



Article

JAVOID, A.^{1*}
MUBEEN, T.¹
BASHIR, U.¹
SHOAIB, A.¹

MANAGEMENT OF PARTHENIUM WEED USING METABOLITES OF *Alternaria japonica*

Manejo da Espécie Infestante Parthenium por Meio de Metabólitos de Alternaria japonica

ABSTRACT - Parthenium (*Parthenium hysterophorus*) is an alien invasive weed infesting many parts of the world including Pakistan. A number of herbicides have been recommended for control of this weed, but these herbicides have issues regarding human health and environmental safety. In the current study, the herbicidal potential of culture filtrates of a fungal species, namely *Alternaria japonica*, was evaluated against parthenium weed. The fungal species was grown in malt extract broth (MEB) and potato dextrose broth (PDB) for 15 days. Culture filtrates were obtained by passing the materials through muslin cloth followed by filtration through filter paper and then through Millipore filter paper. In laboratory bioassays, the effect of original (X) and diluted ($\frac{1}{2}X$) filtrates was studied on seed germination, and shoot and root growth of parthenium. In general, fungal culture filtrates prepared in either of the two growth medium significantly reduced germination and seedling growth of parthenium. However, culture filtrates prepared in potato dextrose broth showed greater herbicidal activity than those prepared in malt extract broth. Foliar spray bioassays were carried out by using culture filtrates of *A. japonica* prepared in potato dextrose broth. In this experiment, 1-, 2- and 3 week old parthenium seedlings were sprayed 4 times with original (X) and concentrated (2X) fungal culture filtrates, with intervals of 4 days. In general, 1 week old parthenium seedlings were highly susceptible to foliar spray of fungal metabolites. The present study concludes that culture filtrates of *A. japonica* prepared in potato dextrose broth contain potent herbicidal constituents for management of parthenium.

Keywords: Asteraceae, congo grass, fungi, mycoherbicide, weed.

RESUMO - A losna-branca (*Parthenium hysterophorus*) é uma espécie invasora não nativa que infesta várias partes do mundo, incluindo o Paquistão. Diversos herbicidas têm sido recomendados para o controle dessa planta daninha, mas eles apresentam riscos à saúde humana e à segurança ambiental. Neste estudo, o potencial herbicida de filtrados de cultura da espécie fúngica *Alternaria japonica* foi avaliado em comparação à planta daninha losna-branca. A espécie fúngica foi cultivada em caldo de extrato de malte (CEM) e caldo de dextrose de batata (CDB) por 15 dias. Os filtrados das culturas foram obtidos pela passagem dos materiais através de um pano de musselina, seguido por filtração em papel-filtro e, em seguida, em papel-filtro Millipore. Nos bioensaios em laboratório, foi observado o efeito dos filtrados original (X) e diluído ($\frac{1}{2}X$) na germinação de sementes, bem como o crescimento do sistema radicular e da parte aérea da losna-branca. Em geral, os filtrados da cultura fúngica preparados em qualquer um dos meios de cultura gerou redução significativa na germinação e no crescimento das mudas de losna-branca. No entanto, os filtrados da cultura preparados em caldo de dextrose

* Corresponding author:
<arshadjpk@yahoo.com>

Received: March 16, 2016

Approved: April 11, 2016

Planta Daninha 2017; v35:e017161195

¹ Institute of Agricultural Sciences, University of the Punjab Lahore, Pakistan.

de batata apresentaram maior atividade herbicida do que os preparados em caldo de extrato de malte. Foram realizados bioensaios foliares por pulverização usando filtrados de cultura de A. japonica preparados em caldo de dextrose de batata. Neste experimento, mudas de losna-branca com uma, duas e três semanas de idade foram pulverizadas quatro vezes com os filtrados inicial (X) e concentrado (2x) da cultura fúngica, com intervalos de quatro dias. Em geral, as mudas de losna-branca com uma semana de idade mostraram alta sensibilidade à aspersão foliar de metabólitos fúngicos. O presente estudo concluiu que os filtrados de cultura de A. japonica preparados em caldo de dextrose de batata contêm componentes herbicidas potentes para o manejo da losna-branca.

Palavras-chave: Asteraceae, capim-do-congo, fungos, mico-herbicida, planta daninha.

INTRODUCTION

Parthenium hysterophorus, belongs to the well-known plant family Asteraceae. It inhabits most parts of the world. It is a noxious weed because of its invasiveness, adaptability to variable environmental conditions, and ability to grow and spread luxuriantly (Kumari, 2014). Its two major synergistically acting allelochemical groups viz. phenolics and sesquiterpene lactones are responsible for reducing seed germination and growth of many plants (Mulatu et al., 2009). This weed poses serious biotic threats to plants as a result of its potential to alter above-ground vegetation and below-ground soil nutrients, and act as an alternate host for crop pests (Timsina et al., 2010). Human health hazards caused by this weed have also reached epidemic proportions with the problems, including allergic effects, skin inflammation, eczema, asthma, diarrhoea, hay fever, black spots, burning, blisters around the eyes etc. Systemic toxicity in livestock has been reported after exposure to and consumption of *parthenium*, which later affects milk and meat quality (Patel, 2014).

Debates are in progress to get rid of parthenium, and many management strategies have been adopted, including chemical herbicides such as glyphosate, atrazine, trifluralin, diphenamid etc. (Naseer-ud-Din et al., 2011), and cropping methods, e.g., hand pulling, mulching, mowing, burning and growing some competitive crops etc. (Khan et al., 2013). Shortcomings regarding chemical herbicides and increased demand of organic farming have shifted scientists' attention towards environmentally-safe weed management methods (Akbar and Javaid, 2013). Various biological weed management options have been applied so far, including the use of natural herbicides from plants (Javaid et al., 2010) and fungi (Akbar and Javaid, 2013) to control weeds. Use of fungal metabolites is regarded as an environmentally safe method to combat troublesome weeds (Farooq et al., 2011). The herbicidal potential of different species of *Fusarium*, *Drechslera*, *Trichoderma* and *Aspergillus* against parthenium has been documented (Javaid and Adrees, 2009; Javaid et al., 2011, 2013, 2014). The genus *Alternaria* of ascomycete fungi is a major phytopathogen, comprised of 299 species, and it is found throughout the world (Kirk et al., 2008). Over 268 metabolites have been reported from different species of *Alternaria*, and most of them showed phytotoxic, cytotoxic, and antimicrobial properties (Lou et al., 2013). Sanodiya et al. (2010) identified tenuazonic acid in *A. alternata* and verified its herbicidal potential against parthenium. The findings of Saxena and Kumar (2010) suggested a significant increase in the mortality of parthenium weed as a result of the application of metabolites of *A. alternata*. Kaur et al. (2016) found that *Alternaria macrospora* was pathogenic to parthenium leaves responsible for causing leaf blight, and its metabolites caused significant damage to the weed. The present research was carried out to assess the herbicidal potential of culture filtrates of *Alternaria japonica* against parthenium weed.

MATERIALS AND METHODS

Laboratory bioassays: Pure culture of *A. japonica* was obtained from the First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of Punjab, Lahore, Pakistan. Two percent malt extract agar medium was used for sub-culturing of the fungus. Fungal metabolites were prepared by inoculating a fungal disc (5 mm) from the periphery of the 7 day old culture to pre-autoclaved 2% malt extract broth (MEB) and potato dextrose broth (PDB) in 250 mL flasks under aseptic conditions. The inoculated flasks were kept in an incubator at 25 °C for two

weeks. After the incubation period, the fungal mat was separated from the broth through a sterilized muslin cloth, followed by further filtration through sterilized filter papers. Filtrates were centrifuged at 600 rpm for 5 minutes and then filtered through Whatman Millipore filter papers. The original fungal metabolites (X) were diluted with autoclaved distilled water to prepare a lower concentration ($\frac{1}{2}X$).

Parthenium seeds were collected from different areas of Punjab University Lahore, Pakistan. They were dried at 45 °C for 2 hours in an oven. Seeds were surface sterilized in sodium hypochlorite solution (2%) for 1 minute and then washed with sterilized water. Twenty-five surface sterilized seeds were placed on pre-sterilized Petri dishes (9 cm diameter) lined with a double layer of filter papers, and moistened with 2.5 mL of original or diluted concentrations of the fungal metabolites. For control, the same amount of distilled water was used. Each treatment was repeated four times. The Petri dishes were kept in a completely randomized design in a growth chamber at 25 °C with a 12 hour light and 12 hour dark period, on a daily basis, for two weeks. Data on seed germination and plant growth were recorded in terms of shoot length, root length, and fresh and dry plant biomass.

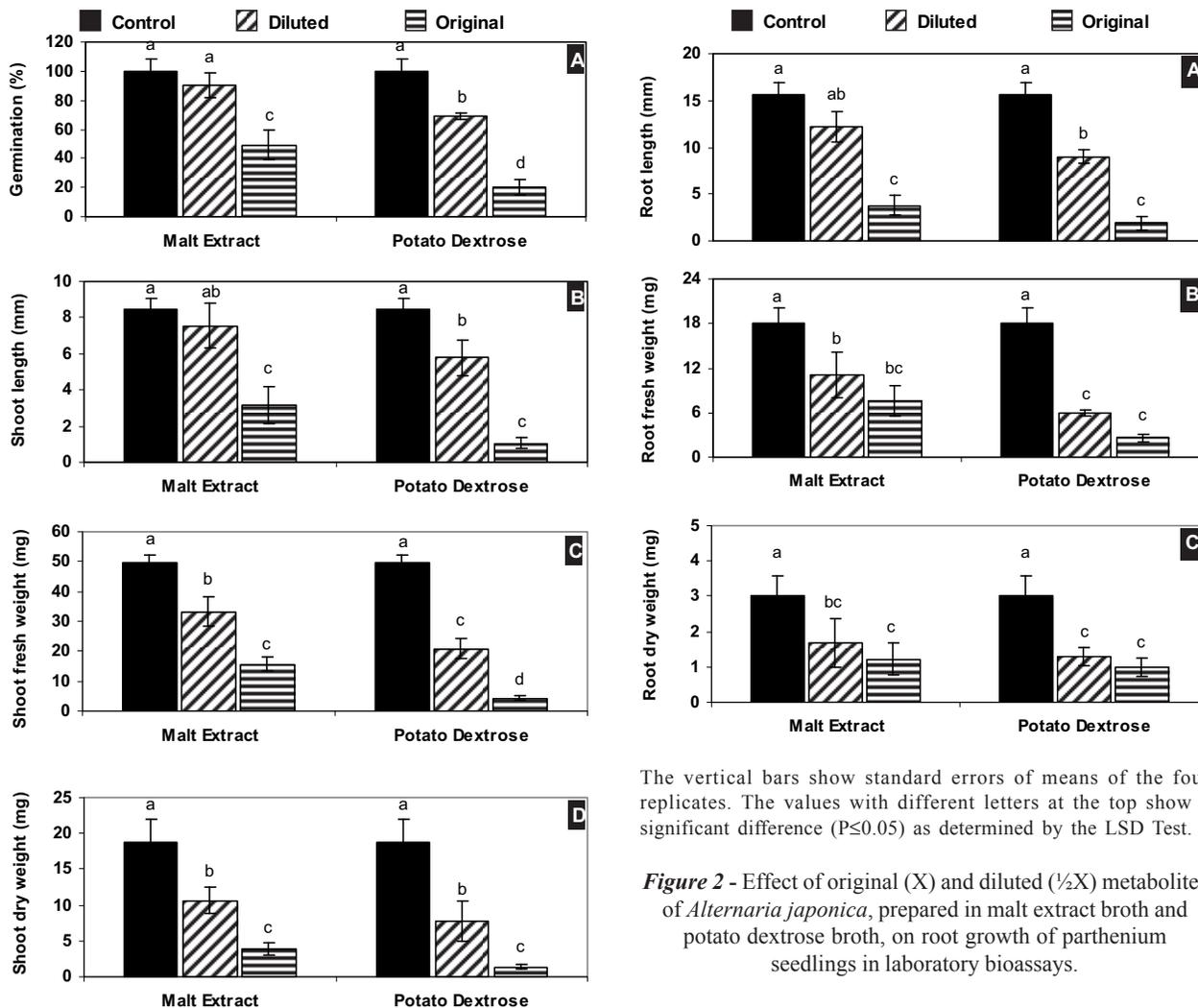
Pot assays: For the pot experiment, PDB was used to make fungal metabolites in a similar method to the one described previously. The experiment was conducted in plastic pots (10 cm diameter) filled with sterilized sandy loam soil followed by sowing of 10 pre-sterilized parthenium seeds in each pot. Foliar spray with original (X) and concentrated (2X) metabolites was carried out on three sets that consisted of 7, 14 and 21 days old plants. Concentrated fungal metabolites were acquired through evaporation of the original fungal metabolites at 45 °C in an electric oven. Pots were sprayed with 2 mL of each original or concentrated metabolites of the fungus while distilled water was sprayed on plants of control treatment. Three sprays were applied on each set every 4 days. The plants were harvested 45 days after sowing and length as well as fresh and dry weight values were recorded for root and shoot.

All the data from laboratory bioassays as well as from foliar spray bioassays were subjected to analysis of variance followed by the LSD test to differentiate the treatment means at 5% level of significance, using the software Statistix 8.1.

RESULTS AND DISCUSSION

Laboratory bioassays: In general, the herbicidal effect of fungal metabolites prepared in PDB was more pronounced on germination and growth of parthenium than those prepared in MEB. The effect of diluted metabolites prepared in MEB was insignificant while the original metabolites significantly reduced germination by 51% compared with the control. On the other hand, both original and diluted metabolites prepared in PDB significantly decreased germination by 31% and 80% compared with the control, respectively (Figure 1A). Some previous studies reveal that metabolites of other *Alternaria* species, especially *A. alternata*, can significantly suppress germination of parthenium seeds, possibly because of the presence of certain herbicidal constituents in these metabolites (Javaid and Adrees, 2009).

The original fungal metabolites prepared in MEB significantly reduced length and biomass of shoot by 63% and 80%, respectively. However, while the diluted metabolites had an insignificant effect on shoot length, they reduced shoot biomass significantly by 43% over the control. On the other hand, shoot length was decreased by 88% and 32%, and shoot dry biomass declined by 93% and 54% because of the original and the diluted fungal metabolites, respectively, prepared in PDB (Figure 1B, D). The effect of the original and diluted metabolites exhibited herbicidal effects against various parameters of root growth which are similar to that of shoot growth (Figure 2A, C). Different herbicidal effects of fungal metabolites in the two growth mediums might have been the result of the formation of different types or quantities of herbicidal constituents in the dissimilar growth medium. The results of some studies carried out previously also support the findings of the present study. Javaid et al. (2013) reported a significant reduction in parthenium seed germination because of metabolites of four *Trichoderma* species prepared in M-1-D medium compared with those prepared in malt extract broth. Likewise, differences in herbicidal activity of various *Drechslera* species prepared in M-1-D medium and malt extract broth were recorded against a problematic weed of wheat, i.e. *Rumex dentatus* (Akbar and Javaid, 2010, 2013).



The vertical bars show standard errors of means of the four replicates. The values with different letters at the top show a significant difference ($P \leq 0.05$) as determined by the LSD Test.

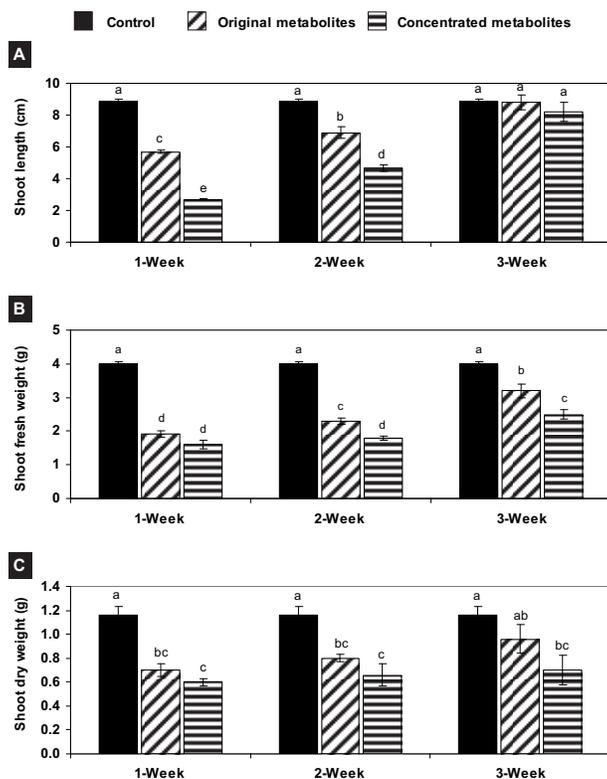
Figure 2 - Effect of original (X) and diluted ($\frac{1}{2}X$) metabolites of *Alternaria japonica*, prepared in malt extract broth and potato dextrose broth, on root growth of parthenium seedlings in laboratory bioassays.

The vertical bars show standard errors of means of the four replicates. The values with different letters at the top show a significant difference ($P \leq 0.05$) as determined by the LSD Test.

Figure 1 - Effect of original (X) and diluted ($\frac{1}{2}X$) metabolites of *Alternaria japonica*, prepared in malt extract broth and potato dextrose broth, on germination and shoot growth of parthenium seedlings in laboratory bioassays.

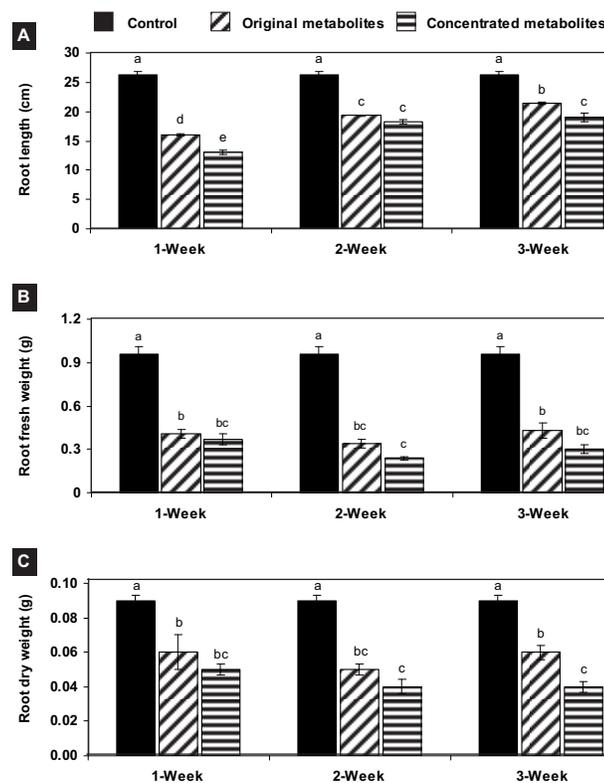
and concentrated metabolites significantly reduced shoot length by 36% and 70% in 1 week old, and 22% and 47% in 2 week old parthenium seedlings on shoot length of 3 week old plants was insignificant. Both the original and the concentrated metabolites significantly reduced fresh and dry biomass of parthenium seedlings. There was 40% and 48% suppression in shoot dry biomass of 1 week old, and 31% and 43% reduction in 2 week old plants, respectively. In 3 week old plants, only the concentrated metabolites showed a significant herbicidal effect and reduced shoot dry biomass by 40% over the control (Figure 3A, C). Both types of metabolites significantly reduced length as well as fresh and dry biomass of roots of parthenium. There was 33% and 44%, 45% and 55%, and 34% and 54% reduction in root dry biomass of 1-, 2- and 3 week old parthenium plants, caused by the original and the concentrated metabolites, respectively (Figure 4A, C). Some previous studies also showed that the herbicidal activity of fungal metabolites decreases with the age of weed plants (Javaid et al., 2011, 2013; Akbar and Javaid, 2013).

The present study concludes that culture filtrates of *A. japonica* contains potent herbicidal constituents for ecofriendly management of parthenium weed. Further research is required to identify the effective herbicidal compounds in these culture filtrates.



The vertical bars show standard errors of means of five replicates. The values with different letters at the top show a significant difference ($P \leq 0.05$) as determined by the LSD Test.

Figure 3 - Effect of original and concentrated metabolites of *Alternaria japonica* prepared in potato dextrose broth on shoot growth of parthenium in pot trials.



The vertical bars show standard errors of means of the five replicates. The values with different letters at the top show significant difference ($P \leq 0.05$) as determined by the LSD Test.

Figure 4 - Effect of original (X) and concentrated (2X) metabolites of *Alternaria japonica* prepared in potato dextrose broth on root growth of parthenium in pot trials.

REFERENCES

- Akbar M., Javaid A. Management of some problematic weeds of wheat by metabolites of *Drechslera* spp. prepared in malt extract. **Pak J Weed Sci Res.** 2010;16:145-51.
- Akbar M., Javaid A. Prospects of using fungal metabolites for the management of *Rumex dentatus*, a problematic weed of wheat. **Int J Agric Biol.** 2013;15:1277-82.
- Farooq M. et al. The role of allelopathy in agricultural pest management. **J Pest Manage Sci.** 2011;67:494-506.
- Javaid A., Adrees H. Parthenium management by cultural filtrates of phytopathogenic fungi. **Nat Prod Res.** 2009;23:1541-51.
- Javaid A., Shafique S., Shafique S. Herbicidal effects of extracts and residue incorporation of *Datura metel* against parthenium weed. **Nat Prod Res.** 2010;24:1426-37.
- Javaid A., Javaid A., Akbar M. Herbicidal potential of culture filtrates of *Drechslera* spp. against *Parthenium hysterophorus*. **Chil J Agric Res.** 2011;71:634-7.
- Javaid A. et al. Effect of culture medium on herbicidal potential of metabolites of *Trichoderma* species against *Parthenium hysterophorus*. **Int J Agric Biol.** 2013;15:119-24.
- Javaid A. et al. Screening of Aspergilli for herbicidal activity of their culture filtrates against *Parthenium* weed. **Pak J Weed Sci Res.** 2014;20:137-44.
- Kaur M. et al. Effects and Management of *Parthenium hysterophorus*: A weed of global significance. **Int Scholarly Res Notices.** 2014;2014:1-12.
- Khan N. et al. Suppressive ability of selected fodder plants on the growth of *Parthenium hysterophorus*. **J Weed Res.** 2013;53:61-8.

Kirk P.M. et al. **Dictionary of the fungi**. 10th ed. Wallingford: CABI, 2008. 22p.

Kumari M. *Parthenium hysterophorus* L.: A Noxious and Rapidly Spreading Weed of India. **J Chem Biol Phys Sci**. 2014;4:1620-8.

Lou J. et al. Metabolites from *Alternaria* fungi and their bioactivities. **Molecules**. 2013;18:5891-935.

Mulatu W., Gezahegn B., Solomon T. Allelopathic effects of an invasive alien weed *Parthenium hysterophorus* L. compost on lettuce germination and growth. **Afr J Agric Res**. 2009;4:1325-30.

Naseer-Ud-Din G.M., Shehzad M.A., Nasrullah H.M. Efficacy of various pre and post-emergence herbicides to control weeds in wheat. **Pak J Agric Sci**. 2011;48:185-90.

Patel S. Harmful and beneficial aspects of *Parthenium hysterophorus*: an update. **Biotechnology**. 2011;1:1-9.

Sanodiya B. et al. Isolation and characterization of Tenuazonic acid produced by *Alternaria alternata*, A potential bioherbicidal agent for control of *Lantana Camara*. **J Plant Prot Res**. 2010;50:133-9.

Saxena S., Kumar M. Evaluation of *Alternaria alternata* ITCC4896 for use as mycoherbicide to control *Parthenium hysterophorus*. **Arch Phytopathol Plant Prot**. 2010;43:1160-4.

Timsina B. et al. Impact of *Parthenium hysterophorus* L. invasion on plant species composition and soil properties of grassland communities in Nepal. **Flora Morphol Distrib Funct Ecol Plants**. 2011;206:233-40.