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SCREENING OF VARIOUS SPECIES OF *ASPERGILLUS* FOR HERBICIDAL ACTIVITY AGAINST PARTHENIUM WEED

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ABSTRACT

Parthenium (Parthenium hysterophorus L.) belonging to family Asteraceae is an aggressive weed that threatens the natural ecosystem in many parts of the world. The present study was carried out to investigate the herbicidal potential of culture filtrates of six species of Aspergillus namely A. fumigatus, A. terreus, A. niger, A. flavus, A. parasiticus and A. spelunceus against parthenium weed in eco-friendly way. Original culture filtrates of A. fumigatus and A. niger considerably decreased parthenium seed germination by 22% and 28%, respectively. Likewise, culture filtrates of A. flavus and A. parasiticus significantly reduced shoot length by 70% and 48%, respectively. Root growth was highly susceptible to fungal culture filtrates exposure. Culture filtrates of all the Aspergillus spp. with the exception of A. spelunceus significantly reduced seedling root length by 43-92% over control. Culture filtrates of A. flavus and A. parasiticus exhibited the best herbicidal activity against shoot and root growth of parthenium.

Key words: Alternative herbicides, fungal metabolites, invasive weed, non-chemical weed management, *Parthenium hysterophorus*.

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INTRODUCTION

Parthenium hysterophorus is a world's seventh most devastating weed of aggressive intenseness and subsequent alarming threat to biodiversity after habitat destruction (Akhter and Zuberi, 2009; Riaz and Javaid, 2012; Masum *et al*., 2013). It caused drastic losses to crop productivity along with ailing effects on human and livestock (Shabbir *et al.,* 2012). Many of its biological and ecological traits amplify its invasiveness and now 30 countries of the globe are considered to be in danger of frantic growth of this weed (Adkins and Shabbir, 2014). In Pakistan, this weed was noticed in 1990 in various

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parts of upper Punjab including districts of Sialkot, Gujranwala, Rawalpindi and others, since then it has invaded different regions of the country at alarming rate (Javaid and Riaz, 2012; Shabbir, 2014).

So far, several management options have been opted to minimize losses by parthenium with inherent limitations in their application (Adkins and Shabbir, 2014). Amongst different methods, use of herbicides has been proved very effective against parthenium (Javaid *et al*., 2006; Javaid, 2007; Shabbir, 2014). However, because of environmental concerns associated with the use of synthetic herbicides and emergence of numerous herbicide-resistant weed biotypes, substantial efforts have been made to design alternative environmental friendly weed-management strategies (Teerarak*aet al.,* 2010; Akbar *et al.,* 2014).

Recent studies show that fungal metabolites can be used as potential alternatives to chemical herbicides for parthenium management. In this connection, Dhanaseeli and Sekar (2004) found considerably greater herbicidal potential of methanolic fractions of different soil fungi against parthenium. Vikrant*et al.* (2006) identified a herbicidal compound i.e. 3-nitro-1,2-benzenedi-carboxylic acid (3-nitrophtaalic acid) from *Phoma herbarum* against parthenium weed. Javaid and Adrees (2009) assessed the herbicidal properties of culture filtrates of different fungal plant pathogens against parthenium. They reported that filtrates of *Cladosporium* sp., *A. alternata* and *D. rostrata* significantly suppressed growth of weed both *in vivo* and *in vitro*. Different species of genus *Drechslera* viz. *D. biseptata*, *D. australiensis, D. holmii* and *D. hawaiiensis* were documented to inhibit up to 90% growth of parthenium (Javaid *et al*., 2011). Recently, Javaid *et al*. (2013) suggested that culture filtrates of different *Trichoderma* species viz. *T. pseudokoningii*, *T. harzianum, T. viride* and *T. reesei* can be used for management of parthenium. Although culture filtrates of a number of fungal groups have been studied for their herbicidal activities, however, studies regarding the herbicidal activities of aspergilli are scarce. The current investigation was, therefore, carried out to appraise herbicidal potential of culture filtrates of six species of *Aspergillus* namely *A. fumigatus, A. terreus, A. niger, A. flavus, A. parasiticus* and *A. spelunceus* against parthenium.

MATERIALS AND METHODS

Preparation of fungal culture filtrates of *Apergillus* spp.

Cultures of six fungal species of genus *Aspergillus* namely *A. fumigatus, A. terreus, A. niger, A. flavus, A. parasiticus* and *A. spelunceus* were obtained from Fungal Culture Bank of Pakistan, University of the Punjab, Lahore, Pakistan. In order to get culture

filtrates of these selected *Aspergillus* species M-1-D broth was prepared following Evidente *et al*. (2006). It contained 1.2 mM $Ca(NO_3)_2$, 30 µM $MnSO_4$, 0.79 mM KNO_3, 3.0 mM $MgSO_4$, 87.6 mM sucrose, 0.87 mM KCI, 22 µM $H3BO_3$, 0.14 mM NaH_2PO_4, 7.4 µM $FeC1_3$, 8.7 µM $ZnSO_4$, 27.1 mM ammonium tartrate and 4.5 µM KI. pH of the growth medium was adjusted at 5.5 using 0.1 M HCl. In each of the six 500 mL flasks, 200 mL medium was autoclaved at 121°C for 20 minutes and cooled at room temperature. Fungal inoculation was done by adding 5 mm discs of actively growing cultures of various selected fungal species and incubated at 25±2 ºC for one month. One month old cultures were filtered and centrifuged at 4000 rpm for ten minutes. These original (100%) filtrates were diluted (50%) by adding appropriate quantity of autoclaved distilled water as described by Akbar and Javaid (2013).

Laboratory bioassays

Seeds of parthenium weed were surface sterilized with 1% sodium hypochlorite for 10 minutes and thoroughly washed with autoclaved water. Twenty five seeds were arranged in sterilized Petri plates (9-cm diameter) lined with sterilized filter papers beds. Each Petri plate was supplemented with 3 mL of diluted or original culture filtrates of aspergilli. Distilled water was used as negative control. Experiment was carried out in a completely randomized design with four replications. Plates were incubated at 25 °C in a growth room with 10 h light period on a daily basis. After 15 days, harvest was taken and data concerning seed germination, and shoot and root length were noted (Javaid and Ali, 2011).

Statistical analysis

Standard errors of means were calculated. Analysis of variance followed by Duncan's Multiple Range Test (Steel and Torrie, 1980) were applied at 5% level of significance to analyze different growth parameters.

RESULTS AND DISCUSSION

Analysis of variance show that there was significant effect of *Aspergillus* species (A), concentration of culture filtrates (C) and well as A × C for germination and root length. However, for shoot length, only the effect of A and A × C was significant (Table-1).

There was a variable effect of culture filtrates of various species of *Aspergillus* on germination of parthenium seeds. The effect of culture filtrates of *A. flavus*and *A. terreus* was insignificant. Conversely, original culture filtrates (100%) of *A. fumigatus* and *A. niger* significantly reduced seed germination by 22% and 28%, respectively, over control. Original culture filtrates of *A. parasiticus* and *A. spelunceus* insignificantly reduced seed germination by 11%

and 16%, respectively over control. It is likely, if these culture filtrates are used in a concentrated form, they may have significant adverse effect on parthenium seed germination (Fig. 1). Earlier there are some reports that demonstrated herbicidal effects of different fungal species on germination of parthenium. Idrees and Javaid (2008) evaluated the herbicidal effect of culture filtrates of many plant pathogenic fungal species and found that filtrates of *Cladosporium oxysporum, Macrophomina phaseolina* and *Fusarium equisetti* exhibited the maximum inhibition against parthenium germination. Likewise, culture filtrates of various species of *Trichoderma* and *Drechslera* have been reported for their potential to suppress parthenium germination (Javaid *et al.,* 2011, 2013). Numerous herbicidal constituents namely holadysenterine, drazepinone and ophiobolin A have been isolated from culture filtrates of fungi especially *Drechslera* spp. (Evidente *et al.,* 2005, 2006; Akbar *et al.,* 2014).

Culture filtrates of the six tested *Aspergillus* spp. showed variable effect on shoot and root length of parthenium seedlings. In general, root growth was more susceptible than the shoot growth. Culture filtrates of *A. fumigatus, A. terreus, A. niger* and *A. spelunceus* either had no effect or stimulated the shoot length. By contrast, these fungal culture filtrates in their original form, variably and significantly reduced root length by 19-69% (Fig. 2 and 3). It can probably be due to the fact that roots are the main avenues that usually uptake phytotoxic compounds from the vicinity and thus show their anomalous growth (Javaid and Shah, 2007).

Culture filtrates of *A. flavus* and *A. parasiticus* showed the maximum herbicidal activity with significant losses in root and shoot length. Original and diluted culture filtrates of *A. flavus* reduced shoot length by 74% and 54%, and root length by 90% and 88%, respectively, over control (Fig. 2 and 3). Original and diluted culture filtrates of *A. parasiticus* significantly suppressed shoot length by 48% and 47%, respectively, over control. Likewise, root length was significantly decline by 92% and 83% by original and diluted culture filtrates, respectively (Fig. 2 and 3). The herbicidal activity of both phylogenetically similar *A. flavus* and *A. parasiticus* could be due to production of variety of hydrolytic enzymes viz. N-acetyl-beta-glucosaminidase, aryl sulfatase, cathepsin (B and D), alkaline proteinase and aminopeptidase (Sharma *et al*., 1989) and toxic metabolites. Moreover, recently Scarpari *et al. (*2014) suggested that the fungal oxylipins (lipid) are generally produced by *Aspergillus* group including *A. flavus* and *A. parasiticus* that may modulate the response of the host defense by inhibiting the production of defense related gene.

CONCLUSION

The present study concludes that growth of root and shoot of parthenium can effectively be controlled in eco-friendly way by using culture filtrates of *A. flavus* and *A. parasiticus.* However, before their large scale application, their effect against other organisms should also be studied. Moreover, further studies are needed for isolation and identification of the effective herbicidal compounds from these culture filtrates. These isolated compounds may be used as analogues for the synthesis of natural product based herbicides.

Table-1. Analysis of variance (ANOVA) for the effect of different concentrations of culture filtrates of *Aspergillus* spp. against *Parhenium hysterophorus*.

Sources of variation	df	Mean Squares		
		Germination	Shoot length	Root length
Aspergillus spp. (A)	5	7401*	74*	150*
Concentration (C)	2	24285*	13ns	790*
A × C	10	1891*	24*	50*
Error	54	54.5	5.4	4.7
Total	71			

* = Significant at P≤0.001, ns = Non-significant

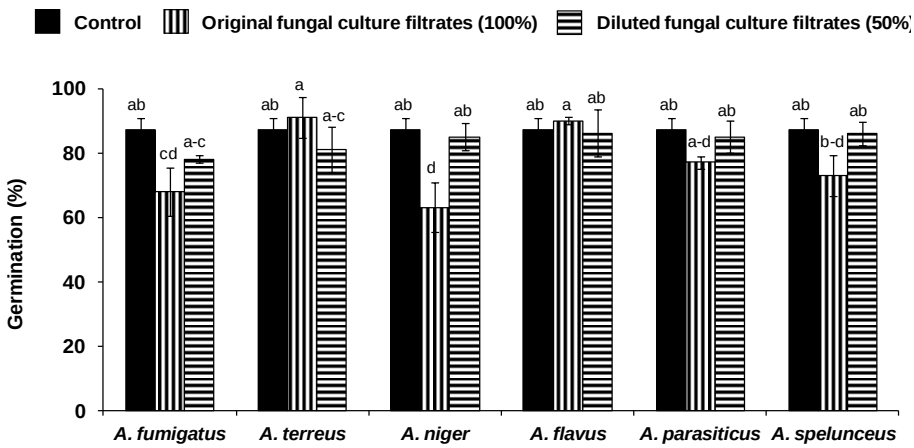

Figure 1. Effect of original and diluted cultural filtrates of six species of *Aspergillus* on seed germination of *P. hysterophorus*. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference as determined by DMR Test at P≤0.05.

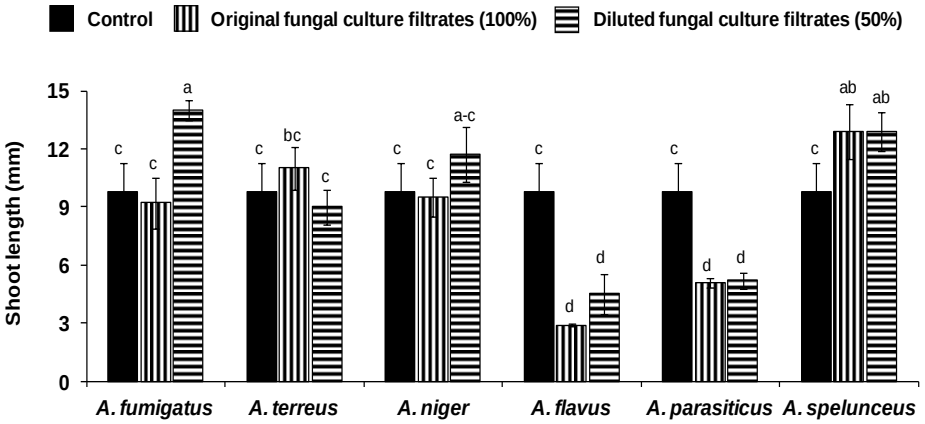

Figure 2. Effect of original and diluted cultural filtrates of six species of *Aspergillus* on shoot length of *P. hysterophorus* seedlings. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference as determined by DMR Test at P≤0.05.

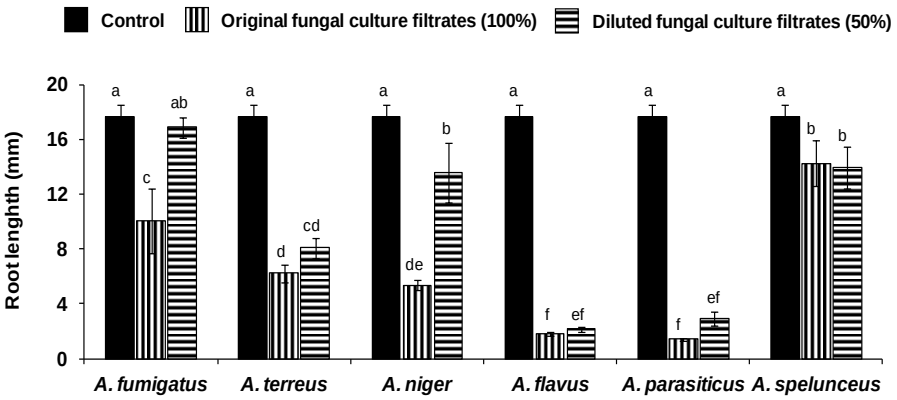

Figure 3. Effect of original and diluted cultural filtrates of six species of *Aspergillus* on root length of *P. hysterophorus* seedlings. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference as determined by DMR Test at P≤0.05.

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