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Article

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HERBICIDAL ACTIVITY OF *Aspergillus niger* METABOLITES AGAINST PARTHENIUM WEED

*Atividade Herbicida dos Metabólitos de **Aspergillus niger** em Losna-branca*

ABSTRACT - Metabolites of *Aspergillus niger,* prepared in malt extract (ME) broth and potato dextrose (PD) broth, were evaluated for their herbicidal activity against a noxious parthenium weed (*Parthenium hysterophorus*). In laboratory assays, original (X) and diluted (½ X) fungal metabolites significantly reduced germination and seedling growth of weed. However, metabolites prepared in ME broth proved to have greater herbicidal activity than metabolites prepared in other growth medium. Original metabolites prepared in ME broth have completely hinder the germination of parthenium seeds; while those prepared in PD broth have reduced germination by 89% over control. In pot trials, one-week, two-week and three-week-old parthenium seedlings were sprayed three times with original and concentrated (2X) metabolites of *A. niger* prepared in ME broth. Plants were harvested after 40 days of sowing. One-week treatment plants were most susceptible to fungal metabolites spray, followed by two-week and three-week treatment plants, respectively. Original and concentrated metabolites have significantly reduced shoot biomass of one-week-old plants by 57% and 68%, and root biomass by 50% and 75%, respectively. The present study has come to the conclusion that *A. niger* metabolites prepared in ME broth can effectively control germination and growth of parthenium.

Keywords: natural herbicide, *Parthenium hysterophorus,* noxious weed.

*RESUMO - Os metabólitos de **Aspergillus niger**, preparados em caldo de extrato de malte (ME) e caldo de dextrose de batata (PD), foram avaliados quanto à sua atividade herbicida contra uma erva partênica nociva (**Parthenium hysterophorus**). Em resenhas de laboratório, metabólitos fúngicos originais (X) e diluídos (½ X) reduziram substancialmente a germinação e o crescimento de plântulas da planta daninha. No entanto, os metabólitos preparados em caldo de ME demonstraram ser superiores na sua atividade herbicida, comparados aos metabólitos preparados em outro meio de crescimento. Os metabólitos originais preparados em caldo ME estagnaram completamente a germinação de sementes de erva partênica, ao passo que os preparados em caldo PD reduziram a germinação em 89%, em relação ao controle. Nos ensaios em vaso, as mudas de erva partênica de uma semana, duas semanas e três semanas foram pulverizadas três vezes com metabólitos originais e concentrados (2X) de A. niger preparados em caldo ME. As plantas foram colhidas após 40 dias de semeadura. As plantas com uma semana de tratamento foram mais suscetíveis à pulverização de fungos metabólitos, seguidas das plantas de duas semanas e de três semanas. Os metabólitos originais e concentrados reduziram significativamente a biomassa de brotos de plantas de uma semana de idade em 57% e 68%, e a biomassa de raízes, em 50% e 75%,*

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*respectivamente. O presente estudo conclui que os metabólitos de **A. niger** preparados em caldo ME podem efetivamente controlar a germinação e o crescimento de ervas partênicas.*

Palavras-chave: herbicida natural, *Parthenium hysterophorus,* erva nociva.

INTRODUCTION

Parthenium is a noxious annual weed that causes destruction in managed as well as natural ecosystems in many countries in the world (Masum et al., 2013; Adkins and Shabbir, 2014). Considered one of the most harmful and invasive terrestrial weed (Hussain et al., 2016), it can adapt to any type of environmental conditions, invade all types of land, and cause heavy losses to plants by reducing their yield, biodiversity, and disturbing their natural ecosystem (Timsina et al., 2011; Kapoor, 2012). It also adversely affects the growth and yield of crop plants (Safdar et al., 2016). The invasive nature of parthenium is due to the presence of parthenin (Verma et al., 2006). This weed also causes devastating effects on humans and livestock (Shabbir et al., 2012). Direct contact of parthenium plants with humans for a long time causes skin disease, dermatitis, and in severe cases, death (Morin et al., 2009).

Many management practices have been used to reduce losses due to parthenium, but with some inherent limitations in their application (Adkins and Shabbir, 2014). Among these methods, the use of herbicides has been proved useful against parthenium (Javaid, 2007; Shabbir, 2014). However, synthetic herbicides create environmental problems and some resistance of weeds against herbicides. Thus, in this situation there has been a need of safe and eco-friendly weed-management strategies (Teeraraka et al., 2010; Akbar et al., 2014). In recent years, some studies have been carried out to control parthenium and other weeds in laboratory, green houses, and under filed conditions by fungal metabolites (Javaid, 2010; Akbar and Javaid, 2015). A metabolite isolated from *Phyllosticta* sp. had significant herbicidal activity (Evidente et al., 2008). According to Javaid and Ali (2011), metabolites of *Trichoderma* species effectively controlled *Avena fatua.* Akbar and Javaid (2012a,b) reported that metabolites extracted from *Drechslera* spp. showed effective control against weeds of wheat, namely *Rumex dentatus*, *Avena fatua* and *Phalaris minor.* Although metabolites of different fungal groups have been used as natural herbicides against parthenium (Javaid, 2010; Javaid et al., 2017), literature discussing the use of metabolites of *Aspergillus niger* as natural herbicide against parthenium is rare. *A. niger* is a fungus ubiquitous in soil that causes black mould on many fruits and vegetables, and spoils preserved foods (Stratford et al., 2016). This present study aims to assess the herbicidal potential of metabolites of *A. niger* against parthenium.

MATERIAL AND METHODS

Culture of *A. niger,* was procured from First Fungal Culture Bank of Pakistan, Institute of Agriculture Sciences, University of the Punjab, Lahore, Pakistan. Subculturing of procured fungal strain was done under laboratory conditions on 2% malt extract agar medium (2 g malt extract, 2 g agar in 100 mL water). After thorough shaking, growth medium was placed in an autoclave at 121 °C for 30 minutes and poured in sterilized Petri plates aseptically after cooling to 70 °C. Fungal culture was inoculated and Petri plates were incubated for one week at 25 °C and stored at 4 °C.

For preparation of fungal metabolites, two liquid growth medium viz. potato dextrose broth (PDB) and malt extract broth (MEB) were used. An amount of 200 mL of each of PDB and MEB was autoclaved in 250 mL volumetric flasks. Flasks were inoculated with 5 mm discs of the selected fungal species and incubated at 25 °C for 15 days. Thereafter, metabolites were filtered through sterilized muslin cloth, followed by filtration through sterilized Whattsman filter paper No. 1. Filtered metabolites were centrifuged for 5 minutes at 600 rpm and then filtered through Millipore filter paper (Akbar et al., 2014).

Parthenium seeds were collected from University of the Punjab Lahore, Pakistan. Seeds were dried in an electric oven at 45 °C. Dried seeds were rubbed for the removal of seed shells. Seeds were surface sterilized with 1% solution of sodium hypochlorite for 10 minutes followed by several washings with distilled water. Pre-sterilized Petri plates (9 cm diameter) were lined with sterilized filter papers. Twenty five surface sterilized seeds were arranged in each Petri plate. Two milliliters of original (X) and diluted (½ X) filtered metabolites of the fungal species prepared in malt extract and potato dextrose broths were poured in each Petri plate. Diluted concentration was made by adding sterilized distilled water in an appropriate amount (Akbar and Javaid, 2013). In control treatment, distilled water was used instead of fungal metabolites. Treatments were replicated 4 times. The experiment was conducted in a completely randomized design in a growth chamber maintained at 25 °C for 10 h light period daily. Harvest was taken after two weeks and data on germination of seeds, root, and shoot length and plants fresh and dry weights were noted (Javaid and Ali, 2011).

Metabolites prepared in malt extract broth exhibited good herbicidal activity in Petri plate experiment so they were selected for further pot experiment. Field soil was filled in 10 cm plastic pots. Twenty seeds of parthenium were sown in each pot. Extra seedlings were removed from each pot after germination and five uniform seedlings were left in each pot for taking accurate data. Pots were divided into three groups to carry out foliar spray on 1 week, 2 week and 3 week old plants. Two different concentrations of metabolites viz. original (X) and concentrated (2X) were used. Concentrated metabolites were prepared by evaporating the water in an electric oven at 45 °C. Both (original and concentrated) concentrations were sprayed on pot grown plants 4 times with a 5 day intervals. Distilled water was sprayed on plants of control treatment. Experiment was conducted under natural conditions.

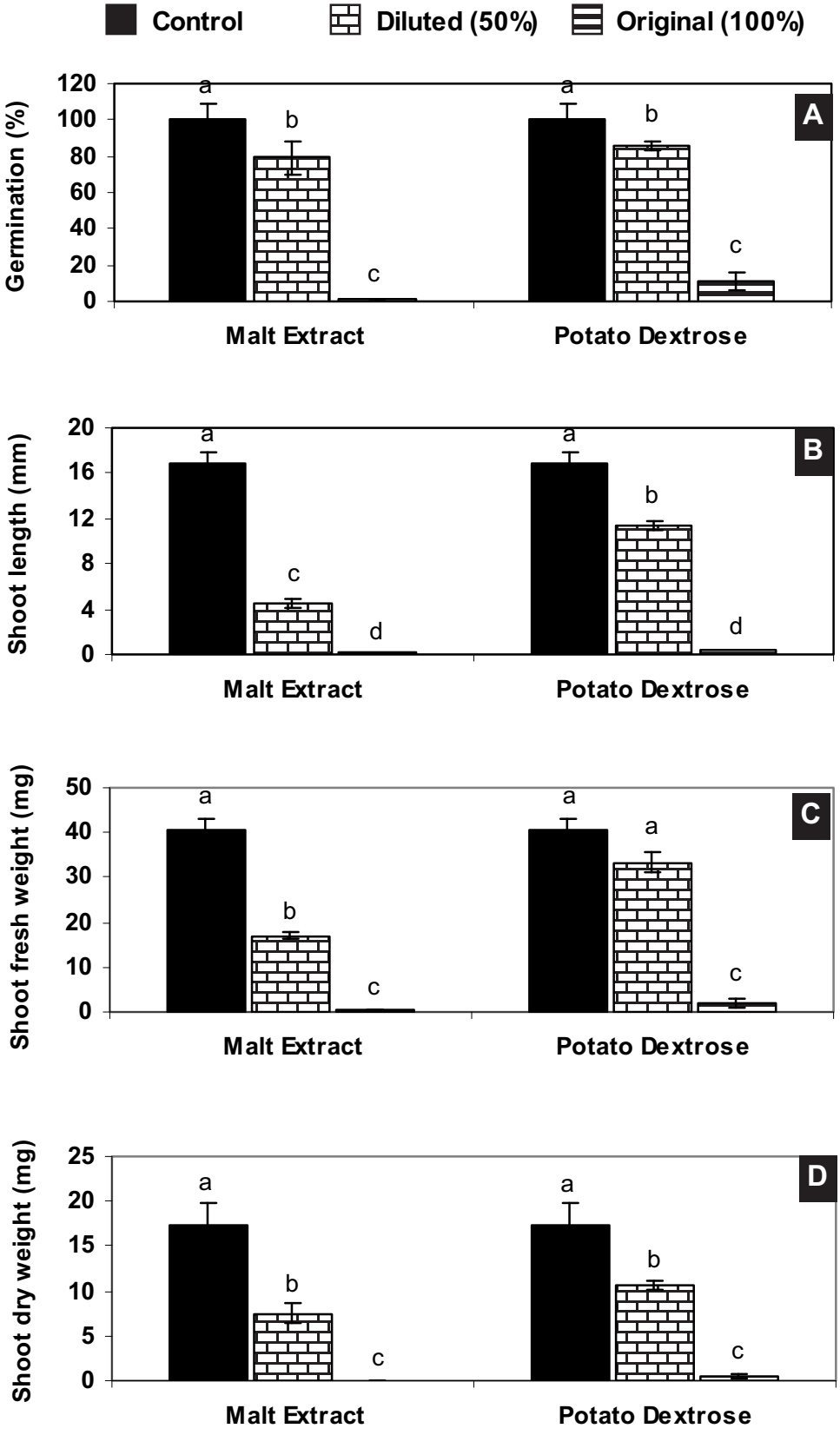
Parthenium plants were harvested after 45 days of sowing. Plants were carefully uprooted and roots were separated from shoots and washed to remove soil particles. Various vegetative growth parameters like shoot growth and root growth, dry weight of seedlings were determined. Data were analyzed by ANOVA followed by LSD test at 5% level of the significance using computer software Statistics 8.1.

RESULTS AND DISCUSSION

Laboratory bioassays

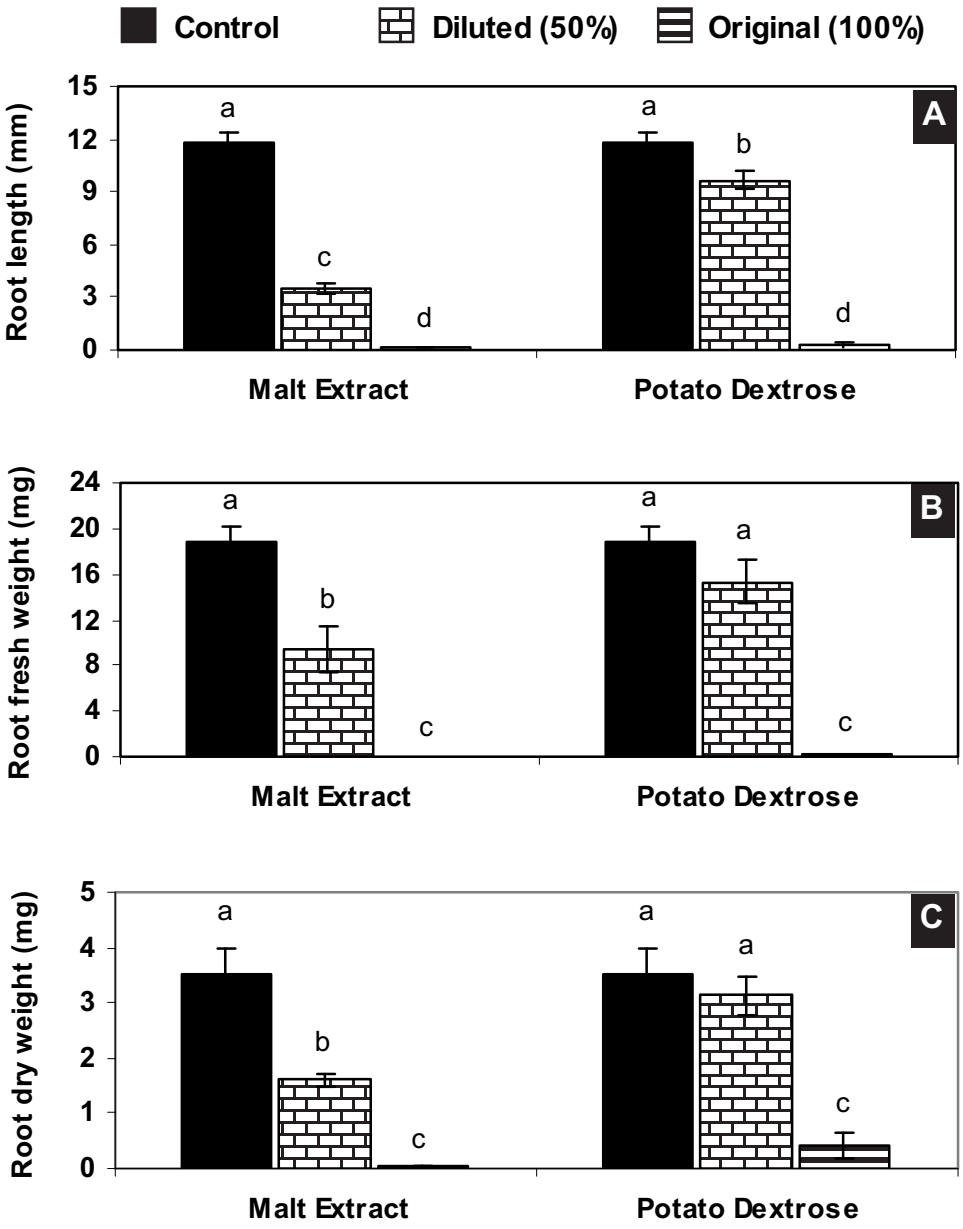
Both original and diluted metabolites of *A. niger* significantly reduced germination of parthenium seeds. However, the inhibitory effect of original metabolites was far better than the diluted ones. Moreover, metabolites prepared in malt extract broth were more inhibitory to germination than those prepared in potato dextrose. There was 99% and 21% reduction in germination due to original and diluted metabolites of *A. niger* prepared in malt extract broth, compared to 89% and 14% reduction due to metabolites prepared in potato dextrose broth, respectively (Figure 1A). Earlier, Javaid et al. (2014) reported that metabolites of other *Aspergillus* species namely *A. fumigatus*, *A. terreus*, *A. flavus*, *A. parasiticus* and *A. spelunceus* reduced germination of parthenium in up to 28%.

The effect of fungal metabolites, prepared in the two growth media, on various parameters of shoot and root growth was similar to that on seed germination. In general, the effect of fungal metabolites prepared in malt extract broth was more severe on shoot and root growth than those prepared in potato dextrose broth. There was 99.4%, 99.3% and 99.4% reduction in length, fresh weight and dry weight of shoot due to original metabolites prepared in malt extract broth. Whereas these parameters were reduced by 97%, 95% and 98%, respectively, due to original metabolites prepared in potato dextrose broth (Figure 1B-D). Likewise, length, fresh weight and dry weight of root were reduced by 99.2%, 99.4% and 99.1% due to original metabolites prepared in malt extract broth and 97.3%, 98.8% and 87.7% due to original metabolites prepared in potato dextrose broth, respectively (Figure 2A-C). Variation in herbicidal activity of fungal metabolites prepared in different growth medium has also been reported in some previous studies. Javaid et al. (2013) found that metabolites of various *Trichoderma* species prepared in M-1-D medium showed greater herbicidal activity against germination and growth of parthenium than those prepared in malt



Values with different letters at their top show significant difference (P≤0.05), as determined by LSD Test.
Vertical bars show standard errors of means of four replicates.

Figure 1 - Effect of original (X) and diluted (½X) metabolites of *Aspergillus niger* prepared in malt extract broth and potato dextrose broth on germination and shoot growth of parthenium seedlings in laboratory bioassays.



Values with different letters at their top show significant difference (P≤0.05), as determined by LSD Test.
Vertical bars show standard errors of means of four replicates.

Figure 2 - Effect of original (X) and diluted (½X) metabolites of *Aspergillus niger* prepared in malt extract broth and potato dextrose broth on root growth of parthenium seedlings in laboratory.

extract broth. Similar variable herbicidal activity was found in metabolites of *Drechslera* species prepared in different growth media against *Rumex dentatus* (Akbar and Javaid, 2010, 2013). Recently, Javaid et al. (2017) reported that metabolites of *Alternaria japonica* prepared in malt extract broth and potato dextrose broth showed different herbicidal activities against parthenium. Variation in herbicidal activities of fungal metabolites prepared in different growth media might be associated with production of different herbicidal constituents or different quantities of the same herbicidal compound in different growth medium.

Pot trials

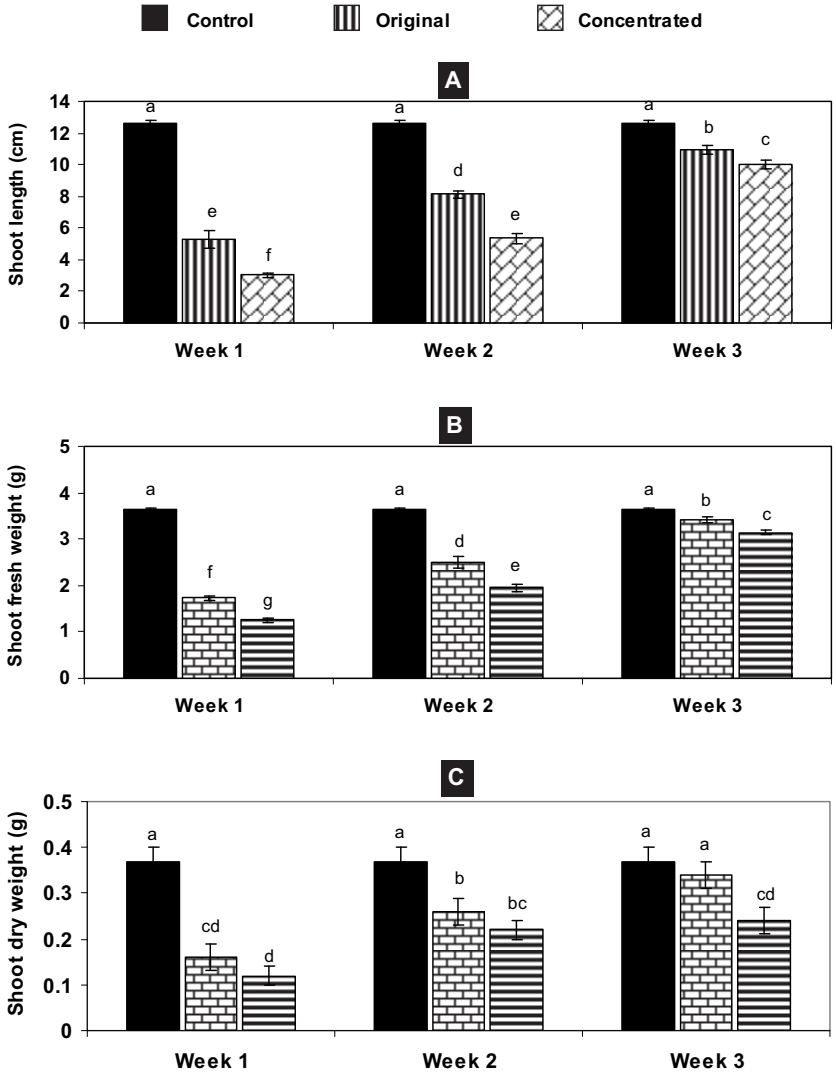
Analysis of variance showed that the effect of age of host at the time of start of fungal metabolites application (W), concentrations of the metabolites (C) as well as W x C were significant

for all the studied parameters of root and shoot growth of parthenium (Table 1). In general, herbicidal activity of the fungal metabolites was reduced with increase in age of host plant. 1 week old parthenium seedlings were highly susceptible to foliar application of fungal metabolites.

Table 1 - Analysis of variance (ANOVA) for the effect of age of host plant (1, 2, 3 weeks) and concentrations of fungal metabolites (original and concentrated) on various parameters of shoot and root growth of pot grown parthenium seedlings

Sources of variation	df	Mean squares					
		Shoot length	Shoot fresh weight	Shoot dry weight	Root length	Root fresh weight	Root dry weight
Age of plant (W)	2	70*	5.55*	0.067*	428*	0.675*	0.0382*
Concentration (C)	2	161*	9.67*	0.073*	1063*	0.699*	0.0178*
W × C	4	19.2*	1.43*	0.018*	108*	0.171*	0.0179*
Error	36	0.365	0.015	0.003	1.07	0.004	0.0026
Total	44						

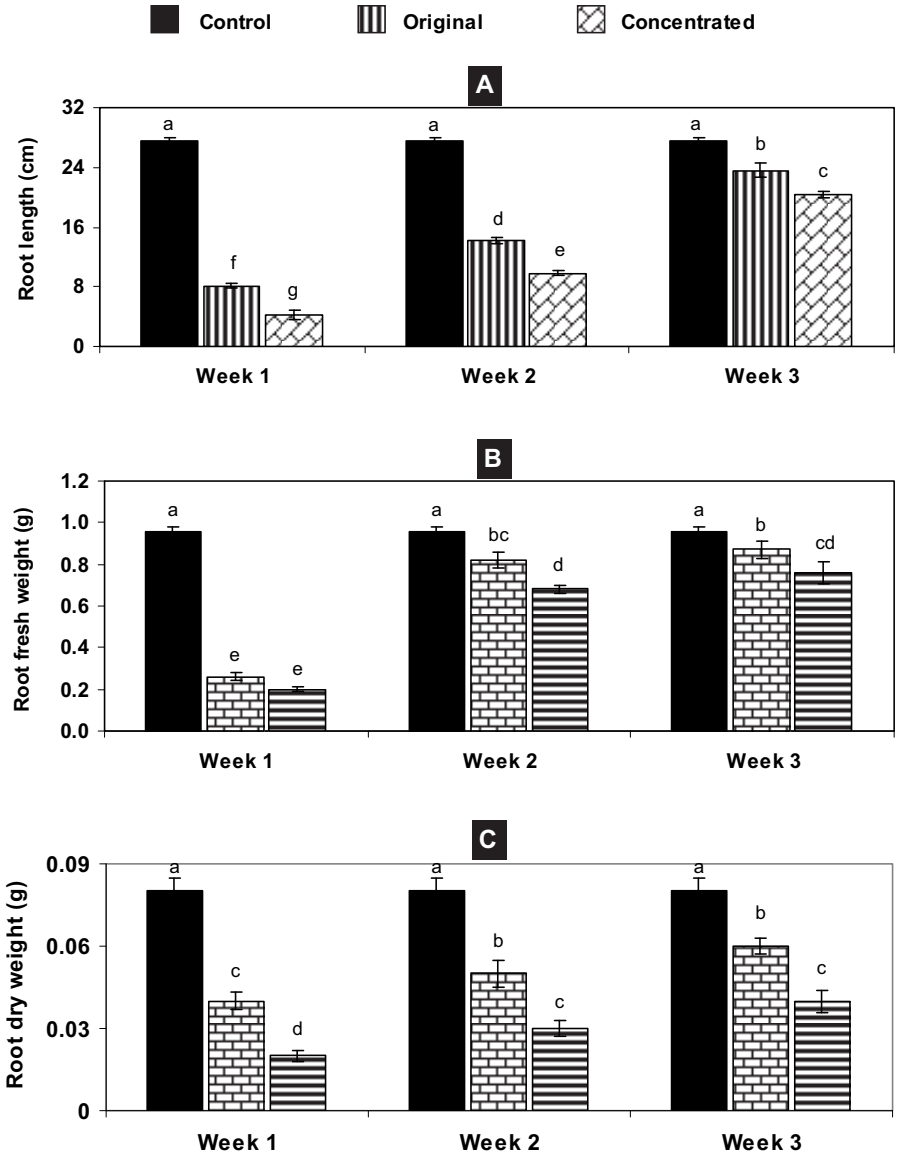
* Significant at P≤0.001.



Values with different letters at their top show significant difference (P≤0.05), as determined by LSD Test.
Vertical bars show standard errors of means of four replicates.

Figure 3 - Effect of foliar spray with original (X) and concentrated (2X) metabolites of *Aspergillus niger* prepared in malt extract broth on shoot growth of pot grown parthenium plants of different ages.

There was 58% and 76% decrease in shoot length, and 57% and 68% decrease in shoot dry weight due to foliar spray of original and concentrated fungal metabolites on 1 week old plants, respectively. On the other hand, original and concentrated fungal metabolites reduced shoot length by 36% and 58%, and shoot dry weight by 30% and 40% over control, respectively, in 2 week old plants. 3 Week old parthenium plants were least susceptible to fungal metabolites where there was 13% and 20% reduction in shoot length, and 8% and 35% suppression in shoot dry weight was recorded over control due to original and concentrated fungal metabolites, respectively (Figure 3). The effect of fungal metabolites on root growth of various ages of parthenium plants was generally similar to that on various parameters of root growth. Root growth in 1 week old plants was the most susceptible to foliar application of metabolites where 71% and 84% reduction in root length, and 50% and 75% reduction in root dry weight over control was recorded due to original and concentrated fungal metabolites, respectively (Figure 4). The results of present study are in line with the findings of earlier studies which also showed that herbicidal activity of fungal metabolites decreased with the age of the weed plants possibly because of increased resistance in old plants (Javaid et al., 2011, 2013, 2016).



Values with different letters at their top show significant difference (P≤0.05), as determined by LSD Test. Vertical bars show standard errors of means of four replicates.

Figure 4 - Effect of foliar spray with original (X) and concentrated (2X) metabolites of *Aspergillus niger* prepared in malt extract broth on root growth of pot grown parthenium plants of different ages.

This present study concludes that metabolites of *A. niger* prepared in malt extract broth possessed pronounced herbicidal activity against parthenium weed. The herbicidal activity of these metabolites is gradually reduced with the aging of the host plant.

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