus is an herbaceous annual or ephemeral member of the Asteraceae. The origin of *P. hysterophorus* is considered to be Mexico, America, Trinidad and Argentina. Within last 100 years, it has found its way to Africa, Australia and Asia. In Australia and India *P. hysterophorus* has achieved the status of "Worst weed". Within last ten years it has become one of the seven most obnoxious weeds of the world (Singla, 1992). *P. hysterophorus* is an allelopathic weed and it inhibits the germination and growth of several crop plants and trees (Basak 1984; Srivastava *et al*., 1985; Dayama, 1986; Swaminathan *et al*., 1990). The allelopathic potential of *P. hysterophorus* weed results from the release of phytotoxic substances such as, ferulic, caffeic, vanillic, chlorogenic, *p*-coumaric and *p*-hydroxybenzoic acids, parthenin, ambrosin and coronopilin (Jarvis *et al.*, 1985).

Aqueous extract of many allelopathic plants are known to exhibit antifungal properties. Hassan *et al*. (1992) reported that leaf extracts of *Datura stramonium* reduced the development of rust pustules on the leaves of wheat. Mughal *et al*. (1996) observed that aqueous leaf extracts of *Allium sativum*, *Datura alba* and *Withania somnifera* inhibited the growth of *Alternaria alternata*, *A. brassicola* and *Myrothecium roridum*. According to Khan *et al*. (1998) aqueous extract of *Allium cepa* exhibited antifungal activity against *Helminthosporium turcicum* and *Ascochyta rabiei* and that of *Calotropis procera* against *Alternaria redicina*. Recently, Bajwa *et al*. (2001) found inhibitory potential in aqueous extracts of three Asteraceous allelopathic species on growth of *Aspergillus niger*. More recently, Bajwa *et al*. (2002) have observed that the aqueous extracts of *Dicanthium annulatum*, *Imperata cylindrical*, *Cenchrus pennisetiformis* and *Desmostachya bipinnata* have potential to control *Fusarium moniliforme* and *F. oxysporum.*

The aims and objectives of the present study were to evaluate the potential of aqueous extract of aerial parts of *P. hysterophorus* to control *in vitro* three pathogenic fungi viz., *Alternaria alternata*, *Fusarium moniliforme* and *Drechslera hawaiiensis*

MATERIALS AND METHODS

Fresh shoots of *P. hysterophorus* were collected from Botanical Garden, University of the Punjab, Lahore and washed thoroughly under running tap water, dried with blotting paper and cut into small pieces. A 70% w/v stock solution of *Parthenium* was obtained by soaking the crushed plant materials in sterilized water for 48 h at room temperature, passing through muslin cloth and finally through Whatman filter paper No.1. The lower concentrations of 10, 20, 30, 50, 60 and 70% were prepared by adding appropriate quantity of distilled water into the stock solution. The extract was stored at 4°C in pre-sterilized flasks. To avoid contamination and prospective chemical alterations, the extract was used within 3-4 days.

Aqueous extract bioassays were carried out in liquid medium. The basal medium employed to grow fungi was 2% malt extract (ME) medium in 250 mL conical flasks. To avoid bacterial contamination, antibacterial Chloromycetin capsules @ 1 capsule 100 mL^{-1} of medium were used. To 80 mL of ME, 20 mL of each of 10–70% extract of *Parthenium* was added. Control received the same quantity of water.

Inoculum discs of 6 mm diameter, obtained from 7-day old actively growing fungal cultures of *A. alternata*, *F. moniliforme* and *D. hawaiiensis* were transferred to flasks aseptically. The flasks were incubated at 25±2°C.

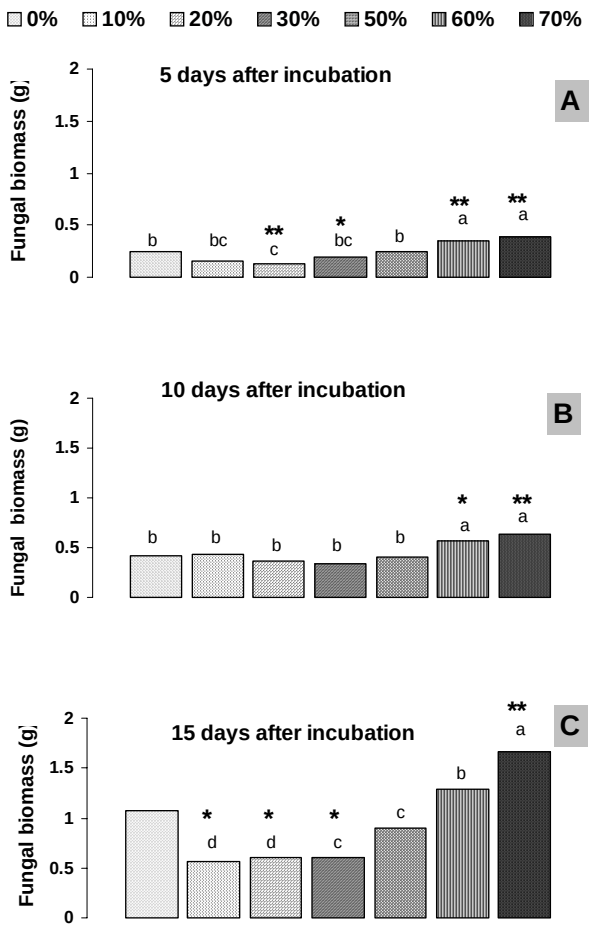
For the assessment of fungal biomass yield, three harvests were designed at intervals of 5-day each. The mycelial biomass from triplicate samples for each treatment was collected on pre-weighed filter papers. Their dry weight yield was determined after 24 h oven drying at 60°C. All the data were analyzed by applying Duncan's Multiple Range (DMR) Test to compare different treatments with one another statistically. The individual treatments were also compared with control for significant/insignificant difference by applying t-test.

RESULTS

Growth response of *D. hawaiiensis* to aqueous extracts of *P. hysterophorus*. The data on dry biomass production in the early growth phase i.e. 5 days after incubation (DAI) revealed an excessive interference of aqueous extract with the growth of fungal test species whereas highly negative response at lower concentrations of 10-30% was evident. A significantly marked positive effect on biomass production was obtained at higher concentrations of 60-70%. The influence of intermediate concentration (50%) was found to be negligible (Fig. 1A). The maximum allelopathic stress was induced by 20% concentration causing a decline of about 46% (Fig. 2A) in the biomass production of *D. hawaiiensis*. It was followed by 10 and 30% concentrations, which revealed a significant reduction in the range of 16-33% in mycelial yield (Fig. 2A). In contrast to lower concentrations, where the allelopathic stress was very obvious in terms of arrested dry biomass increments, it was completely reversed at higher concentrations (50-70%). The mycelial yield of the test fungal species was markedly supported, particularly at 60 and 70% concentrations. A very sharply stimulated growth, even significantly higher than control was recorded in these treatments (Fig. 2A).

At intermediate growth level of 10 DAI, almost similar pattern of inhibition and stimulation in parallel treatments of aqueous extract prevailed. However, the fungal species exhibited the maximum biomass production during this period (between 5-10 days) in all the treatments including control and various dosage of aqueous extract (Fig. 1B). Apparently allelopathic stress was largely over come by the active growth of the fungus at this stage. Although the mycelial yield at 20 and 30% concentrations of aqueous extract was still 12-19% lower than control, the variations were not significant statistically. Biomass production in 10 and 50% concentrations was almost as good as in non-treated control (Fig. 2B). At the higher concentrations (60-70%) statistically significant growth promoting effect was still evident and a stimulus in mycelial biomass production of 36 and 52%, with respect to control,

Fig. 1 (A–C). Effect of different concentrations of aqueous extracts of Parthenium hysterophorus on dry biomass *Drechslera hawaiinsis*. Values with different letters show significant difference (P = 0.05) as determined by DMR Test.*,**, show significant difference from control at 5 and 1% level of significance respectively,as determined by t-test.



was recorded at these concentrations, respectively (Fig. 2B).

In the final phase i.e., after 15 days growth period, the mycelial yield of the fungus species *D. hawaiiensis* was found to be significantly depressed in all experimental treatments as well as in control (Fig. 1C). Consequently, the element of allelopathic stress became even more pronounced at this stage. In the lower regimes of aqueous extract i.e., 10-50%, the mycelial mass production was greatly suppressed. However, statistically significant decline of 17-48%, with respect to control, was recorded at 10-50% concentrations (Fig. 2C).

Growth response of *A. alternata* to aqueous extracts of *P. hysterophorus*. The dry biomass assays of *A. alternata* in the initial period of 5 days revealed a rather erratic pattern of growth. Except for 70%, all regimes of aqueous extract of *P.*

Fig. 2 (A–C). Effect of aqueous extracts of *Parthenium hysterophorus* on percentage reduction in dry biomass production of *Drechslera hawaiinsis.*

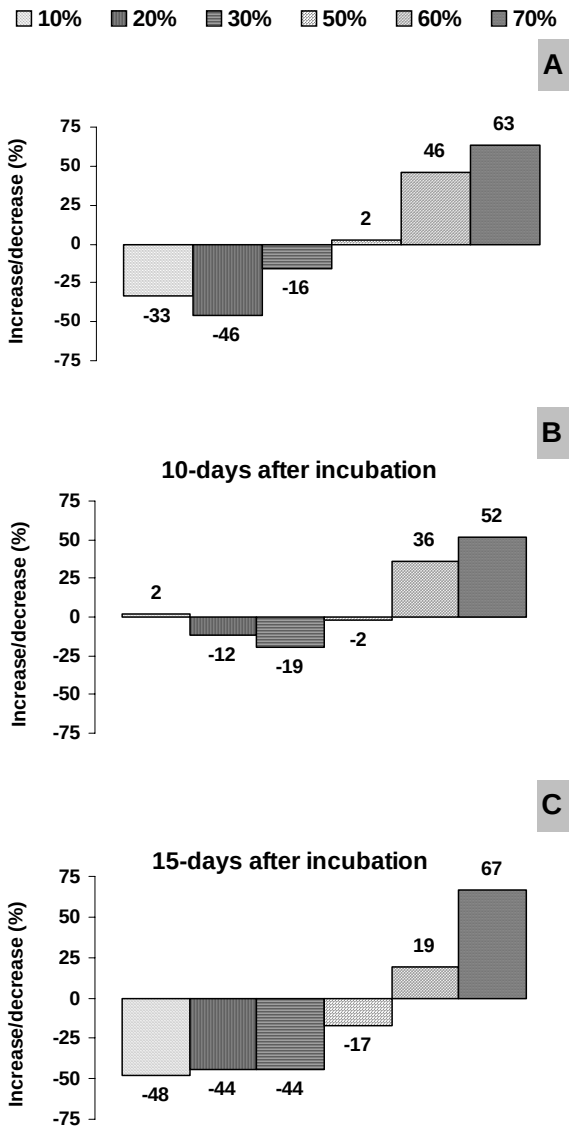
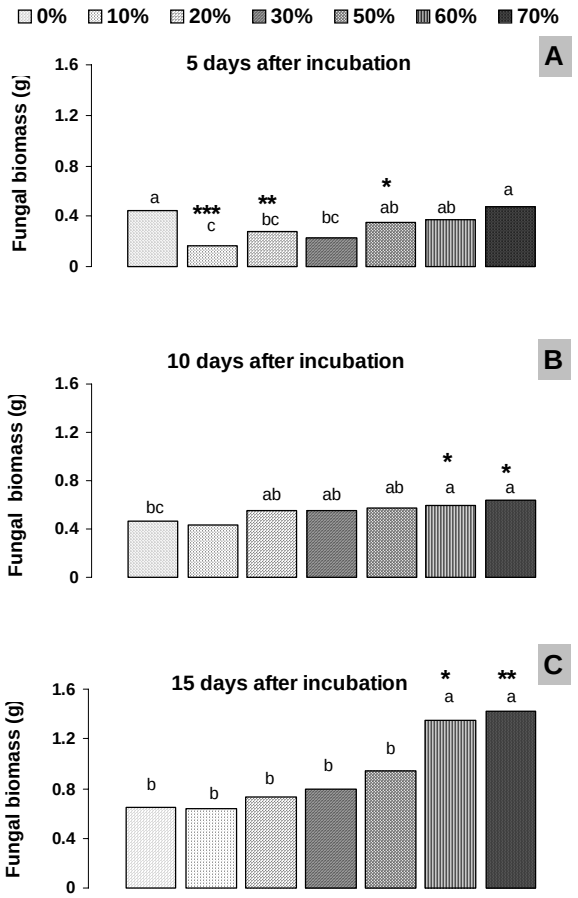


Fig. 3 (A–C): Effect of different concentrations of aqueous extracts of Parthenium hysterophorus on dry biomass of Alternaria alternata.

Values with different letters show significant difference (P = 0.05) as determined by DMR Test.

*,**, show significant difference from control at 5 and 1% level of significance respectively,as determined by t-test

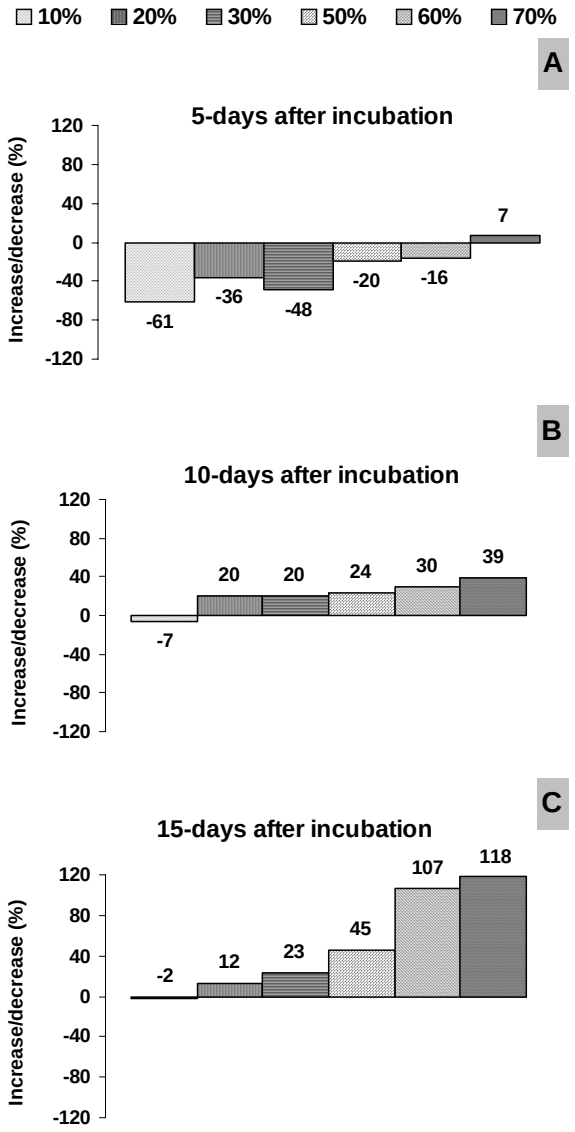


hysterophorus caused considerable inhibition in mycelial biomass production of the fungus. However, the statistically significant reduction in growth was evidenced at lower concentrations (10-50%) in comparison to control. The mycelial growth of *A. alternata*, was only slightly enhanced at 70%, the stimulus being statistically non-significant (Fig. 3A & 4A).

Mycelial yield assays in the middle phase i.e. 10 DAI revealed that the species by this stage had acquired growth maxima in all the treatments. Almost negligible biomass increments were obtained by the species in case of control treatment. By apparent alleviation of potential stress factor, the biomass production in all aqueous extract treatments was sharply enhanced with the exception of 10% concentration where the mycelial yield was highly stimulated (Fig. 3B). At 10% concentration, although statistically insignificant, the mycelial production was lower than control but in contrast to that at 60 and 70% concentrations it was significantly higher (Fig. 3B).

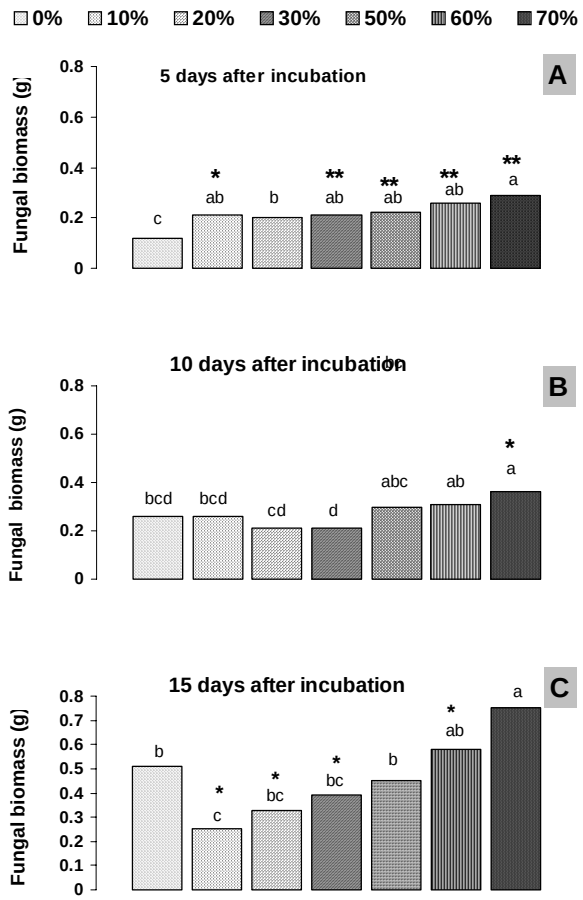
At 15 days stage, again biomass production was found to be highly depressed in all treatments in general. However, it was relatively more sharply declined in control and lower concentration (10-50%). Dry biomass yield was still significantly higher (12-118%) than control. The pattern of gradually higher production of biomass in response to increasing concentrations of aqueous extract was clearly indicated (Fig. 3 C & 4C)

Fig. 4 (A–C). Effect of aqueous extracts of *Parthenium hysterophorus* on percentage reduction in dry biomass production of *Alternaria alternata.*



Growth response of *F. moniliforme* to aqueous extracts of *P. hysterophorus.* Periodic biomass assays of *F. moniliforme* in various treatments clearly represented a slightly variable pattern of growth in response to aqueous extracts of *P. hysterophorus* (Fig. 4 A-C). It is evident from the biomass assessment data at 5-day stage that mycelial growth of fungus species was very slow in the control medium. In contrast to that all employed concentrations of aqueous extract provided a considerable boost in biomass production and statistically significant stimulation in mycelial yield was achieved in these treatments (Fig. 5A).

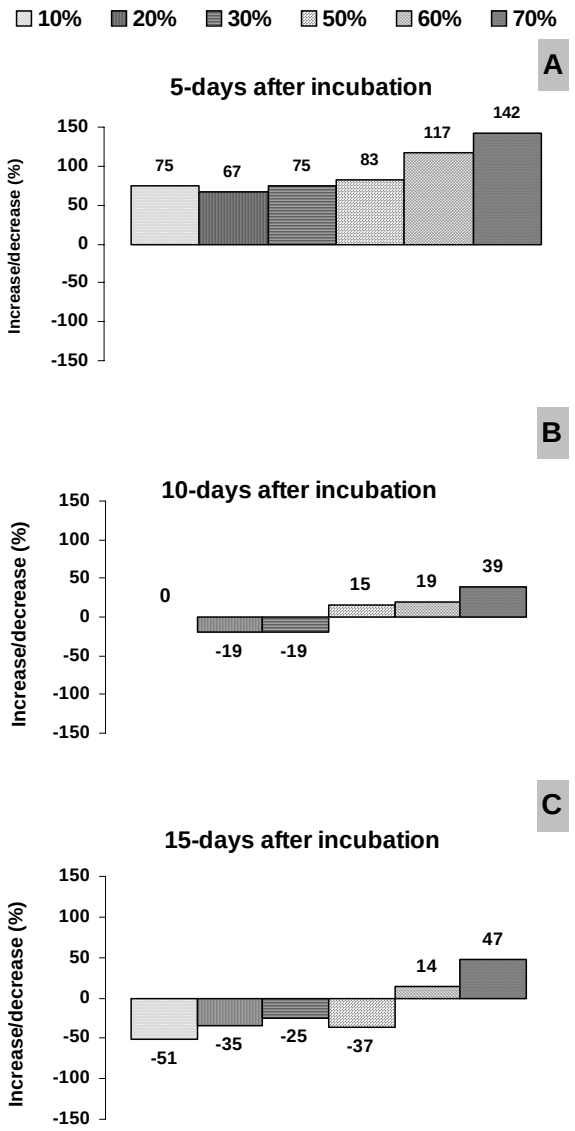
Fig. 5 (A–C). Effect of different concentrations of aqueous extracts of *Parthenium hysterophorus* on dry biomass of *Fusarium moniliforme*. Values with different letters show significant difference (P = 0.05) as determined by DMR Test. *,**, show significant difference from control at 5 and 1% level of significance respectively,as determined by t-test.



After an initial lag phase, the growth of the test fungus species was gradually activated in the next interval of 5-10 days. In aqueous extract treatments acquisition of further biomass increments was either very slow or it became almost static. Arrest in biomass production was clearly evident at 20 and 30% concentrations. Biomass production however was still significantly higher at 70% concentration in comparison to control (Fig. 5B).

Biomass assay in the later phase revealed that the rate of mycelial growth yield of *F. moniliforme* in control was further improved while in the case of lower concentrations of aqueous extract the further input of biomass increments was relatively slow (Fig. 5C). It resulted in 25-51% depression in mycelial yield at 10-30% concentrations. Slight decline in biomass growth recorded at 50%, was statistically non-significant. The rate of biomass production

Fig. 6 (A–C). Effect of aqueous extracts of *Parthenium hysterophorus* on percentage reduction in dry biomass production of *Fusarium moniliforme*



at this stage was comparatively high at 60 and 70% than all other treatments. However, due to enhance growth rate in control treatment, the significantly improved biomass production was only evident at highest concentration of aqueous extract i.e., 70% (Fig. 6C).

DISCUSSION

In the present study, aqueous shoot extract of *Parthenium hysterophorus* was used against a variety of pathogenic fungi. The results of this conceptual study clearly reflects that this weed has inherent ability to induce allelopathic effects on mycelial growth rate and consequently on proliferation of these fungi. The relative intensity of this effect, however, varies with the species involved, as well as the concentration of the shoot extract employed.

It is evident from the study that average mycelial growth rate per day was significantly inhibited by allelochemicals specifically at lower concentrations of aqueous shoot extract. These results are supported from previous investigations in which leaf extracts of *Datura stramonium* have been shown to cause considerable decline in the development of rust pustules on leaves of wheat (Hassan *et al.,* 1992). There are other evidences showing similar effects against known pathogenic fungi (Mughal *et al.,* 1996; Khan *et al.,* 1998), which further confirm the presence of antifungal agents in the test species.

Greater inhibition of fungal growth of *D. hawaiiensis* at lower concentrations of aqueous extract was observed as a general trend at each harvest stage. In this regard lower concentrations of 10-50% of shoot aqueous extract of *Parthenium* displayed maximum inhibition against *D. hawaiiensis*. But the higher concentrations of 60 and 70% were found to be ineffective in terms of antifungal activity. These findings are in line with the previous observations that *Trachyspermum ammi* (ajwain) oil was found to be found toxic against pathogenic fungi and caused growth reduction in *A. alternata, Aspergillus flavus* and *A. fumigatus* even at as low concentrations as 0.003% but in contrast to that at 0.12% concentration this oil was ineffective against fungal species (Kazmi *et al.,* 1993). The enhancement of biomass production of *D. hawaiiensis* at higher concentration of shoot extracts may be attributed to detoxifying ability of the fungi, to allelochemicals (Sicker, 1998). It may be due to the ability of some allelochemicals to enhance the growth of mycoflora (Mughal *et al*., 1996) or ability of particular species to exploit them as source of nutrition.

In the case of *A. alternata*, the comparative effectiveness of aqueous extracts of selected test species revealed that 10-60% concentrations were relatively more allelopathic as compared to other species while 70% concentration supported the mycelial biomass production during the early phase. *A. alternata* however exhibited high resistance and less susceptible against allelopathic stress of *P. hysterophorus.* Consequently, no antifungal effects were witnessed against this species during the later growth stages. These findings are in line with those of Mughal *et al.* (1996) who have reported little or no antifungal activity of both leaf and stem extracts of *Senebiera didyma* against *Alternaria alternata, Alternaria brassicola* and *Myrothecium roridum*. In contrast to this, Dixit and Tripathi (1975) have observed stimulatory instead of retarding effect of *Brassica juncea* and *B. pekinensis* against *Fusarium nivale* and *Cephalosporium* sp.

Periodically, varied growth response was exhibited by *Fusarium moniliforme.* The increase in growth rate at lower

concentrations during early phase was followed by severe detrimental effects at the same concentrations of shoot extract of *Parthenium hysterophorus* in the later growth phases. This indicates a species-specific behaviour whereby *F. moniliforme* exerted resistance against toxicity initially. However, it was later broken down due to extended exposure to allelochemicals. Vir and Sharma (1985) have reported that 10% concentration of neem oil gave 100% inhibition of mycelial growth in *Aspergillus niger*, *Drechslera rostrata* and *M. phaseolina*. Similarly, leaf extract of *Chenopodium murale* and *Cannabis sativa* reduced mycelial growth of *Ascochyta rabiei* significantly (Khan *et al.*, 1998). The higher concentrations during middle and later growth period exhibited a marked enhancement in fungal biomass production probably because the fungal metabolites either might have denatured the allelochemicals or higher plant cell contents provided extra nutritional resources. These results are supported by the fact that the allelopathic substances have selective effects, depending upon their concentrations, either inhibitory or stimulatory to the growth of companion or subsequent crops or weeds (Paruis *et al.*, 1985; Cheema, 1988).

The present study signifies the importance of aqueous extracts of this allelopathic weed as potential agent to be manipulated for biological control of pathogenic fungi, only when these extracts are used at lower concentrations; whereas, at higher concentrations a potent increase in biomass production may prove to be beneficial for mass production of mycoherbicides to control the weeds of economically important crops.

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