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Antifungal Activity of Allelopathic Plant Extracts III: Growth Response of Some Pathogenic Fungi to Aqueous Extract of *Parthenium hysterophorus*

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Abstract: The use of allelopathic plant extracts as biological control agents is being popularized in recent years. The present research work was designed to evaluate the potential of aqueous extracts of allelopathic weed, ***Parthenium hysterophorus***, against three pathogenic fungi viz. ***Drechslera tetramera***, ***Aspergillus niger*** and ***Phoma glomerata***. The test fungal species were grown in 100 ml liquid malt extract medium containing 20 ml of each of 0, 10, 20, 30, 50, 60 and 70% w/v shoot extract of ***Parthenium hysterophorus***. Fungal growth was monitored periodically after 5, 10 and 15 days of incubation. A highly contrasting response was exhibited by the test pathogens to employed extract treatments. The lower concentrations of 10, 20 and 30% extract exhibited antifungal activity resulting in a pronounced decrease in fungal biomass production. The response to extract was species-specific. ***D. tetramera*** was the most susceptible while ***P. glomerata*** was found to be least susceptible to the applied aqueous extracts. The higher concentrations of 50, 60 and 70% extract markedly enhanced the fungal biomass production at all the harvest intervals.

Key words: Allelopathy, *Parthenium hysterophorus*, *Drechslera tetramera*, *Aspergillus niger*, *Phoma glomerata*

Introduction

Fungi rank second only to insects as a cause of plant diseases, which result in heavy loss of plant products. Pathogenic fungi alone cause nearly 20% reduction in the yield of major food and cash crops (Agrios, 2000). One-third global agricultural production is reportedly destroyed each year by different pests and diseases (Maqbool ***et al.***, 1988). To avoid the implication of yield losses due to plant diseases, variety of control measures presently are in use. In physical methods, use of sunlight and UV radiations etc. are included, while the most commonly known means of controlling fungal diseases in fields and green houses and sometimes in storage is through the use of chemical compounds that are toxic to fungi. No doubt the use of chemicals has been found very effective in controlling plant fungal diseases but some major problems threaten to limit the continued use of fungicides. Firstly, some fungi have developed resistance to chemicals. This necessitates higher dosage or the development of new chemicals to replace those to which fungi are resistant. Secondly, some fungicides are not readily biodegradable and tend to persist

for years in the environment. This leads to third problem, the detrimental effects of chemicals on organisms other than target fungi (Brady, 1984). Because of these problems associated with the use of chemicals, researches are now trying to use environmentally safe alternative methods of fungal control.

Aqueous extract of many allelopathic plants are known to exhibit antifungal properties. Allelochemicals reduce the germination of spores and mycelial growth of pathogenic fungi. Many research workers have tried to find safe and economical control of plant diseases by using extracts of different plant parts (Bhowmick and Chaudhary, 1982; Vir and Sharma, 1985; Jeyarajan ***et al.***, 1988; Swada ***et al.***, 1971; Singh ***et al.***, 1980; Sumbali and Mehrotra, 1981; Narayansamy and Krishnamohan, 1988). Studies have been carried out to screen different plants for their antifungal and antibacterial properties against different plant pathogens. Among these, Neem (***Azadirachta indica)*** is important. It mainly contains compounds known as limonoids, which possess antifungal activities (Singh ***et al.***, 1980; Jayarajan ***et al.***, 1987; Eswarmurthy ***et al.***, 1988; Loke, 1990; Kazmi ***et al.***, 1993). Leaf decoction of ***Acacia nilotica, Calotropis procera, Datura stramonium*** and ***Dadonea viscosa*** were found to be effective in suppressing uredospore germination on detached leaves of wheat (Bhatti, 1988). Hassan ***et al.*** (1992) reported that leaf extracts of ***Datura stramonium*** reduced the development of rust pustules on the leaves of wheat. According to Kazmi ***et al.*** (1993) there was total growth inhibition (***in vitro***) when oil of Ajwain was used against ***Alternaria alternata, Aspergillus flavus*** and ***A. fumigatus***. Mughal ***et al.*** (1996) observed that aqueous leaf extracts of ***Allium sativum*** and ***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) 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***. More recently Bajwa ***et al.*** (2002) found inhibitory potential in aqueous extracts of three Asteraceous allelopathic species on growth of ***Aspergillus niger***.

The aims and objectives of the present study were to evaluate the potential of aqueous extract of aerial parts of ***Parthenium hysterophorus*** to ***in vitro*** control of three pathogenic fungi viz. ***Drechslera tetramera, Phoma glomerata and Aspergillus niger***.

Materials and Methods

Fresh healthy aerial parts i.e. leaf, flower and stem of ***Parthenium hysterophorus***, growing as wild in the premises of Punjab University, were collected. These were washed thoroughly under running tap water, dried with blotting paper and were 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 in the stock solution. The extract was stored at

4°C in pre-sterilized flasks. To avoid contamination and prospective chemical alterations, the extract was generally 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 used in 250 ml conical flasks. To avoid bacterial contamination, antibacterial Chloromycetin capsules 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 healthy growing fungal cultures of ***Aspergillus niger***, ***Drechslera tetramera*** and ***Phoma glomerata*** 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 test (DMR) to compare the different treatments with one another statistically. The individual treatments were also compared with control for significant/insignificant difference by applying t-test.

Results

Effect of aqueous extracts of *Parthenium* on biomass production of *Drechslera tetramera*

The dry biomass production response of ***Drechslera tetramera*** was variable when grown in the presence of different concentration of aqueous extracts of ***P. hysterophorus*** (Fig. 1 A-C). The lower concentrations of 10, 20 and 30% of extracts of ***Parthenium*** were found to be inhibitory for fungal growth. The lowest concentration of 10% of aqueous extract exhibited a persistent negative effect on the fungal biomass production for the entire incubation period i.e. up to 15 days. A highly significant (P = 0.01 and 0.001) reduction in biomass of fungus was obtained in this treatment as compared to control (Fig. 1 A-C). The losses recorded in fungal dry biomass were 29, 20 and 36% after 5, 10 and 15 days of incubation (Fig. 2). Negative effect of 30% aqueous extract of ***Parthenium*** was also highly significant (P = 0.01 and 0.001) as compared to control, 5 and 15 days after incubation with an insignificant impact on fungal growth after 10 days of incubation (Fig.1). The losses in fungal dry biomass ranged from 41% after 5 day of incubation to 19% after 15 days of incubation (Fig. 2). The dry biomass was insignificantly different from control up to 10 days after incubation when the fungus was grown in the medium containing 20% aqueous extract of ***Parthenium***. However at final growth stage i.e. 15 days after incubation a highly significant (P = 0.01) effect was evident resulting in 25% loss in dry biomass of the test fungal species (Fig. 1, 2).

In contrast to lower concentrations of 10-30% the higher concentrations of aqueous extract of ***Parthenium*** viz., 50, 60 and 70% promoted the fungal biomass production (Fig.1). The effect of these higher concentrations was more pronounced and highly significant (P = 0.01 and 0.001) after 10 days than after 5 days incubation period (Fig. 1). However, after 15 days of incubation the effect of these higher concentrations was insignificant as compared to control (Fig. 1C). The highest increase of 51% in fungal biomass was recorded in 70% extract after 5 days incubation followed by 48% in the same treatment after 10 days of incubation (Fig. 2). The fungal biomass

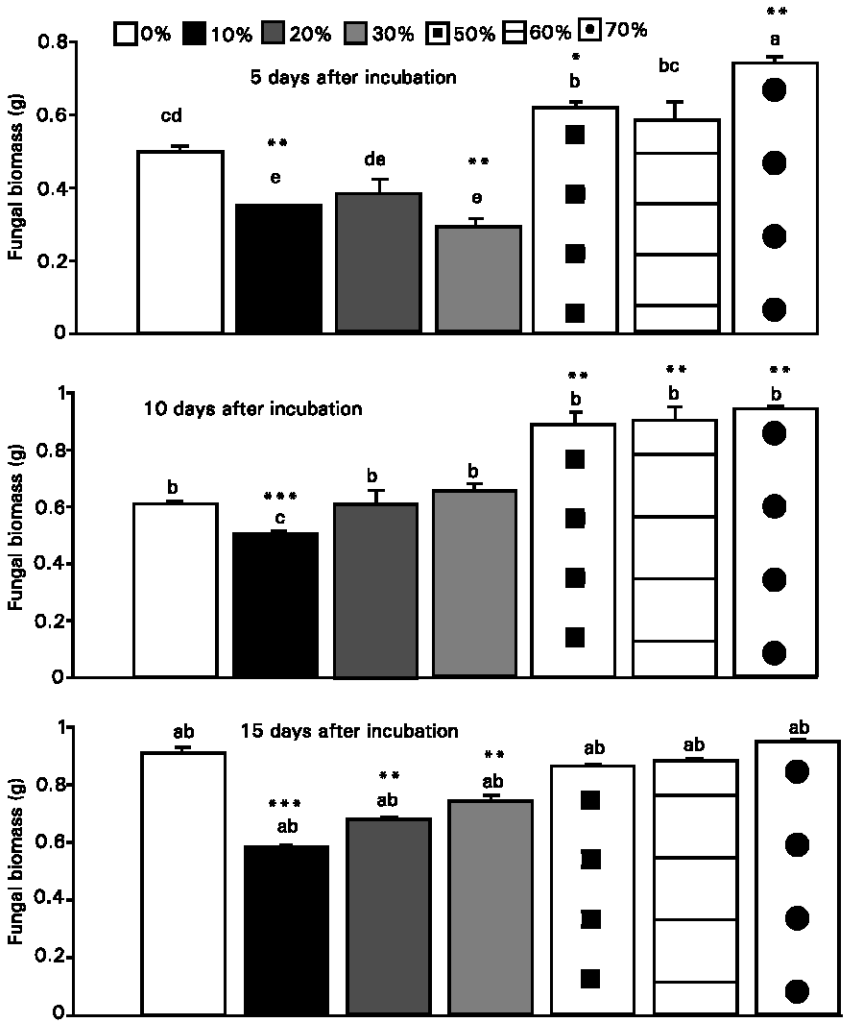

Fig. 1a-c: Effect of different concentrations of aqueous extracts of ***Parthenium hysterophours*** on dry biomass production of ***Drechslera tetramera***

Vertical bars shoe standard errors of means of three replicates

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

production in control increased gradually up to 15 days while in 50-70% extract the fungus attained the maximum growth after 10 days of incubation and there was not any pronounced change in dry biomass beyond that (Fig.1).

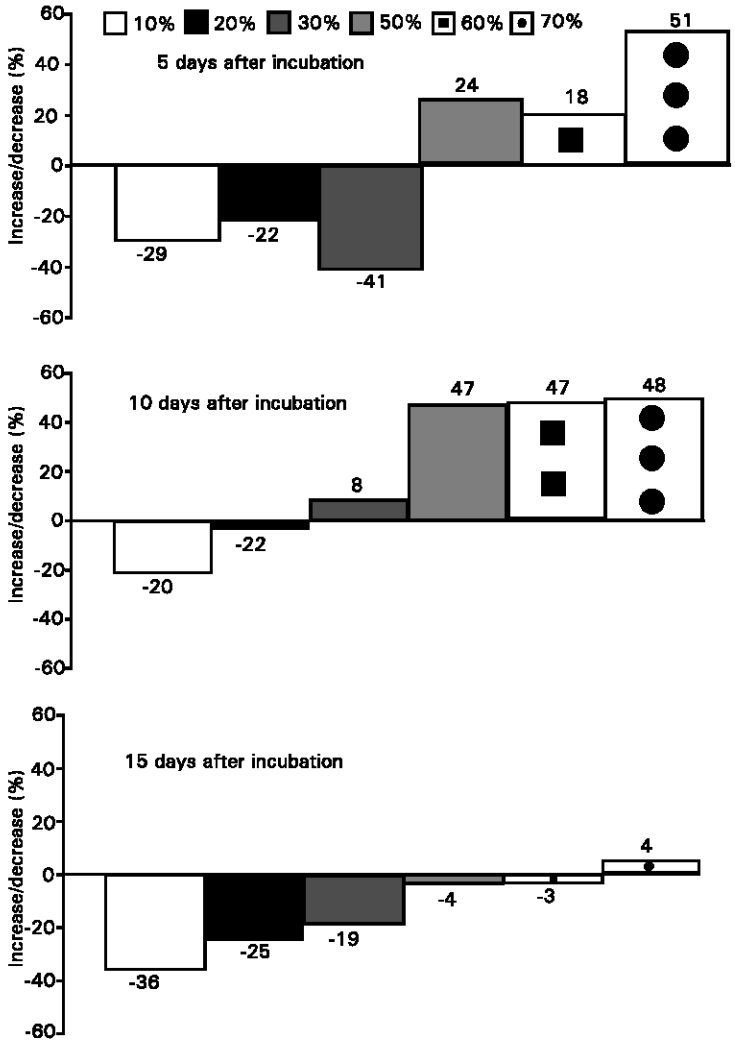

Fig. 2a-c: Effect of aqueous extracts of *Parthnium hysterophorus* on percentage losses in dry biomass production of *Drechslera tetramera*

Effect of aqueous extract of *Parthenium hysterophorus* on biomass production of *Aspergillus niger*

Mycelial growth in terms of dry biomass production in *Aspergillus niger* showed marked variation when grown in different concentrations of aqueous extracts *of Parthenium hysterophorus* at all the three harvest intervals (Fig. 3). When fungus was subjected to 5% aqueous extract, a persistent and significant decrease in fungal biomass was observed. Effect was more pronounced 5 days after incubation with a 29% reduction in dry biomass (Fig. 3). In 10%

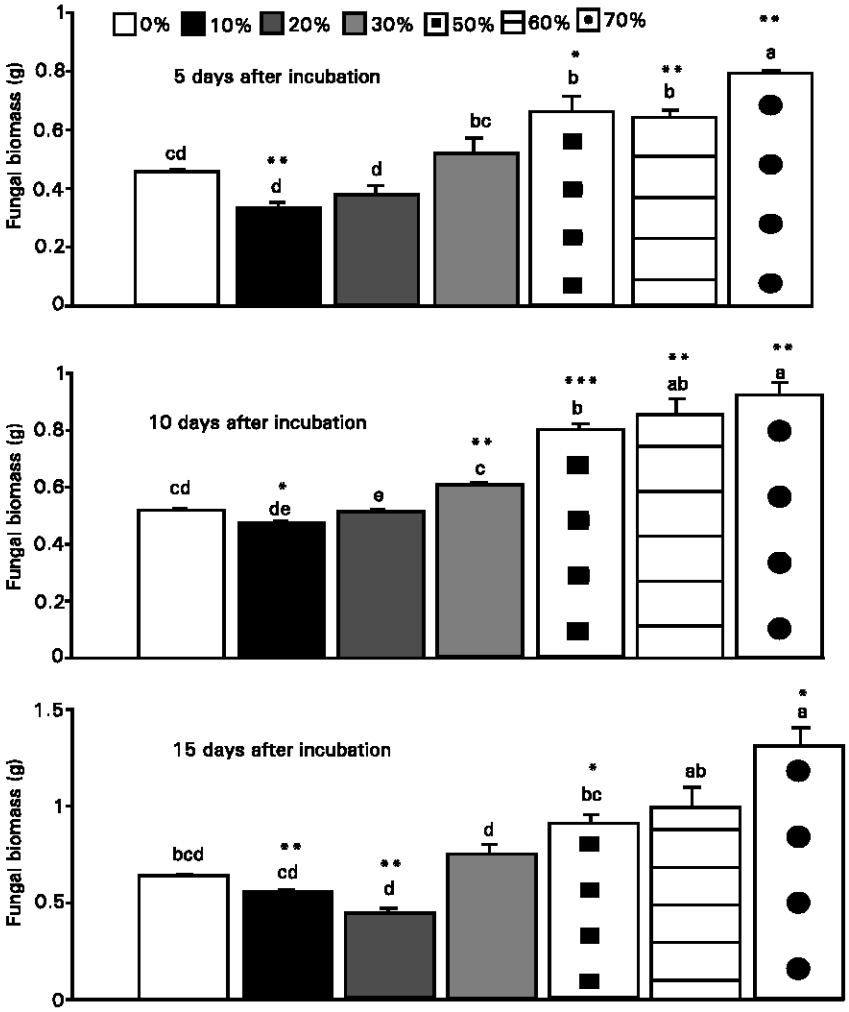

Fig. 3a-c: Effect of different concentrations of aqueous extracts of ***Parthenium hysterophorus*** on dry biomass production of ***Aspergillun niger***

Vertical bars shoe standard errors of means of three replicates

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

extract the fungal mycelial production exhibited an insignificant decrease upto 10 days growth followed by a highly significant (P = 0.01) reduction in biomass as compared to control, at final growth interval i.e. 15 days after incubation (Fig. 3). ***Aspergillus niger*** when grown in higher concentrations of 30-70% extract of ***Parthenium,*** showed stimulation in biomass production. The fungal biomass exhibited a gradual increase in higher concentrations. In 30% extract the positive

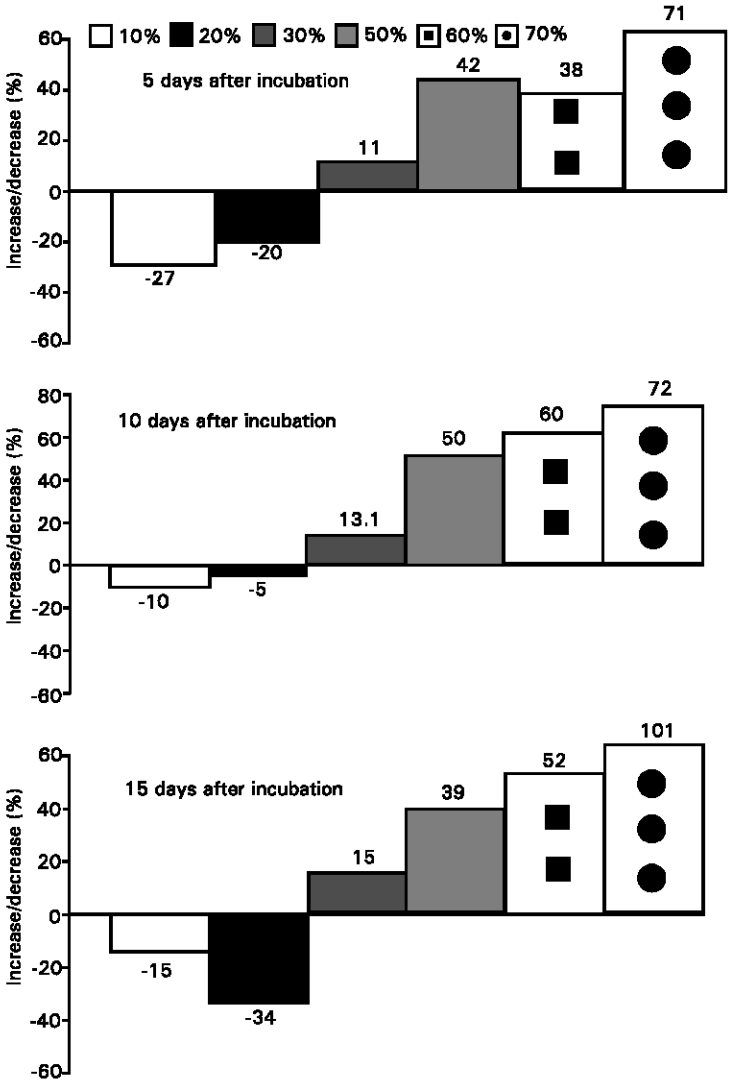

Fig. 4a-c: Effect of aqueous extracts of ***Parthnium hysterophorus*** on percentage losses in dry biomass production of ***Aspergillus niger***

effect of extract was insignificant at first harvest interval but significant at later two growth stages. Effect of 50-70% extracts was persistent and exhibited a significant difference from control, at all the three growth stages. The increase in fungal biomass due to 50, 60 and 70% extracts was 42-50, 38-60 and 71-101%, respectively, at different growth stages. Maximum enhancement was observed in 70% extract at last harvest interval (Fig. 4). Dry biomass of the test fungal species was significantly higher in 50-70% extracts as compared to lower concentration of 10 and 20% at all the three harvests (Fig. 3, 4).

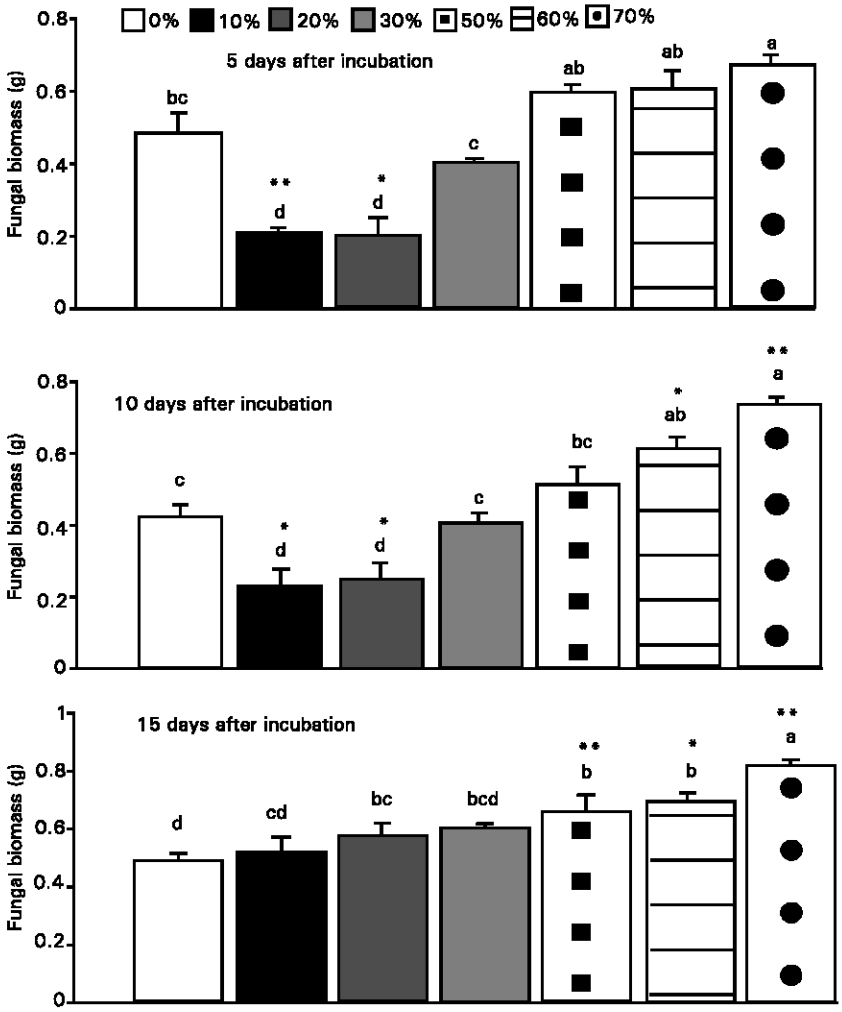

Fig. 5a-c: Effect of different concentrations of aqueous extracts of *Parthenium hysterophours* on dry biomass production of *Phoma glomerata*

Vertical bars shoe standard errors of means of three replicates

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

Effect of aqueous extracts of *Parthenium* on biomass production of *Phoma glomerata*

A variable response of biomass production in *Phoma glomerata* was recorded to aqueous extracts of *Parthenium hysterophorus* at different harvest intervals (Fig. 5). *Phoma glomerata* when grown in either 10 or 20% extract of *Parthenium* exhibited a significant reduction in biomass up to 10 days growth stage as compared with control. The reduction in biomass ranged

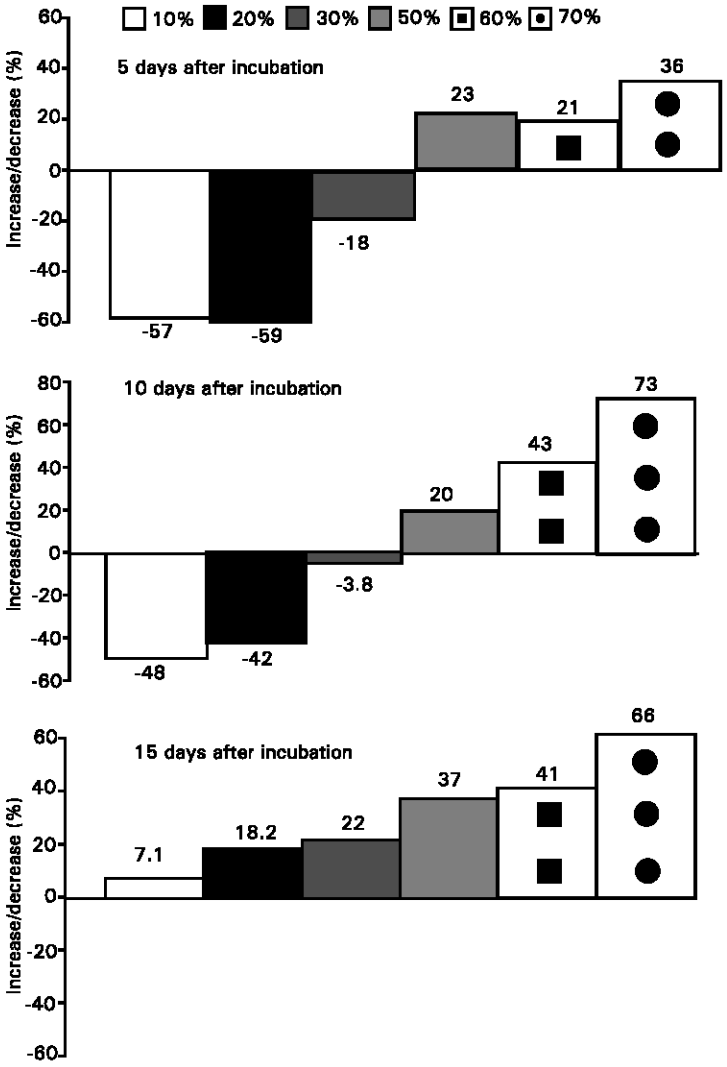

Fig. 6a-c: Effect of aqueous extracts of ***Parthnium hysterophorus*** on percentage losses in dry biomass production of ***Phoma glomerata***

from 42 to 59% due to 10 and 20% extracts at first two harvest intervals (Fig. 5, 6). In 30% extract there was an insignificant decrease in fungal biomass up to 10 days growth. At final harvest interval i.e. 15 days after incubation there was a gradual increase in fungal biomass due to application of higher concentrations. However, the positive impact of extracts was insignificant up to 30% and significant (P = 0.05, 0.01) there after. The insignificant effect of 10-30% extract ranged from 7.1-22% and significant effect of higher concentration ranged from 37-66% (Fig. 5, 6).

Discussion

The results obtained in the present study revealed that generally the lower concentrations of 10, 20 and 30% aqueous extracts of ***Parthenium*** reduced the fungal biomass production in all the test pathogenic fungal species especially at initial growth stage. In contrast, the higher concentrations of 50, 60 and 70% of aqueous extracts of ***Parthenium*** invariably enhanced the fungal biomass, production in all the three test species. However, the dry biomass production response to lower and higher concentrations of extracts varied in different test species and at different growth stages. The lowest concentration of 10% of ***Parthenium*** extract caused a persistent negative impact on growth of ***Drechslera tetramera*** and ***Aspergillus niger***. The losses in dry biomass range from 20-36% and 10-27% in ***Drechslera tetramera*** and ***Aspergillus niger*** respectively. Adverse effect of 20% concentration of extract was significant only at final harvest interval i.e., 15 days after incubation in both ***Drechslera tetramera*** and ***Aspergillus niger***. In contrast significant negative impact of this concentration was observed at 5 and 10 days harvest intervals in ***Phoma glomerata***. Similar inhibition in biomass production has also been reported by many earlier workers in other fungal species due to aqueous extracts of allelopathic plants. The leaf extract of ***Datura stramonium*** considerably declined the development in rust pustules on leaves of wheat (Hussain ***et al.***, 1992). Similarly Rafiq ***et al.*** (1984) have reported a marked suppression in growth of ***Helminthosporium turcicum***, ***H. maydis*** and ***H. carbonum*** due to aqueous leaf extracts of ***Anagalis arvensis***. According to Khan ***et al.*** (1998) aqueous extracts of ***Allium cepa*** exerted antifungal activity against ***Helminthosporium turcicum*** and ***Ascochyta rabiei*** and that of ***Calotropis procera*** against ***Alternaria radicina***. Recently Bajwa ***et al.*** (2001) found inhibitory potential in aqueous extracts of three asteraceous allelopathic species against growth of ***Aspergillus niger***. The reduction in dry fungal biomass in the presence of allelochemicals could probably be attributed to the rate of mitosis (Cornman, 1946) and enhanced cellular respiration (Singh and Kohli, 1999).

The higher concentrations of 50, 60 and 70% aqueous extract of Parthenium enhanced the fungal biomass invariably in all the three fungal test species. Generally there was a gradual increase in fungal biomass by increasing the concentration of the extract. The optimal range of 4-6 pH has been reported for the test fungal species area known to show their best growth in pH range of 4-6 (Domsch ***et al.***, 1980). Since the aqueous extract of ***Parthenium*** contains phenolic acids, the enhanced growth in higher concentrations of the extract might be due to low pH level of the medium. Recently Shafique (2002) has reported a similar stimulatory effect of 50-70% concentrations of ***Parthenium*** on the growth of ***Drechslera hawaiinsis***, ***Alternaria alternata*** and ***Fursarium*** moniliforme. In contrast to fungal growth response to allelopathic extracts, in higher plants the growth have been shown to be stimulated by lower concentrations while higher being inhibitory (Shaukat ***et al.***, 1983; Narwal, 1994).

The response of fungal dry biomass to the allelopathic stress was found to be species specific. ***Phoma glomerata*** was found to be highly susceptible to lower concentrations of 10 and 20% extract where by a biomass loss of up to 59% was recorded. In ***Drechslera tetramera*** and ***Aspergillus niger*** the growth losses were 36 and 34% respectively in lower concentrations. Similarly in highest concentration of 70% extract, the maximum enhancement of 101% was

recorded in ***Aspergillus niger*** followed by 73 and 51% in ***Phoma glomerata*** and ***Drechslera tetramera***, respectively. This varied susceptibility to extract could be due to inherent difference in physiological and morphological characteristics of various species involved (Shaukat ***et al.***, 1983). Toxicity is assumed to be associated with the presence of strong electrophilic or nucleophilic system. Action by such systems on specific positions of proteins or enzymes would alter their configuration and affect their activity (Macias ***et al.***, 1992).

The present study concludes that the lower concentrations of 10-30% of aqueous extract of ***P. hysterophorus*** can be effectively employed to control to pathogenic fungi. The higher concentrations of the extract are stimulatory to the pathogenic fungi. This aspect would greatly be helpful in biological weed control through mycoherbicides where mass production of the candidate fungal agent is required.

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