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Antifungal Activity of Allelopathic Plant Extracts I. Effect of Aqueous Extract of Three Allelopathic Asteraceous Species on Growth of *Aspergillii*

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Abstract: Effect of aqueous root and shoot extract of three allelopathic plants of family Asteraceae viz., *Helianthus annuus* L., *Ageratum conyzoides* L. and *Cirsium arvense* (L.) Scop., on growth of three pathogenic fungi namely *Aspergillus niger* Van Tieghem, *A. fumigatus* Fresenius and *A. nidulans* Eidam was studied in Hagem's liquid nutrient medium. Root extract of *H. annuus* suppressed the growth of *A. niger* and *A. fumigatus* very effectively, *A. conyzoides* reduced the growth of *A. fumigatus* to a certain extent while the aqueous extracts of *C. arvense* induced lowest toxicity against the fungal species. *A. nidulans* exhibited complete resistance against all allelopathic extracts tested.

Key words: Allelopathic extract, Asteraceae, *Aspergillus* spp.

Introduction

Fungi rank second only to insects as a cause of plant diseases, which result in heavy loss of plant products. Although chemical control of fungi is the best way to protect plants 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 resistance. 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 the target fungi (Brady, 1984). Considering the high cost of chemical pesticides and their side effects, alternative methods such as biological control of plant diseases have been suggested (Mulder, 1979). Allelopathy has a wide range of influences in various disciplines of agriculture such as agronomy, horticulture, forestry, plant pathology and weed sciences (Rice, 1984). Generally plants exhibit allelopathy by releasing phytotoxins from leaves, stems, roots, rhizomes, flowers, fruits, seeds and glandular trichomes (Horsely, 1977; Sterling *et al*., 1987; Khan and Jahan, 1988; Kuti *et al*., 1990). All basic plant processes such as hormonal balance, chlorophyll production, respiration, photosynthesis, plant water relations and permeability may be affected by allelochemicals (Yamane *et al*., 1992). Aqueous extracts of many allelopathic plants are known to exhibit antifungal properties. Allelochemicals reduce the germination of spores and mycelial growth of pathogenic fungi. In recent years extensive studies have lead to reveal various functional aspects of allelochemicals. Dwivedi and Dubey (1986) observed that volatile fractions of *Azadirachta indica* and *Eucalyptus globulus* were effective in suppressing the sclerotial germination of *Macrophomina phaseolina*. 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). Mughal *et al*. (1996) reported that aqueous leaf extracts of *Allium sativum*, *Datura alba* and *Withanea somnifera* inhibited the growth of *Altemaria altemata*, *A. brassicola* and *Myrothecium roridum*. According to Khan *et al*. (1998) aqueous extract of *Allium cepa* exhibited antifungal activity against *Helminthosproium turcicum* and *Ascochyta rabiei* and that of *Calotropis procera* against *Altemaria radicina*. The present investigations were, therefore, designed to study the effect of aqueous root and shoot extracts of three asteraceous plant species viz, *Helianthus annuus*, *Ageratum conyzoides* and *Cirsium arvense* on growth of three pathogenic species of genus *Aspergillus* namely *A. niger*, *A. fumigatus* and *A. nidulans*.

Materials and Methods

Fresh plant materials of three allelopathic plant species of family Asteraceae namely *Cirsium arvense*, *Ageratum conyzoides* and *Helianthus annuus* were collected. Plants were washed thoroughly, roots and shoot were separated, cut into fine pieces and air dried. Both root and shoot materials were surface sterilized root and shoot materials were crushed thoroughly under aseptic conditions and soaked in sterilized water @ 20 g/100 ml of water for 24 hours and filtered. Filtrates were preserved at 4°C. To avoid any prospective chemical alterations the extracts were generally used within a week.

Hagem's nutrient medium was used for fungal growth. Twenty percent w/v extract of root and shoot was diluted to 15, 10 and 5% w/v extracts by adding sterilized water. 80ml of autoclaved Hagem's Nutrient Medium was poured in 250 ml flasks and 20 ml of sterilized 5, 10, 15 and 20% w/v root and shoot extracts were added to make the volume 100ml. Control received the same amount of sterilized water.

Discs of 6 mm diameter of each of the three test fungal species viz., *Aspergillus niger*, *A. fumigatus* and *A. nidulans* were transferred aseptically into the media flasks of different concentrations. Flasks were incubated at 25±3°C. Growth of fungal species was assessed periodically at intervals up to two weeks. Fungal biomass from each flask was filtered on pre-weighed filter paper, oven dried at 60 °C for 24 hours and weighed. All the data were analyzed statistically by LSD method.

Results

Growth response of *A. niger* to allelopathy: The periodic growth assays of *Aspergillus niger* against the aqueous shoot extracts of three test species of the family Asteraceae are presented in Fig.1. The dry weight determination in varying concentrations of 0-20% revealed that the lowest and highest (5% and 20%) concentrations of both *Ageratum conyzoides* and *Helianthus annuus* induced a significant initial inhibition in the growth of *A. niger* which persisted over the growth period and the species exhibited highly marked sensitivity against 20% concentration of *Helianthus annuus*. The intermediate concentrations of 10% and 15% apparently were supportive and specifically maximum benefit was attained in the early growth phase.

In contrast to *Ageratum conyzoides* and *Helianthus annuus*,

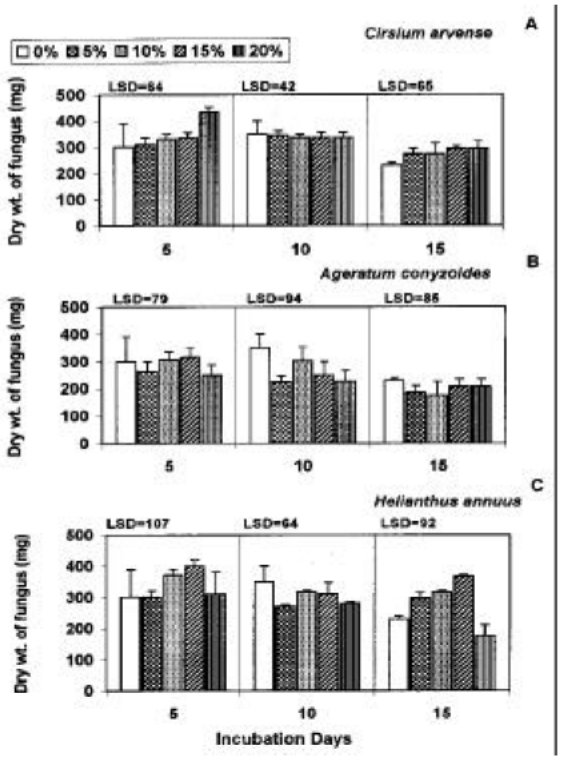

Fig. 1: (A-C) Effect of shoot extracts of *Cirsium arvense* *Ageratum conyzoides* and *Helianthus annuus* on dry biomass production of *Aspergillus niger*.

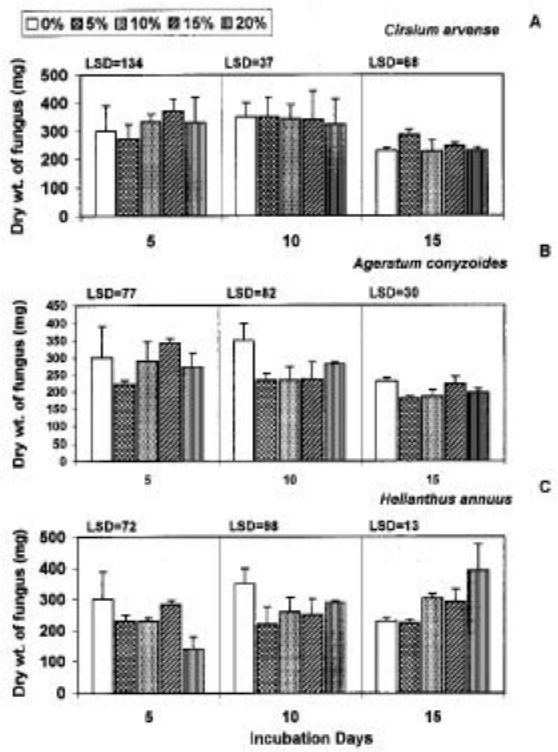

Fig. 2: (A-C) Effect of root extracts of *Cirsium arvense* *Ageratum conyzoides* and *Helianthus annuus* on dry biomass production of *Aspergillus niger*.

neither of the concentration of aqueous shoot extract of *Cirsium arvense* caused any adverse effect on the growth of *A. niger*. All concentration provided early stimulus in the biomass production, specifically, a highly significant response was evident in highest concentration of 20%. The maximum possible growth by the test species being achieved over 10 days in 0-15% concentrations and seemingly up to 5 days in 20% concentration. The limiting factor of nutrition arrested further growth increments. Therefore, any significant response to treatment was not evident in the later phase.

The assessment of growth of *A. niger*, exposed to various dilutions of aqueous root extracts from different allelopathic agents, in general represented a varied response (Fig. 2) as compared to that exhibited against aqueous shoot extract of the respective species. While the 5% and 20% concentrations of aqueous root extract of *Ageratum conyzoides* induced almost parallel inhibition in dry mass production, growth stimulus of the level initially evidenced in 10% and 15% concentrations of aqueous shoot extract was also indicated in this case. At 10 days interval, the growth was further declined in 10 and 15% concentrations, further increments in 5% and 20% were almost negligible and a significant decline also occurred in 15% concentration. So that by the last harvest, dry weight of fungus was found to be declined in all the treatments in comparison to control.

As far as the sensitivity of the fungus against aqueous root extract of *Cirsium arvense* is concerned, only 5% concentration induced slight inhibition in growth while a small non-significant enhancement was observed in higher concentrations. By the second harvest interval, the dry mass production in all treatments as well as control was parallel. In further 5 days, the stress on growth was found to have no more significant effect on growth of *A. niger* by aqueous root extract concentrations of 10%.

A more contrasting effect on growth of *A. niger* was exhibited by aqueous root extract of *Helianthus annuus*. Whereas the dosage of 15% aqueous shoot extract provided an early stimulus, all other aqueous root extract treatments caused severe inhibition and consequently growth decline in this phase, with a highly marked toxicity in 20% concentration. However, from 10 days onward non or very little growth improvement occurred in 5-15% treatment, growth stress in 20% was relieved greatly and a sharp, gradual increase in growth was evident in later phase indicating a contrasting response as compared to aqueous shoot extract.

Growth response of *Aspergillus fumigatus* to allelopathy: The growth bioassays of *A. fumigatus* in response to aqueous shoot extract of various allelopathic plant species (Fig. 3) expressed almost similar pattern of effects as was evidenced previously in *A. niger*. The early growth was makedly declined against all concentrations of shoot extracts of both *Helianthus annuus* and *Ageratum conyzoides* while it slightly improved in *Cirsium arvense*. As far as individual allelopathic potential of the specie is concerned, the aqueous shoot extract of *Ageratum conyzoides* at 20% level caused a relatively more pronounced toxic effect on initial growth of the species which was found to be relieved slowly in the later intervals. An over all decline of 18-56% in dry biomass production was occurred against this concentration. The rest of the employed treatments of *A. conyzoides* caused slow gradual depression

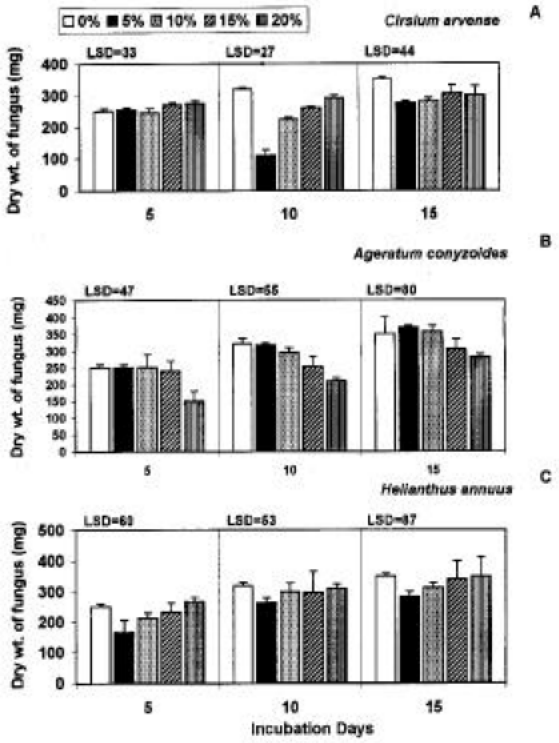

Fig. 3: (A-C) Effect of shoot extracts of *Cirsium arvense* *Ageratum conyzoides* and *Helianthus annuus* on dry biomass production of *Aspergillus niger*.

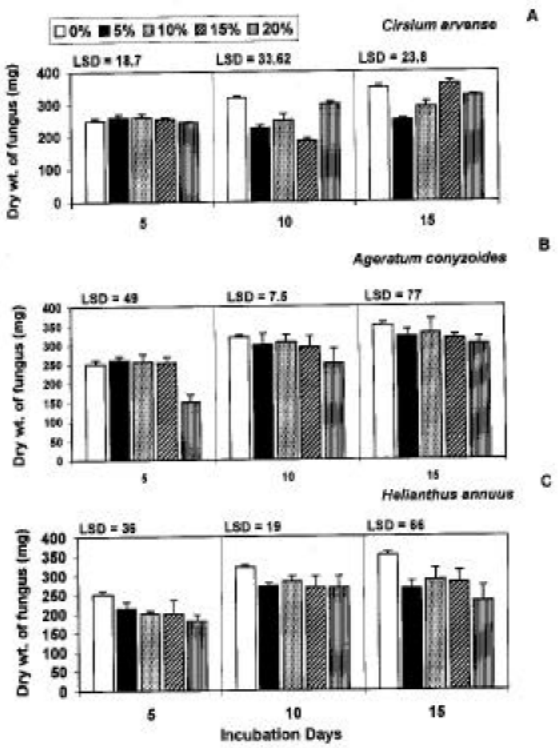

Fig. 4: (A-C) Effect of root extracts of *Cirsium arvense* *Ageratum conyzoides* and *Helianthus annuus* on dry biomass production of *Aspergillus niger*.

in fungal growth, proportionate directly to the levels of concentrations of aqueous shoot extract.

The response of *A. fumigatus* against aqueous shoot extract of *Helianthus annuus* was highly contrasting as compared to that of *A. conyzoides* upto first harvest, interval of 5 days. The biomass production was significantly declined in all applied concentrations of 5-15% and specifically, more in 20%. From 5 days onward the allelopathic stress of the treatments was no more evident. As a consequence the fungal biomass increased exclusively over the last growth interval so much so that at the last harvest stage the dry weight gain was found to be stimulated in higher doses of (15% and 20%).

No significant influence either on initial or final biomass production of *A. fumigatus* was observed against aqueous shoot extract of *Cirsium arvense*. However, in the intermediate stage of growth, a rather delayed toxic effect and consequent decline in growth inversely proportional to the levels of employed concentrations of aqueous shoot extract were quite evident in 5-10% treatments. In 20% concentration as the possible maximum dry weight was almost attained in the early phase, no further considerable variation was observed in this treatment.

The results obtained on comparative dry weight gain of *A. fumigatus*, after being exposed to variable concentrations of aqueous root extract of different allelopathic agents are summarized in Fig. 4. It showed that irrespective of treatments, the maximum biomass input, in general was occurred in the early growth phase between 5-10 days, very little or no further growth increments apparently were added in the last phase.

As far as, the specie to specie effectivity is concerned the *A. conyzoides* and *C. arvense* as well as *H. annuus* induced influence almost identical to aqueous shoot extract of the respective species with only slight and relatively insignificant variations. As in the case of *Ageratum conyzoides* the concentration based variations in 5-15% treatments were not evident from the middle phase onwards. Similarly the toxic effects of *Helianthus annuus* diappeared to some extent in this phase of fungal growth. The 5-15% concentrations of *Cirsium arvense* induced some stress on the growth of *A. fumigatus* in the middle phase and specifically sharp decline was observed in 15% concentrations as against 5% concentration in the case of aqueous shoot extract. However, incredibly sharp increase in the same concentrations lead to significant improvement in dry weight gain. The 20% concentration was found to be least effective and periodic, gradual and normal increase parallel to control was very obvious.

Growth response of *Aspergillus nidulans to* allelopathy: *Aspergillus nidulans* was highly resistant to shoot extracts of all the three test species (Fig. 5). The addition of relatively increasing or decreasing concentrations of aqueous shoot extract caused parallel proportionate depression or stimulus in growth of the fungus. A specifically significant growth enhancement was achieved against supplementations of shoot extract of *Cirsium arvense*. An apparent exception to widely expressed effectivity of the test species, however, was evidenced in response to *Helianthus annuus* at the later growth intervals. Seemingly in 5-15% concentrations of aqueous shoot extract the growth of fungus was declined

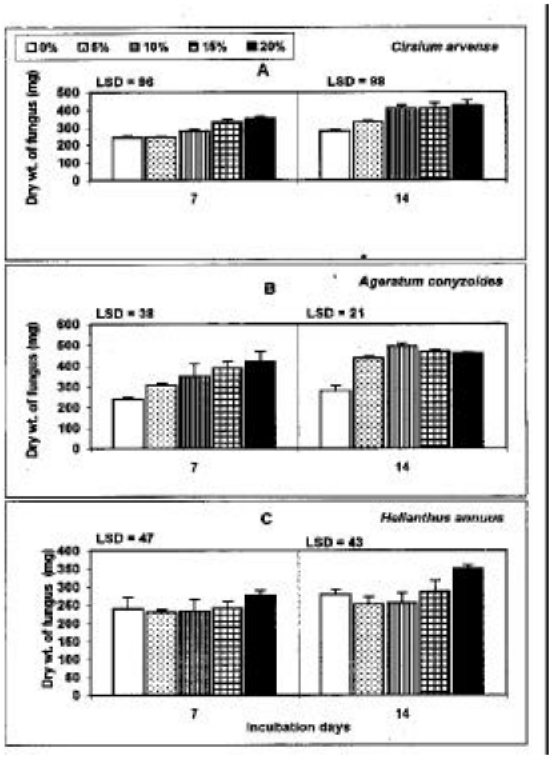

Fig. 5: (A-C) Effect of shoot extracts of *Cirsium arvense* *Ageratum conyzoides* and *Helianthus annuus* on dry biomass production of *Aspergillus niger*.

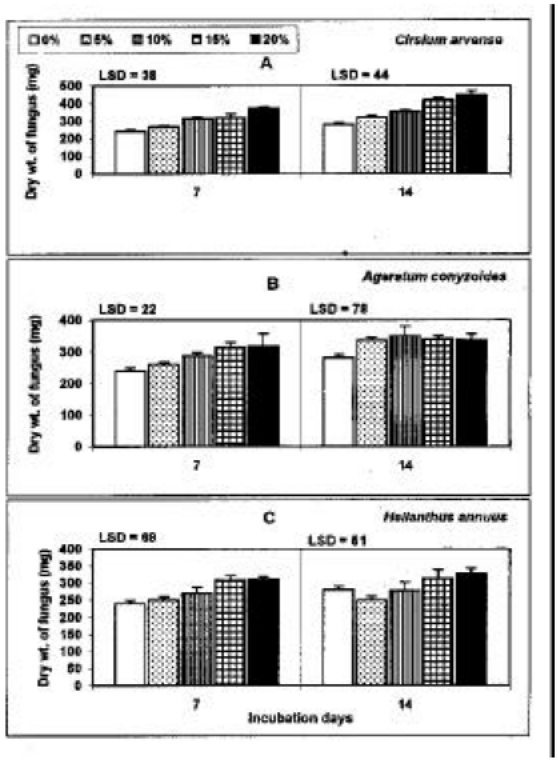

Fig. 6: (A-C) Effect of root extracts of *Cirsium arvense* *Ageratum conyzoides* and *Helianthus annuus* on dry biomass production of *Aspergillus niger*.

proportionately with increase in dosage, the overall depression being statistically insignificant.

The aqueous root extract of various test species induced parallel and proportionate corresponding effect on the growth of fungus in variously amended extracts as evidenced previously against aqueous shoot extracts of respective species (Fig. 6). The growth of fungus in general was stimulated and the level of stimulus, parallel to various concentrations of aqueous root extract was very obvious.

Discussion

In the present study aqueous root and shoot extracts of three plant species of family Asteraceae viz., *Ageratum conyzoides*, *Helianthus annuus* and *Cirsium arvense* exhibited a marked variability in their effect on growth of three pathogenic species of genus *Aspergillus* namely *Aspergillus niger*, *A. fumigatus* and *A. nidulans*.

Present investigations revealed that the growth of *A. fumigatus* was significantly checked by aqueous root and shoot extracts of *Ageratum conyzoides*. Similar trend of growth was observed for *A. niger* and *A. fumigatus* under the influence of root extract of *Helianthus annuus*. These findings are in line with the previous results of Singh *et al.* (1980) who reported antifungal activity of neem extracts against *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*. Fungicidal effects of *Allium sativum* and *A. cepa* against many fungal genera has also been reported by Misra and Dixit (1977). Similar effects of *Datura stramonium* against a variety of plant pathogens has previously been observed by many workers (Misra, 1978; Bhowmick and Choudhary, 1982; Jaiswal *et al.*, 1984; Bhatti, 1988). The inhibition of mycelial growth and parallel decrease in biomass may be attributed to the presence and detrimental effects of allelochemicals on cell division, cell elongation and nutrient uptake respectively (Blake, 1985).

Contrary to the detrimental effects of shoot and root extracts of *A. conyzoides*, aqueous solutions of *Cirsium arvense* enhanced the growth of *A. niger* and *A. fumigatus* while growth of *A. nidulans* was improved by aqueous extracts of all selected plant species. Similar stimulating effects of *Brassica juncea* and *B. pekinensis* against *Cephalosporium sacchari* and *Fusarium nivale* were observed by Dixit and Tripathi (1975).

It is evident from the results of present study that the aqueous root and shoot extracts of *A. conyzoides* and *C. arvense* were similar in their antifungal activity. The different parts of same plant may behave differentially as in case of *H. annuus* against *A. niger*. In this regard Mughal *et al.* (1996) found that the growth of *Alternaria alternata* was significantly retarded by leaf extract of *Withania somnifera*, while the root extracts exhibit no such effect.

Growth response of selected fungi towards aqueous extracts of test plants was found to vary at different growth stages. Aqueous shoot and root extracts of *H. annuus* suppressed the fungal growth in early phase but during the later growth period the effect was not much pronounced. Similarly normal fungal growth comparable to control was observed in all the cases during later period of study. For their periodically varied growth responses, it seems probable that lower growth during

early period owes to the higher concentration of allelochemicals in the medium causing growth check. On the other hand similar pattern of growth as that of control in all the treatments during later period of growth was due to the fact that, as the time passed products of fungal metabolites denatured the allelochemicals along with decomposition and autophagy, fungi due to lack of nutrients resulting in the production of smaller fungal biomass.

Greater inhibition of fungal growth at higher concentration of aqueous plant extracts was a general trend during this study. In this regard higher concentration (20%) of both root and shoot aqueous extracts of *Ageratum conyzoides* displayed maximum inhibition against *A. fumigatus*. This is in conformity with the earlier findings of Mughal *et al.* (1996) who observed that high concentration of plant extracts induced maximum retardation in fungal growth. Similar responses have also been reported by Singh *et al.* (1980) which indicated that concentration of allelochemicals has considerable effect.

It is concluded that aqueous extracts of different plants or plant parts may be used as biofungicides against a variety of pathogenic fungi. However the efficiency of such extracts depends greatly upon the resistance offered by the different fungal species. Among the three selected plant species, the aqueous root extracts of *Helianthus annuus* proved to be the most effective against *A. niger* and *A.fumigatus* while inefficiency of root and shoot extracts of *Cirsium arvense* was quite evident. To generalize these findings allelopathy of test plants needs to be investigated against a wide range pathogenic fungi to develop sufficient understanding to utilize the appropriate concentrations and to specify host pathogen combinations.

References

Bhatti, M.H.R., 1988. Antifungal properties of plant leaf decoction against leaf rust of wheat. Pakistan J. Bot., 20: 259-263.

Bhowmick, B.N. and B.K. Chaudhry, 1982. Antifungal activity of leaf extract of medicinal plants on *Alternaria alternata*. Ind. Bot. Rep., 1: 164-165.

Blake, N.E., 1985. Effect of allelochemicals on mineral uptake and associated physiological processes. In: The Chemistry of Allelopathy. Biochemical Interactions among Plants (Thompson, A.C., ed.), pp:161. American Chemical Washington.

Brady, N.C., 1984. *The nature and properties of soil*. MacMillan Publishing Company, New York, pp:528.

Dixit, S.N. and S.C. Tripathi, 1975. Fungistatic properties of some seedling extracts. Curr. Sci., 44: 279-280.

Dwivedi, R.S. and R.C. Dubey, 1986. Effect of volatile and non-volatile fractions of two medicinal plants on germination of *M. phaseolina* sclerotia. Trans. Br. Mycol. Soc., 87: 326-328.

Horsley, S.B., 1977. Allelopathic interference among plants. II. Physiological modes of action. *Proc. 4th North American Forest Biology Workshop* (H.E. Wilcox and A. Hamer, eds.), pp:93-136. Syracuse University Press, Syracuse, N.Y.

Jaiswal, S., A. Batra, and B.K. Mehta, 1984. The antimicrobial efficiency of root oil against human pathogenic bacteria and phytopathogenic fungi. Phytopathelegische Zeitschrift., 109: 90-93.

Khan, M.J. and B. Jahan, 1988. Allelopathic potential of senesced anthers of Bombax ceiba L., inhibiting germination and growth of Lettuce seeds. Pak. J. Bot., 20: 205-212.

Khan, T.Z., Nasir, M.A. and S.A.A. Bokhari, 1998. Antifungal properties of some plant extracts. Pak. J. Phytopathol., 10: 62-65.

Kuti, J.O., B.B. Jarvis, N.R. Mokhtari and G.A. Been, 1990. Allelochemicals regulation of reproduction and seed germination of two Brazillian Baccharis species by phytotoxic trichothecenes. J. Chem. Ecol., 16: 3441-3453.

Misra, S.B. and S.N. Dixit, 1977. Screening of some medicinal plants for antifungal activity. Geobios, 4: 129-132.

Misra, S.B., 1978. Antifungal activity of vapours of some plant extracts. Acta Botanica Indica, 6: 118-121.

Mughal, M.A., T.Z. Khan and M.A. Nasir, 1996. Antifungal activity of some plant extracts. Pak. J. Phytopathol., 8: 46-48.

Mukerjee, S.R., R.P. Nath and M.G. Haider, 1979. Effect of smut disease on the jaggery quality of sugarcane. Ind. Phytopathol., 32: 627-628.

Mulder, D., 1979. Soil disinfestation. Elsvier Scientific Publication 10, Amsterdam, 368 pp.

Rai, R.A., 1973. Chemical control of powdery mildew of pea. Ind. Phytopathol., 26: 537-539.

Rice, E.L., 1984. *Allelopathy*, 2nd Ed. Academic Press Inc., Orlando, Florida, USA.

Singh, U.P., H.B. Singh and R.B. Singh, 1980. The fungicidal effect of neem extracts on some soil borne pathogens of gram (*Cicer arietinum*). Mycologia., 72: 1077-1093.

Sterling, T.M., R.L. Houtz, and A.R. Putnam, 1987. Phytotoxic exudates from Velvetleaf (*Abutilon theophrasti*) glandular trichomes. Am. J. Bot., 74: 543-550.

Yamane, A., H. Nishimura and J. Mizutani, 1992. Allelopathy of yellow field cress (*Rorippa sylvestris*). J. Chem. Ecol., 18: 683-691.