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*Full Length Research Paper*

# Antifungal activity of different extracts of *Ageratum conyzoides* for the management of *Fusarium solani*

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***Ageratum conyzoides* L. is potential allelopathic weed very useful for its antifungal and antimicrobial activity. Being environmentally safe and friendly, it has the potential to substitute synthetic fungicides. The current study, therefore, was designed to evaluate the *in vitro* efficacy of aqueous methanolic and *n*-hexane extracts of *A. conyzoides* against the pathogenic fungi, *Fusarium solani* Mart. (Sacc.), isolated from roots of egg plant *(Solanum melongena).* The target fungus was exposed to various concentrations (2, 4 and 6% w/v) of aqueous, methanolic and *n*-hexane extracts of Inflorescence, leaf, stem and root. All the employed concentrations of extracts of four plant parts significantly suppressed the growth of the target fungal pathogen. The *n*-hexane extracts of leaf and inflorescence caused highly significant reduction of 84% in growth of *F. solani* followed by stem and root extracts which caused which caused 80% and 72% reduction in growth, respectively. The same pattern in growth reduction was observed in methanolic and aqueous extracts. Among the four parts of the tested weed, different concentrations of the methanolic extract of leaf were found to be highly effective in controlling target fungal species resulting in up to 78% reduction in fungal biomass over control followed by inflorescence (74% reduction), stem (63% reduction) and root (59% reduction) at highest used concentration. In case of aqueous extracts, the maximum reduction was observed in leaf extract (72%) followed by inflorescence, stem and root, respectively.**

**Key words:** *Ageratum conyzoides*, aqueous extract, *Fusarium solani*, *n*-hexane extract, methanolic extract.

## INTRODUCTION

The fungi are the second most important disease causing organisms which causes severe crop losses all over the world. One third global agriculture production is destroyed each year by different pest and diseases (Zahid et al., 2012). Mostly, different chemicals are used to control the diseases. The use of chemicals has been found very effective in controlling fungal diseases but some major problems threatened to limit the continued use of fungicides.

The synthetic fungicides usually take long periods of time to be degraded completely causing heavy toxicity to human beings and domestic animals (Rajkumar et al., 2005). Some fungi have developed resistance to chemicals which become difficult to control. In nature, many secondary metabolites play an important role in the protection of the plants as antibacterial, antiviral, antifungal and insecticidal agents (Hajlaoui et al., 2009).

Extracts of many allelopathic plants are known to exhibit antifungal properties. The active ingredients found in allelopathic plants can be synthesized, or used in the form of extracts. The plant extracts are rapidly degraded in soil by reducing the impact on the environment, and they can have an effective role in sustainable agriculture (Cho et al., 2006).

*Ageratum conyzoides* belongs to family Asteraceae, which is a potential allelopathic weed (Batish et al., 2009). A wide range of allelochemicals including alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated from *A. conyzoides* (Okunade, 2002). Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities (Kamboj and Saluja, 2008). The crude plant extract also showed insecticidal and pesticidal activities against various types of insects and pests (Vyas and

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Mulchandani, 1980). The allelopathy of *A. conyzoides* can be used in various agricultural practices and to control diseases. Studies on the allelopathy of volatile substances of *A. conyzoides*, an important weed in South China, show that the volatiles of its fresh leaves significantly inhibited the seedling growth of all test plants. Joint effect of allelopathic potential and some meteorological factors such as sunshine, time, temperatures and rainfall are the important factors that dominated *A. conyzoides* in natural communities (Fei and Kong, 2002). It has been reported by Batish et al. (2009) that growth of rice has been suppressed due to the release of phenolic allelochemicals and residues of *A. conyzoides* and not because of alteration in the nutrients of the soil. Thus allelopathy plays an important role in root mediated interference of *A. conyzoides*.

*A. conyzoides* (billy goat weed), caused inhibition of *Raphanus sativus* L. (radish) germination and growth. The leaf exhibited a greater suppression than the stem and root. Three phenolic compounds were identified in the leaf, stem and root exudate which includes gallic acid, coumaric acid and protocatechuic acid, while catechin was found only in the stem. *A. conyzoides* can be used as natural herbicides to control weeds in fields to reduce the consumption of synthetic herbicides (Tran et al., 2004**).** *A. conyzoides* also has phytotoxic effects on chickpea (*Cicer arietinum*) (Batish et al., 2006). In the present study, an attempt was made to investigate the causal organism of wilt disease of *Solanum melongena* and to explore the antifungal activity of aqueous and organic solvent extracts of different parts of *A. conyzoide*s against *Fusarium solani.*

## MATERIALS AND METHODS

### Isolation of target fungal species

For the isolation of the pathogen, the roots of egg plant (*S. melongena*) were cut into 5 mm long and 2 to 3 mm thick pieces. Pieces were surface sterilized with 1% NaOCl solution for about 1 min which was then followed by three washing with sterilized water. Surface sterilized pieces were then transferred to malt extract agar (MEA) media in 9 cm Petri plates. Plates were incubated at 30 ± 2°C and observations were made daily for emergence of culture. Identification was done by observing the morphological characters (colony colour, shape, texture etc). Further authentic identification was done by First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan (FCBP).

### Selection of test plant

*A. conyzoides* was taken for investigation of its antifungal activity against the fungus (*F. solani*), because of its allelopathic nature. Plant was collected in spring season from the premises of grave yard in front of Institute of Agricultural Sciences Quaid-e-Azam campus, University of the Punjab, Lahore. The collected plant material was washed with tap water and surface sterilized with 1% sodium hypochlorite solution followed by washing with sterilized water. Plants were then dried in the sunlight, crushed and stored in the polythene bags for further process.

### Laboratory bioassay

### Aqueous extract preparation

Twenty grams of each dried plant parts (inflorescence, leaf, stem and root) in the powder form were soaked in 100 ml of sterilized water at room temperature for 24 h in order to prepare 20% w/v plant extract. Extracts were then filtered through muslin cloth followed by Whatman filter paper No. 1. The 20% solution was designated as stock solution, from which lower concentrations of 2, 4 and 6% were made by diluting with appropriate amount of distilled water. The extract was stored at 4°C. Control medium received 80 ml of broth methanolic extract (ME) and 20 ml of distilled water with no plant extract. Actively growing discs of *F. solani* were prepared by using a presterilized cork borer having 5 mm diameter and were then transferred to presterilized flasks aseptically. Flasks were incubated at 30 ± 2°C for 10 days in an incubator shaker. Each treatment was in triplicate. After 10 days, biomass of fungi was collected on pre-weighed filter paper. The fungal biomass was dried in an oven at 60°C for 24 h (Bajwa et al., 2006).

### Organic solvent extract preparation

Methanolic and *n*-hexane was used for organic solvent extract bioassay. Twenty grams dried powder of each plant parts viz; inflorescence, leaf, stem and root were soaked separately in 100 ml of *n*-hexane for about 24 h to prepare 20% w/v extract in 250 ml flask. Organic solvent was allowed to evaporate at room temperature and their volume raised up to 100 ml by adding distilled water. Extract was then filtered through muslin cloth followed by Whatman filter paper No. 1 (Alkhail, 2005). The same procedure was done for the preparation of Malt Extract.

### Antifungal bioassays

Aqueous solvent extract bioassays were carried out in liquid medium. The 2% malt extract medium was prepared in 250 ml conical flask. Chloromycetin capsule was added to 1 capsule 250 mg $ml^{-1}$ of medium to avoid contamination. In 80 ml of ME, 20 ml of each of 2, 4 and 6% extract of *A. conyzoides* was added. Control medium received 80 ml of broth ME and 20 ml of distilled water with no plant extract.

### Data collection and statistical analysis

In laboratory bioassays the percentage reduction in fungal biomass due to various concentrations of the extracts over control was calculated by applying the following formula:

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

The experiment was conducted using a completely randomized design. Standard errors of means of three replicates were computed through Microsoft Excel. All the data was subjected to Duncan's multiple range test (Steel and Torrie, 1997) using COSTAT.

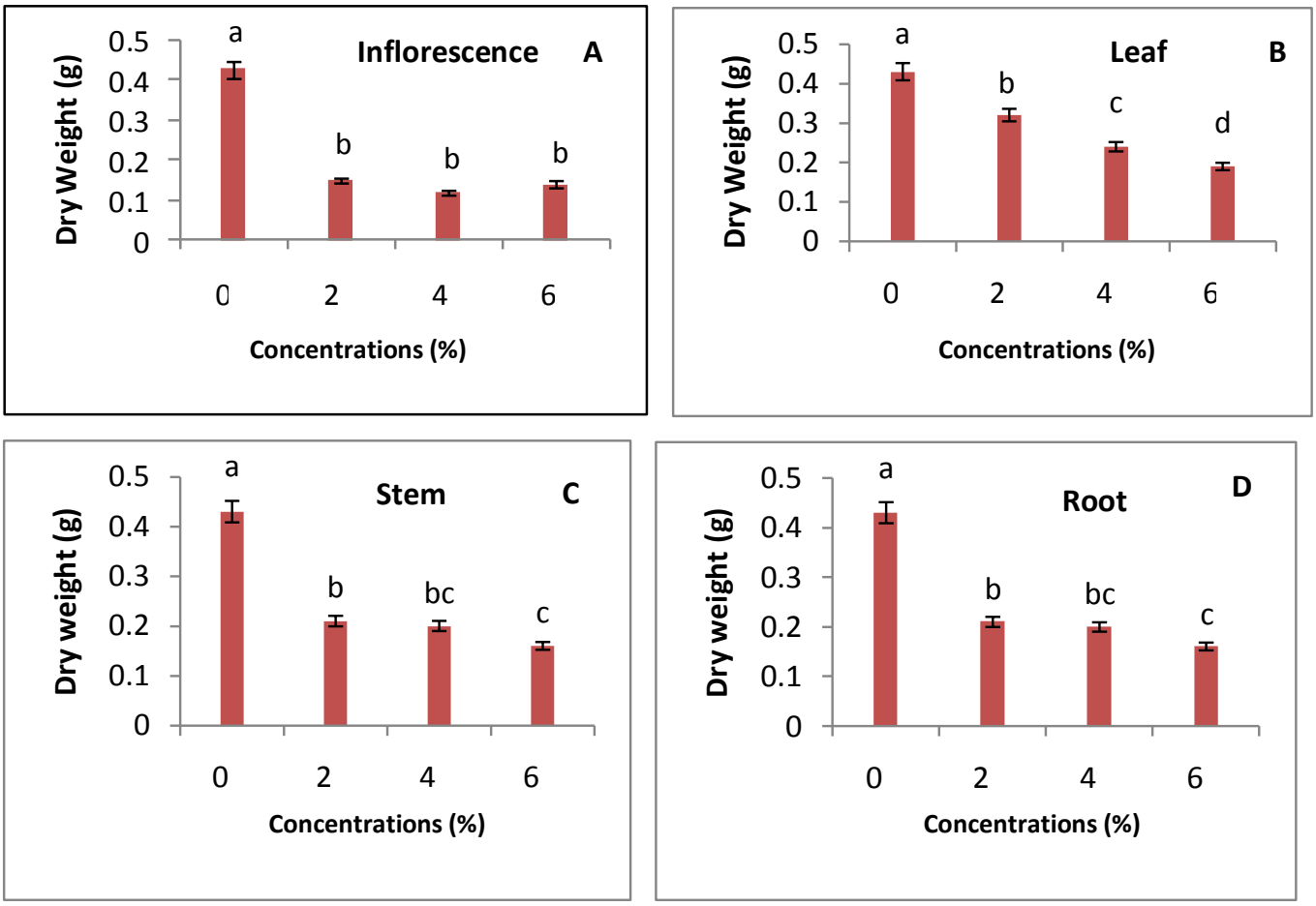


**Figure 1.** Effect of aqueous extracts of inflorescence, leaf, stem and root of *Ageratum conyzoides* on dry weight production of *Fusarium solani* .Vertical bars show standard errors of means of three replicates. Bars with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's multiple range test.

## RESULTS

### Effect of aqueous extract of *A. conyzoides* on the growth of F. solani

The potential of *A. conyzoides* allelochemicals to reduce fungal biomass production of *F. solani* was evaluated. Data analyzed statistically revealed that higher concentration of 6% of all four plant part extracts of *A. conyzoides* significantly reduced biomass production of *F. solani*.

### Effect of inflorescence extract

The aqueous extract of inflorescence induced fungal biomass inhibition up to 42 in 2% concentration. However, extract of 4% concentration caused substantial decrease up to 49%. At 6% extract concentration, maximum fungal biomass reduction (56%) occurred. Higher extract concentration exhibited more inhibitory activity as compared to lower concentrations (Figure 1A).

### Effect of leaf extract

In case of aqueous extract of leaf, the highest concentration (6%) showed a significant decrease in biomass production at 72%. The reduction of 67% in biomass production was at 4%, whereas 65% reduction was recorded in 2% concentration (Figure 1B).

### Effect of stem extract

As far as aqueous extract of stem was concerned, the results show less biomass reduction as compared to leaf and inflorescence. In case of 2% extract concentration, a decline of 26% in biomass production was evidenced. Fungal biomass decreased significantly at 44 and 59% against 4 and 6% stem extracts, respectively (Figure 1C).

### Effect of root extract

The aqueous extract of *A. conyzoides* root showed greater inhibitory effects in higher concentration of 6% that significantly decreased fungal biomass production by 63%. Decrease in 4% extract concentration claimed up to 54%. Minimum reduction of 51% was exhibited in biomass production under 2% root extract of *A. conyzoides* (Figure 1D). Comparison among aqueous extract concentration of *A. conyzoides* parts was found to be dosage-

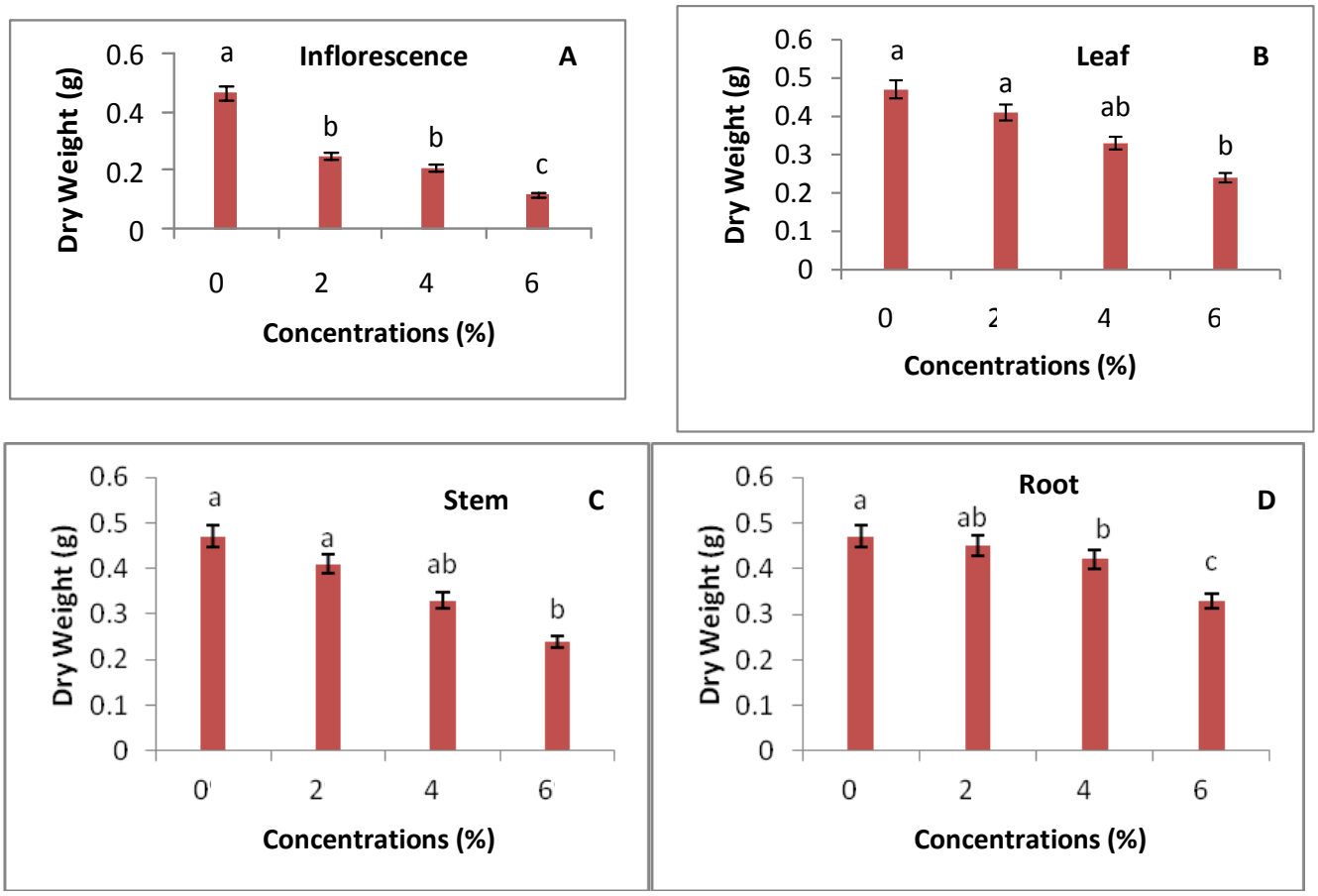


**Figure 2.** Effect of methanolic extracts of inflorescence, leaf, stem and root of *Ageratum conyzoides* on dry weight production of *Fusarium solani*. Vertical bars show standard errors of means of three replicates. Bars with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's multiple range test.

dependent as the lowest tested concentration inhibited less biomass production, whereas at highest concentration maximum biomass reduction was observed.

### Effect of methanolic extract of *A. conyzoides* on the growth of *F. solani*

#### Effect of inflorescence extract

Data records on the effect of inflorescence methanolic extract of *A. conyzoides* on the growth of *F. solani* showed a gradual decrease in fungal biomass production with the increase in extract concentration. Significant reduction of 74% in fungal biomass was recorded at the highest concentration of 6% as compared to the control. Least growth inhibition up to 47% was observed at 2% concentration of methanolic extract and a reduction of 55 to 4% extract concentration was observed. So, all the concentrations of methanolic extract showed significant antifungal effect against *F. solani* (Figure 2A).

#### Effect of leaf extract

Methanolic leaf extract bioassays showed significant reduction up to 78% in fungal biomass at higher concentration of 6%. At 4%, comparatively low decline in fungal biomass was observed, that is, 74%, whereas in the lowest concentration of 2%, fungal growth was inhibited up to 72%. In general it was observed that all the concentrations exhibited significant inhibitory effect on fungal biomass production as compared to control (Figure 2B).

#### Effect of stem extract

As compared to leaf, stem methanolic extracts exhibited less reduction in fungal biomass production. Maximum decrease of 63% in biomass production was observed in 6% concentration of methanolic as compared to control. Whereas 54% decrease in biomass production was observed at 4% and decline of 51 to 2% methanolic extract concentration of stem (Figure 2C).

#### Effect of root extract

The effect of root methanolic extract of *A. conyzoides* exhibited less decrease in fungal biomass as compared to inflorescence, leaf and stem, respectively. As 59% reduction was observed in 6% concentration as compared to the control. The lowest concentration of 2% showed 26% reduction whereas in 4% concentration biomass decline was 44% (Figure 2D).

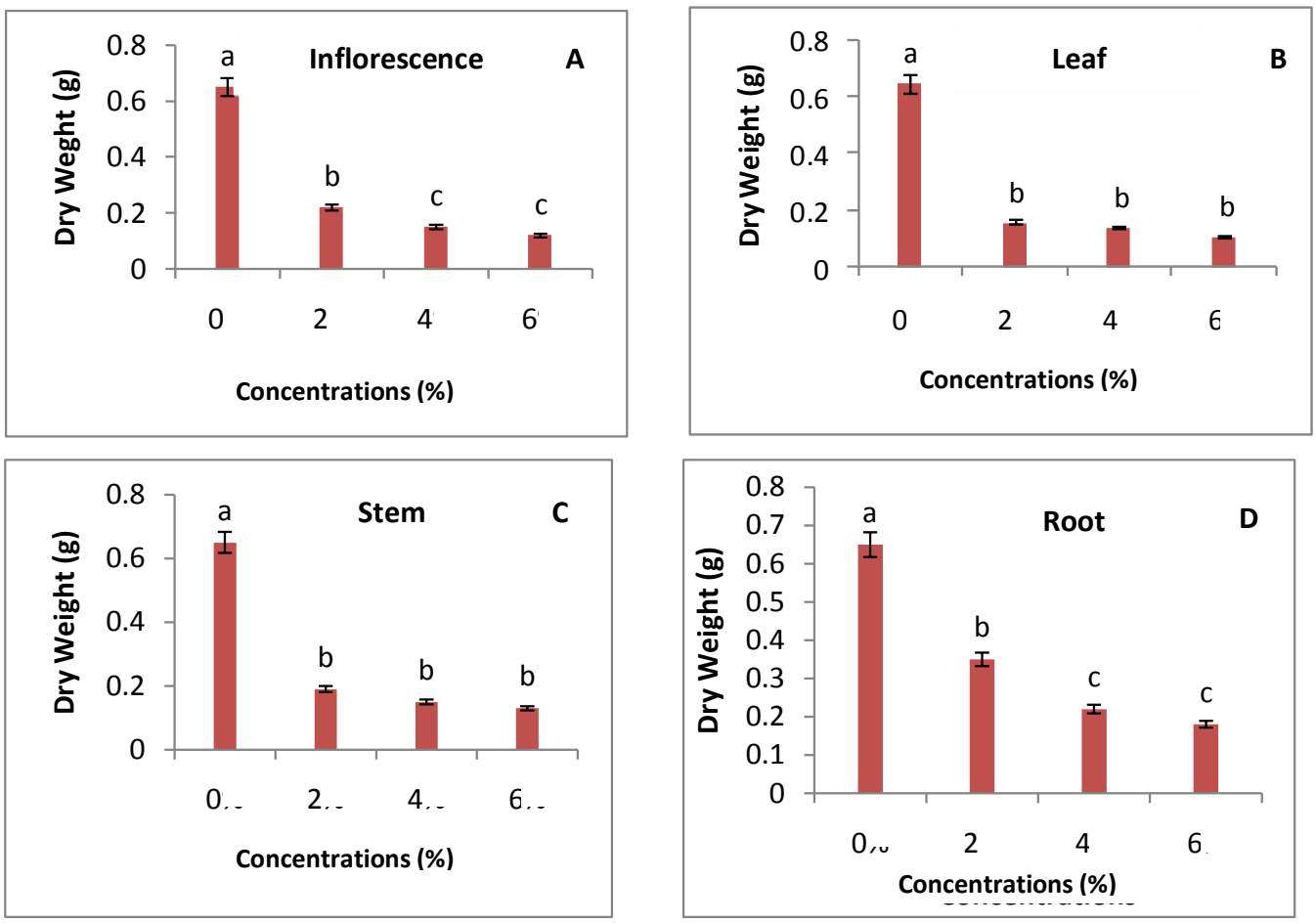


**Figure 3.** Effect of *n*-hexane extracts of inflorescence, leaf, stem and root of *Ageratum conyzoides* on dry weight production of *Fusarium solani*. Vertical bars show standard errors of means of three replicates. Bars with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's multiple range test.

**Effect of *n*-hexane extract of *A. conyzoides* on the growth of *F. solani***

Efficacy of *n*-hexane extracts of dry inflorescence, leaf, stem and root of *A. conyzoides* to influence the growth of *F. solani* was estimated and general impact was found to be more conspicuous in higher concentrations as compared to the lower ones.

**Effect of inflorescence extract**

In case of inflorescence *n*-hexane extract, a significant reduction in fungal biomass production was observed in all extract concentrations. There was a decrease of 82% in biomass production at the highest concentration of 6%. Relatively less inhibition of fungal biomass production was recorded in 4% which was 77%. In the case of 2%, *n*-hexane extract of inflorescence exerted less influence on fungal biomass reduction as compared to 4 and 6% was witnessed (Figure 3A).

**Effect of leaf extract**

The allelopathic efficacy of *n*-hexane leaf extract against reduction in fungal biomass was also found to be highly pronounced. 78% reduction in biomass production was observed in 2% leaf extract of *n*-hexane. Further increase in extract concentration resulted in parallel greater reduction in biomass production of fungi, that is, 80 to 4% and 83 to 6% *n*-hexane extract concentration (Figure 3B).

**Effect of stem extract**

The potential of *n*-hexane stem extract of *A. conyzoides* against the biomass production of *F. solani* was evaluated. In case of stem *n*-hexane extract, a persistent pattern was evidenced. The highest concentration, that is, 6% caused significant reduction in fungal biomass production which is 80%. Relatively low declines in fungal biomass production, that is, 77 and 71% were observed at 4 and 2% stem extract, respectively (Figure 3C).

**Effect of root extract**

In the case of root *n*-hexane extract, maximum decline of 72% in fungal biomass was observed at 6%. A decrease of 66% was evidenced in 4% stem extract. Fungal biomass reduction decreased significantly by 46% in 2% root extract as compared to control (Figure 3D).

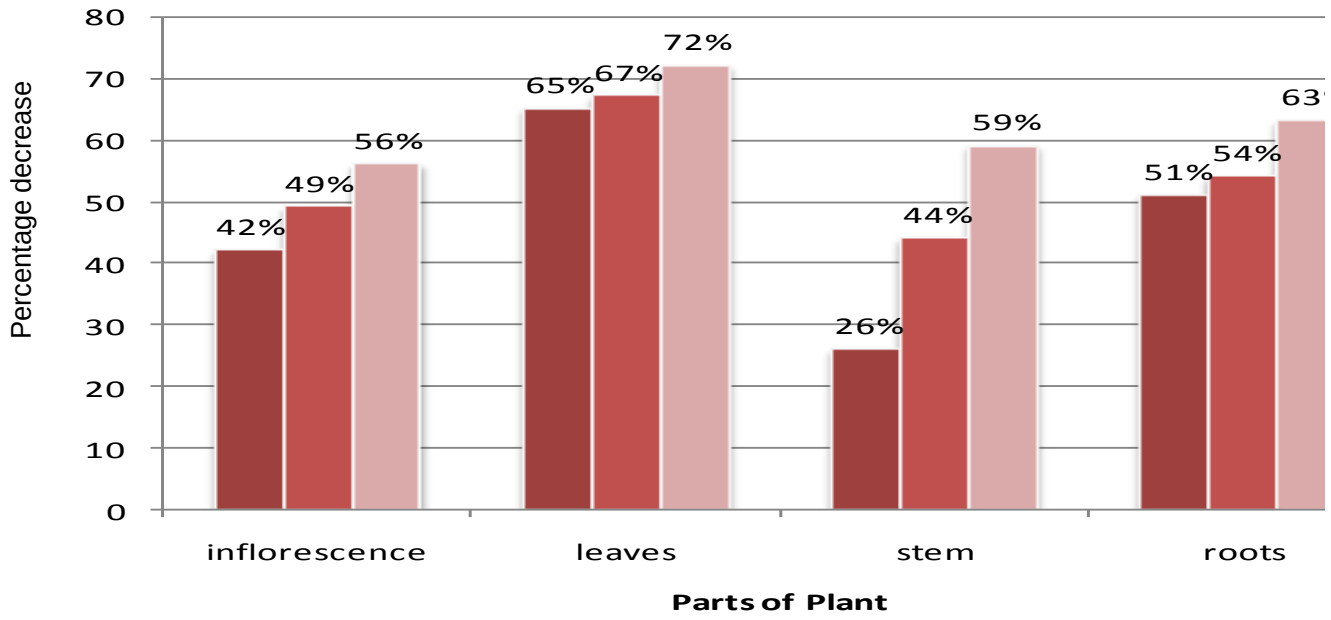


**Figure 4.** Percentage increase/decrease in biomass of *Fusarium solani* due to different concentrations of aqueous extracts of inflorescence, leaf, stem and root of *Ageratum conyzoides* against control.

## DISCUSSION

The present study was planned to evaluate the antifungal potential of an allelopathic plant *A. conyzoides*. It was checked for its antifungal and allelopathic effect against *F. solani*, a devastating pathogen on variety of economic plants. All vegetative plant parts for example, inflorescence, leaf, stem and roots of *A. conyzoides* were used in dried and crushed form to control the growth of *F. solani*, cause of wilt and rot diseases. All the aerial parts and root of *A. conyzoides* showed significant decline in the growth of fungal species of *F. solani*. Among the aqueous extracts of the all parts of *A. conyzoides*, leaf showed maximum reduction of 72% at the highest concentration of 6% (Figure 4). *A. conyzoides* L. extracts are known to possess pharmacological and biocidal activity. It was investigated that the growth of *Aspergillus fumigatus* was significantly checked by aqueous root and shoot extracts of *A. conyzoides*.

In a similar study, higher concentration of both root and shoot of aqueous extracts of *A. conyzoides* displayed maximum inhibition against *A. fumigatus* (Bajwa et al., 2001). The findings in this study are also in line with the earlier findings of Mughal et al. (1996) who observed that high concentrations of plant extracts induced maximum retardation in fungal growth. Target fungus was also subjected to methanolic extracts of different plant parts. Among the four parts of the tested weed, different concentrations of the methanolic extract of leaf were found to be highly effective in controlling target fungal species resulting in up to 78% reduction in fungal biomass over control followed by inflorescence (74% reduction), stem (63% reduction), root (59% reduction) at highest concentration (Figure 5).

Volatiles from macerated green leaf tissue of *A. conyzoides* were also effective against *Aspergillus parasiticus* (Patil et al., 2010). In general, methanolic leaf extract of *A. conyzoides* caused more pronounced inhibition for growth of *F. solani* because leaf exhibits higher allelopathic potential as compared to inflorescence, stem and root respectively. Antifungal activity of test plant parts in different solvents was evident (Einhelling, 1996; Willis, 1999). *n*-hexane extracts of inflorescence, leaf, stem and root also showed same pattern of results as methanolic and aqueous extracts. *n*-hexane extracts of these plant parts showed significant decline in fungal growth. Minimum reduction in fungal biomass was noticed in root (46 to 72%), whereas leaf showed maximum fungal biomass reduction (78 to 83%). Inflorescence and stem also showed fungal biomass reduction (66 to 84% and 71 to 80%), respectively (Figure 6). Although all the *n*-hexane extracts of inflorescence, leaf, stem and root showed significant reduction in biomass production; *n*-hexane leaf extract showed the maximum reduction.

Bioactivity of a secondary metabolite (a chromene) isolated from the shoots of *A. conyzoides* against some plant pathogenic fungi was reported. The crude *n*-hexane extract completely inhibited the growth of *Rhizoctonia solani* and *Sclerotium rolfsii*. Crude or refined extracts from *A. conyzoides* offer the possibility of biocontrol of plant pathogenic fungi (Iqbal et al., 2004). It is evident from the present findings that leaf extract of *A. conyzoides* possesses fungitoxic potential wort exploiting for the biological management of plant diseases caused

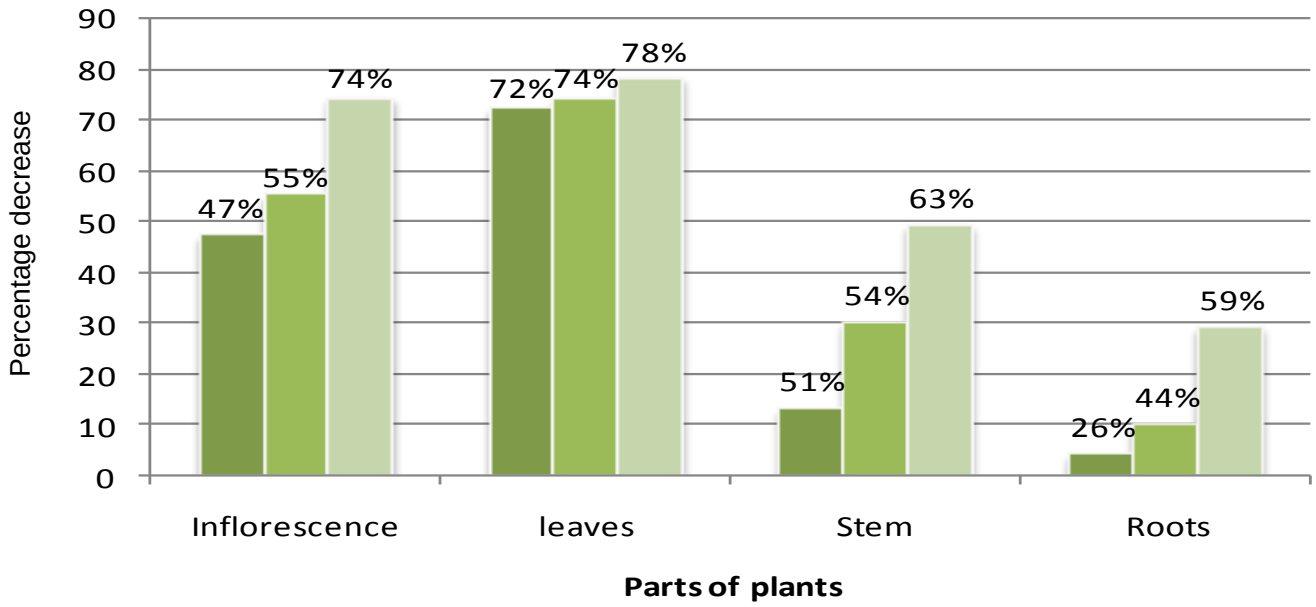


**Figure 5.** Percentage increase/decrease in biomass of *Fusarium solani* due to different concentrations of methanolic extracts of inflorescence, leaf, stem and root of *Ageratum conyzoides* against control.

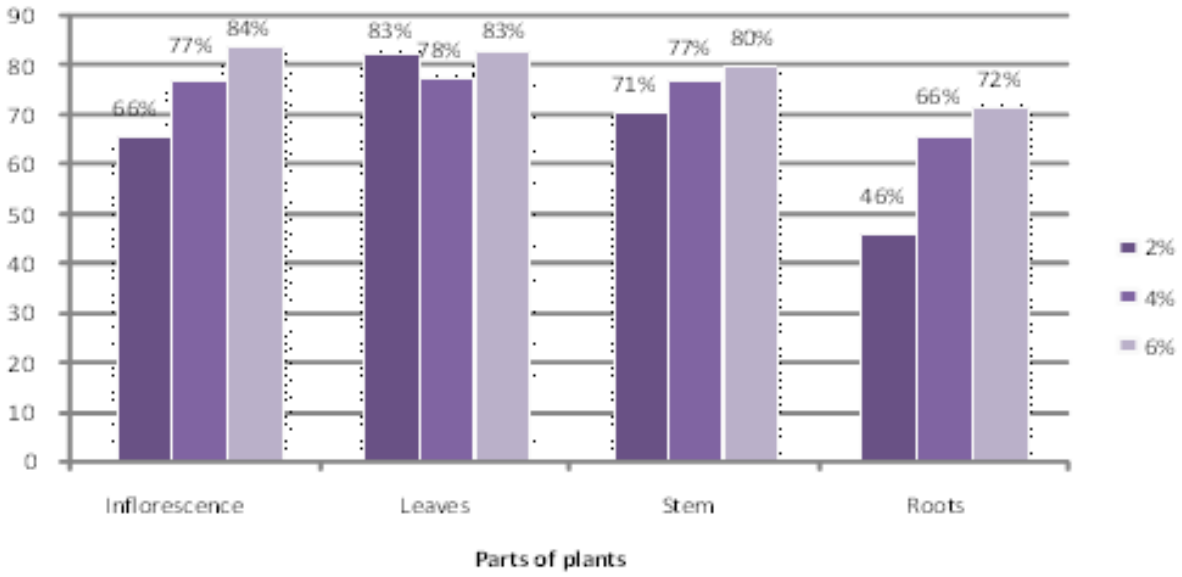


**Figure 6.** Percentage increase/decrease in biomass of *Fusarium solani* due to different concentrations of *n*-hexane extracts of Inflorescence, leaf, stem and root of *Ageratum conyzoides* against control.

by pathogenic fungi. Earlier, Ramos et al. (2007) reported growth reduction of mycelia of p*hytophthora* on neem leaf extract media. The results show that leaf extract of *A. conyzoides* was more effective in causing growth inhibition in test fungus as compared to the other parts (inflorescence, stem and root), and this may be due to presence of more allelochemicals in leaf as compared to other parts. According to Tran et al. (2004), three phenolic compounds were identified in the leaf, stem and root of *A. conyzoides* including gallic acid, coumallic acid and protocatechuic acid and catechin wasfound only in the stem. Three additional allelochemicals were found in the leaf consisting of p-coumaric acid, sinapic acid and benzoic acid. The greater number of allelochemicals found in the leaf might result in the stronger inhibitory activity then stem and root.

## Conclusion

This study suggests that *A. conyzoides* has great allelopathic potential. All the parts of *A. conyzoides* have fungitoxic chemicals against *F. solani*, the wilt causing pathogen. Aqueous and organic solvent extracts of *A. conyzoides* greatly reduced the biomass of *F. solani*, which can be used for the disease management. Further investigation of the isolation of active antifungal compound should be done from different parts of the *A. conyzoides*, and the isolated antifungal compounds should be checked against other pathogenic fungi to control the different diseases.

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