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Full Length Research Paper

Antifungal potential of *Parthenium hysterophorus* L. plant extracts against *Fusarium solani*

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Fusarium wilt, caused by *Fusarium solani* (Mart.) Sacc, is an economically important disease of potato in Pakistan. Recent studies have shown that plant pathogens can be controlled by plant products as these are biodegradable, exhibit structural diversity and complexity. Presently, antifungal bioassays were conducted to confirm mycotoxic potential of root, shoot and leaf of *Parthenium hysterophorus* L. against *F. solani* FCBP-434 using 1 to 4% concentrations of aqueous, methanol and n-hexane extracts. Bioassays revealed that growth of *F. solani* was greatly inhibited at 1 and 2% concentrations of aqueous and methanol leaf and stem extracts while 3 and 4% concentrations of n-hexane extract proved to be more effective in suppressing growth. Among root extracts, higher concentrations of aqueous and n-hexane exhibited more promising results by causing reduction of 85 and 74%, respectively; whereas, in methanol extract again, lower concentrations were more inhibitory. The study concludes that aqueous and organic extracts of *P. hysterophorus* have potential to obstruct dreadful effect of pathogenic fungi by suppressing their growth. *P. hysterophorus* conferred vital and surprisingly stable compounds having inhibitory potential against *F. solani* FCBP-434.

Key words: Aqueous and organic solvents, biological control, Fusarium wilt, potato, *P. hysterophorus*.

INTRODUCTION

Potato (*Solanum tuberosum* L.) ranks third among food crops after wheat and rice and fifth in total production in Pakistan. It is considered as 'king' of vegetables. Potato production in Pakistan has increased many folds but its per acre yield is far less than in other parts of the world (Malik, 1995). Among the various factors responsible for its low per acre production, potato diseases are considered to be the most important. Fusarium wilt is a fungal disease caused by several species of *Fusarium*, including *Fusarium eumartii* Carpenter, *Fusarium oxysporum* Schlecht., *Fusarium avenaceum* (Corda ex Fr.) Sacc., and particularly *Fusarium solani* (Mart.) Sacc.. In order to prevent these plant diseases and to protect the crop plants against pathogens, chemical control methods are in practice. In view of the high cost of chemical pesticides and their hazardous consequence, use of biodegradable material like fresh plant extracts from different parts gained importance during last three decades for plant disease control (Bajwa et al., 2007b; Shafique et al., 2011). Plant extracts and essential oils show antifungal activity against a wide range of fungi (Masoko et al., 2007). Therefore, the development of biopesticides has been focused as a viable pest control strategy in recent years. Among these *Parthenium hysterophorus* L. (Asteraceae), a major weed in Pakistan, is known for its antimicrobial efficacy (Bajwa et al., 2004, 2007a). The allelopathic and antifungal potential of *P. hysterophorus* results from the release of phytotoxic substances such as caffeic, ferulic, vanillic, chlorogenic, *p*- coumaric and parthenin, *p*-hydroxybenzoic acids, ambrosin and coronopilin (Jarvis et al*.,* 1985). The antifungal activities of root and shoot extracts of two Asteraceous plant species including *P. hysterophorus* and *Ageratum conyzoides* were determined against

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Macrophomina phaseolina (Tassi) Goid., the cause of charcoal rot disease of *Helianthus annus* L. A measured reduction in *M. phaseolina* biomass was observed due to aqueous extracts of different concentrations (Bajwa et al*.,* 2007a). The objective of the present study was to evaluate the potential of plant extracts of leaves, stem and roots of *P. hysterophorus*, for antifungal activity against *F. solani* by using food poisoning method.

METHODOLOGY

Procurement and maintenance of cultures

The pure cultures of *F. solani* FCBP- 434 were obtained from first Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore. They were maintained and subcultured monthly on malt extract agar medium (Merck, Germany) at 4°C.

Preparation of plant extracts

Aqueous extract of water soluble ingredients of dried leave, stem and root was prepared according to Bajwa et al. (2007b). While the method of Alkhail (2005) was followed for the preparation of extract of different plant parts in methanol and n-hexane. The lower concentrations of 1, 2, 3 and 4% of aqueous, n-hexane and methanol extracts of plant parts were prepared by adding appropriate quantity of sterilized distilled water.

To make methanol and n-hexane control, 2 ml of methanol and n-hexane were added to sterilize distilled water to make final volume 100 ml, in respective flasks.

Plant extract bioassays

Extract bioassays were carried out in malt extract liquid medium according to Bajwa et al. (2006). The mycelial biomass from triplicate samples for each treatment was collected on pre-weighed filter papers after 10 days. Their dry weight yield was determined after 24 h oven drying at 60°C.

Data analysis

All the data was subjected to analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to delineate mean differences (Steel and Torrie, 1980) using computer software SPSS and COSTAT.

RESULTS

Antifungal activity of leaf extract of *P. hysterophorus*

Response of F. solani FCBP-434 to aqueous leaf extracts of P. hysterophorus

The data on dry biomass production by 7 to 10 days after incubation (DAI) revealed an excessive interference of aqueous extract with the growth of test fungal species. All the concentrations significantly decreased the fungal biomass as compared to control. The biomass decline was observed at lower concentrations of 1 to 2% while a marked increment in biomass production was obtained at higher concentrations of 3 to 4%, but it was still significantly lower than control (Figure 1). Thus 1% concentration exhibited the most promising results as it caused about 85% reduction in fungal biomass while 62, 52 and 41% decline in fungal biomass was depicted by 2, 3 and 4% aqueous extract, respectively.

Response of F. solani FCBP-434 to methanol leaf extracts of P. hysterophorus

The response of *F. solani* in terms of dry biomass production was variable when grown at different concentrations of methanol extract of *P. hysterophorus.* All the concentrations significantly reduced the fungal biomass production but in erratic pattern (Figure 1). Amongst these 2% concentration was the most effective in suppressing the biomass production up to 76% followed by 3, 4 and 1% concentrations which caused decline in fungal biomass production up to 67, 42 and 43% respectively.

Response of F. solani FCBP-434 to n-hexane leaf extracts of P. hysterophorus

A gradual and significant decrease in dry fungal biomass production with an increase in concentration of n-hexane extract was observed (Figure 1). The maximum antifungal stress was induced by 4% concentration causing a decline of about 66% in the biomass production of *F. solani*. It was followed by 3, 2 and 1% concentration which revealed a significant reduction, in the range of 56, 50 and 44%, respectively.

Antifungal activity of stem extract of *P. hysterophorus*

Response of F. solani FCBP-434 to aqueous stem extracts of P. hysterophorus

All the concentrations of the extracts significantly reduced the *in vitro* growth of the target fungal pathogen similar to aqueous leaf extract, but the suppressive effect of extract decreased with the increase in concentration (Figure 2). Maximum inhibition in fungal biomass production (70%) was recorded in 1% extract treatment. Effect of other extract concentration was also significant but less pronounced as compared to 1% concentration however it was effective as compared to control. There was 48 to70% reduction in fungal biomass production as noticed

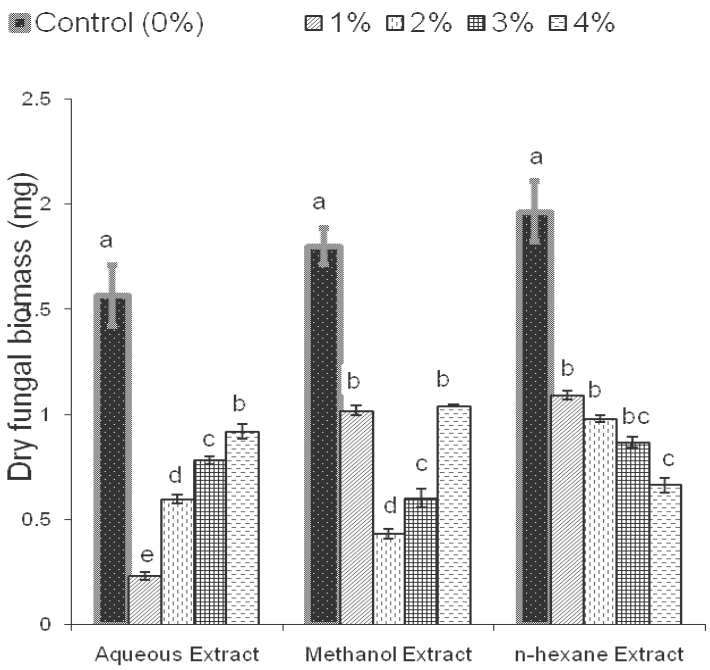

Figure 1. Effect of different concentrations of aqueous, methanol and n-hexane leaf extracts of *P. hysterophorus* on dry biomass production of *F. solani* FCBP-434. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P = 0.05) as determined by DMR test.

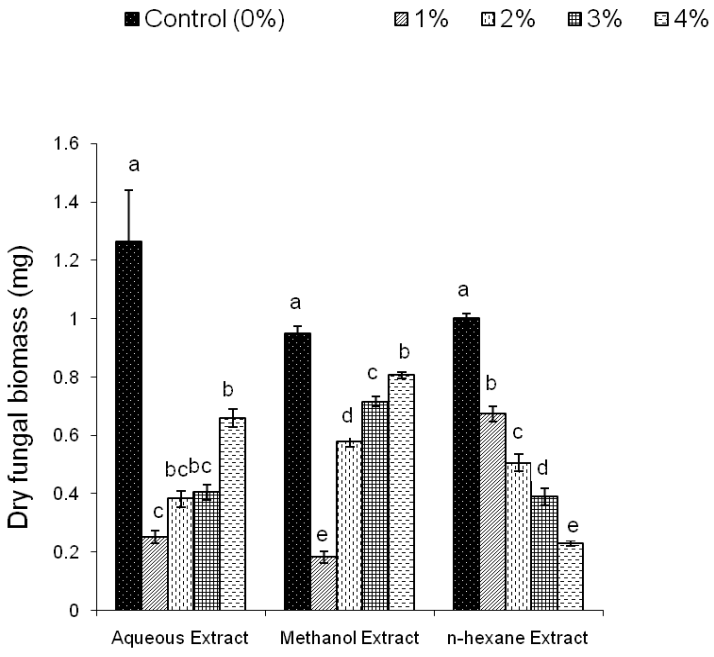

Figure 2. Effect of different concentrations of aqueous, methanol and n-hexane stem extracts of *P. hysterophorus* on dry biomass production of *F. solani* FCBP-434. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P = 0.05) as determined by DMR test.

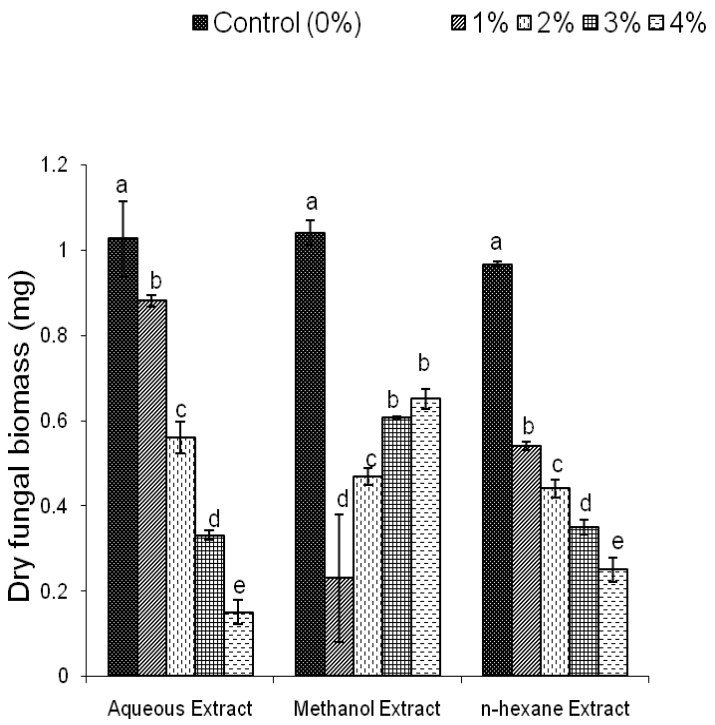

Figure 3. Effect of different concentrations of aqueous, methanol and n-hexane root extracts of *P. hysterophorus* on dry biomass production of *F. solani* FCBP-434. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P = 0.05) as determined by DMR test.

due to 1 to 4% concentrations of the extracts.

Response of F. solani FCBP-434 to methanol stem extracts of P. hysterophorus

All regimes of methanol extract of *P. hysterophorus* caused considerable inhibition in biomass production of the fungus. The maximum inhibition in fungal growth was evidenced by 1% concentration which decreased the biomass production up to 80%. While all the other concentrations (2 to 4%) proved less toxic against the target pathogen and caused about 15 to 38% reduction in biomass production, respectively (Figure 2).

Response of F. solani FCBP-434 to n-hexane stem extracts of P. hysterophorus

A variable response of dry biomass production in *F. solani* FCBP-434 was recorded to n-hexane extract of *P. hysterophorus* in different concentrations but similar to leaf n-hexane extract. *F. solani* FCBP-434 exhibited a significant reduction when exposed to different concentrations of extracts compared to control. The reduction in biomass ranged from 33 to 77% (Figure 2).

Antifungal activity of root extract of *P. hysterophorus*

Response of F. solani FCBP-434 to aqueous root extracts of P. hysterophorus

All the concentrations of the aqueous root extracts significantly reduced the *in vitro* growth of the target fungal pathogen in quite different way as compared to aqueous leaf and stem extracts as a significant and gradual decline in biomass production was recorded with concentration increment (Figure 3). The fungal biomass suppressed at lower concentrations (1 to 2%) in a range of 14 to 45%. While higher concentrations (3 to 4%) proved the most effective for the suppression of fungal pathogen as these caused a reduction up to 67 to 85%, respectively.

Response of F. solani FCBP-434 to methanol root extracts of P. hysterophorus

The response of fungus in terms of dry weight varied with

different concentrations of the extract. In methanol treatment, the lowest concentration (1%) was the most effective in reducing and suppressing the target fungal pathogen as compared to higher concentrations. The percent inhibition at this concentration was up to 77%. Further increase in extract concentration led towards the decrease in efficacy of plant extract. The fungal biomass increased at higher concentrations (2 to 4%) but still a significant inhibition in biomass production was detected with reference to control (Figure 3).

Response of F. solani FCBP-434 to n-hexane root extracts of P. hysterophorus

Studies were conducted to evaluate the effect of n-hexane root extract of *P. hysterophorus* on the growth of *F. solani* FCBP-434 and the results are compiled in Figure 3. A similar kind of suppressive effect of various concentrations was recorded against target fungal pathogen as exhibited by n-hexane leaf and stem extract. All the concentrations significantly reduced the fungal biomass production. Amongst these 4% concentration was the most effective in suppressing the biomass production up to 74%. It was followed by 3, 2 and 1% concentrations causing inhibition of 63, 54 and 44%, respectively (Figure 3).

DISCUSSION

In present study the inhibitory effect of extracts of *P. hysterophorus* was investigated against pathogenic isolate *F. solani* FCBP-434. The inhibitory effect of the plant extracts might be attributed to the presence of antifungal compounds in different plant parts' extracts viz. parthenin in *P. hysterophorus.* It is also possible that the extract inhibited or altered the mode of action of their biological chemicals.

It is obvious from the study that mycelial growth was significantly inhibited by antifungal compounds specifically at lower concentrations of aqueous leaf and stem 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 studies showing similar effects against known pathogenic fungi (Mughal et al., 1996; Khan et al*.,* 1998), which further confirms the presence of antifungal compounds in the test species. There was 41 to 85% and 48 to 80% reduction in fungal growth due to various employed concentrations of leaf and stem extract. Jain (2005) found *P. hysterophorus* to completely inhibit the growth of *F. solani*. Further increase in extract concentration exhibited significant difference in antimycotic activity as compared to 1% extract. In case of aqueous root extract of *P. hysterophorus,* phytotoxins or antifungal compounds inhibited the fungal biomass at higher concentration. In case of root extract, inverse pattern of antimycotic activity was observed as 4% concentration of root organic extract was found to be the most effective in suppressing the growth of *F. solani.* The variation in antifungal activity of the shoot and root extracts may be attributed to the different chemical nature of the compounds present in these plant parts (Afsharypuor et al., 1995).

Greater inhibition (42 to 76%) of fungal growth of *F. solani* at medium concentrations (2 to 3%) of leaf methanolic extract was observed. While in case of stem and root methanolic extracts, lower concentrations (1 to 2%) were more effective as compared to higher concentrations (3 to 4%). 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 llelochemicals to enhance the growth of mycoflora (Mughal et al., 1996) or ability of particular species to exploit them as source of nutrition. The pattern of gradually higher production of biomass in response to increasing concentrations of aqueous extract was also similar to investigation of Bajwa et al*.* (2004).

In case of n-hexane extract of all three plant parts that is, leaf, stem and root, the comparative effectiveness of n-hexane extracts of selected test species revealed that higher concentrations were relatively more allelopathic as compared to lower concentrations. These results are supported by the fact that Vir and Sharma (1985) have also employed 10% concentration of neem oil that exhibited 100% inhibition in *Aspergillus niger, Drechslera rostrata* and *Macrophomina phaseolina.* Thus it can be recommended that the use of *P. hysterophorus* against *F. solani* give better results as they are biologically based and environmental safe alternatives. The results of present study can be further exploited for formulating integrated disease management and is an important step in developing plant based fungicides based on field trail and toxicological experiment.

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