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EFFECTS OF *PENICILLIUM EXPANSUM* METABOLITES ON GERMINATION AND GROWTH OF *PARTHENIUM HYSTEROPHORUS* L.

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ABSTRACT

Parthenium (*Parthenium hysterophorus* L.), an exotic weed of Mexican origin, has invaded most of the terrestrial ecosystems and field crops in Punjab, Khyber Pakhtunkhwa and Kashmir during the last 30 years. In an attempt to control it under *in vitro* conditions, metabolites of a fungal species *Penicillium expansum* were tried as natural herbicides. For this purpose, the fungal species was grown in malt extract broth (MEB) and potato dextrose broth (PDB) for two weeks and the filtrates (metabolites) were tested for their herbicidal activity, both in original (100%) and diluted (50%) forms. For comparison, a control (water), and the two growth media (in 100% and 50%) were also used. In general, the effect of growth media, in either of the two concentrations, was insignificant on germination and various growth parameters of the weed, over control. By contrast, both the concentrations of fungal metabolites significantly suppressed germination and growth of parthenium. Metabolites prepared in PDB were more herbicidal in nature than those prepared in MEB. There was 66–100%, 93–100%, 92–100% and 95–100% reduction in germination, shoot length, root length and plant dry weight of parthenium due to metabolites prepared in PDB as compared to 24–37%, 54–68%, 74–88%, and 73–75% reduction in these parameters due to metabolites prepared in MEB, respectively.

Keywords: Asteraceae, Fungal metabolites, Herbicidal activity, Invasive weed.

INTRODUCTION

Parthenium is an annual herbaceous weed, native to Mexico and other parts of Americas, and is included among the top invasive weeds (Costello *et al.*, 2022). It has spread in some parts of Pakistan at an alarming extent (Javaid *et al.*, 2022a). Presently, it is found in more than 80 countries with pantropical distribution (Shi *et al.*, 2015). It is considered as one of the most problematic weeds in Australia, Southeast and Southern Asia, the Middle East, the Pacific, and Southern and Eastern Africa (Adkins and Shabbir, 2014). A recent prediction shows that due to climate change, 21 countries such as China, Bulgaria, Netherlands, Brunei, South Korea and New Zealand, which are presently in a category of low habitat suitability for parthenium, will be changed into a category of moderate to very high suitability till the years 2081–2100 (Adhikari *et al.*, 2023). It is a well-known fact that parthenium has a significant adverse effect on the growth and yield of crops as well as on natural ecosystems (Boja *et al.*, 2022; Masum *et al.*, 2022), the rearing of animals and their feed, and on the health of humans such as problems in breathing, contact dermatitis, asthma and bronchitis (Kaur *et al.*, 2021).

The most common practice for control of weeds is the use of synthetic herbicides. Parthenium can also be controlled by a number of herbicides such as chwastox, bromoxynil+MCPA, glyphosate, metribuzin, triasulfuron + terbutryn and others (Javaid *et al.*, 2006; Khan *et al.*, 2012). However, use of these herbicides is nowadays is being discouraged due to their ill effects on health and environment, and development of resistance in various weed species (Bruggen *et al.*, 2018). Scientists are trying to explore ecofriendly alternative strategies to control parthenium (Javaid *et al.*, 2020). Among others, exploration of natural herbicidal constituents from fungi for control of parthenium and other weeds is gaining importance nowadays (Akbar *et al.*, 2014; Javaid *et al.*, 2022b). Metabolites of *Alternaria japonica* (Javaid *et al.*, 2017) and *Aspergillus niger* (Bashir *et al.*, 2018) declined germination and growth of parthenium both *in vitro* and *in vivo*. Similar herbicidal activities have also been shown by the metabolites of *Macrophomina phaseolina*, *Cladosporium oxysporum* and *Fusarium equisetti* (Idrees and Javaid, 2008). Thus, this study was undertaken to investigate the *in vitro* herbicidal potential of metabolites of *P. expansum* against parthenium.

MATERIALS AND METHODS

Preparation of *P. expansum* metabolites

An authentic culture of *P. expansum* was obtained from Fungal Culture Bank of Pakistan and refreshed it. Two growth media namely PDB and MEB were prepared by adding 2 g of each in 100 mL distilled water and autoclaved at 121 ºC for half an hour. After cooling, each media flask was inoculated with fresh culture of *P. expansum.* The flasks were incubated for 2 weeks at 28 ºC and after completion of this incubation period, fungal biomass was separated by filtration while the filtrates were collected and stored at 4 ºC for use in the laboratory bioassays. These original filtrates/metabolites were designated as 100% concentration, which were also diluted to 50% by adding distilled water.

Laboratory bioassays

Laboratory bioassays were carried out in 9-cm diameter Petri plates. A single filter paper was placed in each Petri plate and 25 parthenium seeds were spread with uniform distance from each other. A volume of 2.5 mL of the filtrates was poured in each plate, covered with a lid and the plates were arranged in a completely randomized design on a bench for 10 days. There were four replicates of each treatment. Distilled water was added in control treatment as a substitute of fungal metabolites. In order to rule out any effect of growth media, treatments with original (100%) and diluted (50%) growth media were also included. On day 10, germinated seeds in each plate were counted and the data were transformed to percentage germination. Lengths of roots and shoots of the seedlings were separately measured with a scale, and averaged shoot/root lengths for each replicated plate were calculated. All the plants from each plate were collectively weighed in fresh as well as in dried form.

Statistical analysis

Data concerning germination and various plant growth parameters were analyzed by two-way ANOVA. Treatment means were separated by applying LSD test ($P \leq 0.05$) using software STATISTIX 8.1.

RESULTS AND DISCUSSION

Effect of *P. expansum* metabolites on germination of parthenium

There was a significant difference ($P \leq 0.001$) in germination between the two growth media (Table 1). However, original and diluted concentrations of both the growth media had insignificant effect on germination of seeds as compared to control. On the other hand, there were drastic negative effects of both the concentrations of metabolites prepared in the two types of growth media (Fig. 1A & 2A). Likewise, metabolites of other *Penicillium* species namely *Penicillium citrinum* and *Penicillium crustosum* also suppressed germination of this weed (Javaid *et al.*, 2021; Khan *et al.,* 2022). In general, PDB was superior over MEB with respect to production of herbicidal metabolites by the *P. expansum* during 14-day incubation period. Original metabolites of PDB completely controlled germination of parthenium. Fungal metabolites prepared in PDB reduced germination by 66–100% as compared to 24–37% decline in germination because of metabolites prepared in MEB (Table 2). Similar differential activity of herbicidal metabolites of some other fungal species, prepared in different growth media, has also been demonstrated in some earlier studies. *Alternaria citri* metabolites in PDB reduced parthenium germination by 92% as compared to 43% reduction due to metabolites in MEB (Javaid *et al.,* 2022b). Similarly, metabolites of *P. crustosum* and *P. citrinum* in PDB reduced parthenium germination by 62% and 57% as compared to metabolites of these fungi in MEB that reduced 33% and 31% germination over control, respectively (Javaid *et al.,* 2021; Khan *et al.,* 2022). By contrast, metabolites of *Aspergillus niger* in PDB were less herbicidal against parthenium than those prepared in MEB (Bashir *et al.*, 2018). It clearly demonstrates that herbicidal potential of fungal metabolites varies with fungal species and type of growth media. For a better herbicidal activity of metabolites of different fungal species, a correct combination of the fungus and the growth media should be known.

Effect of *P. expansum* metabolites on shoot length of parthenium

The effect of growth media (G), metabolite's concentration (C) and G × C was significant ($P \leq 0.001$) for shoot length (Table 1). Metabolites of *P. expansum* from both the growth media significantly ($P \leq 0.05$) declined the length of parthenium seedlings over control. The two concentrations of each of the two growth media did not show any pronounced effect on shoot length (Fig. 1B & 2B). Original and diluted metabolites in PDB were more herbicidal in nature than those prepared in MEB causing 93–100% and 54–68% suppression in the length of parthenium seedlings, respectively (Table 2). Likewise, metabolites of *P. crustosum* and *P. citrinum* in PDB reduced shoot length of parthenium by 87–88% and 80–89%, and metabolites in MEB reduced this parameter by 49–74% and 70–

77%, respectively (Javaid *et al.,* 2021; Khan *et al.,* 2022). Earlier, similar effects of metabolites of *Fusarium solani* and *F. oxysporum* were also recorded on shoot length of this plant species (Javaid and Adrees, 2009).

Table 1. Two-way ANOVA table for comparison of herbicidal activity of fungal metabolites prepared in malt extract broth and potato dextrose broth.

Sources of variation	**Df**	**Mean Squares**				
		Germination	Shoot length	Root length	Fresh weight	Dry weight
Growth media (G)	1	4461*	1.29*	0.223 ns	5423*	75.9ns
Concentration (C)	4	9328*	8.09*	0.661*	121578*	10797*
G × C	4	2491*	0.38*	0.046 ns	2488*	384*
Error	40	48.3	0.04	0.053	196	34.7
Total	49					

* Significant at $P \leq 0.001$; ns: Non-significant

Table 2. Percentage decrease/increase in germination and growth of parthenium weed over control due to metabolites of *Penicillium expansum* (PE) prepared in potato dextrose broth and malt extract broth.

Treatments	**Decrease (-) / increase (+) over control (%)**				
	Germination	**Shoot length**	**Root length**	**Fresh biomass**	**Dry biomass**
Metabolites prepared in malt extract broth (MEB)					
MEB (100%)	-7	-2	+28	-4	-3
MEB (50%)	-7	-2	+17	-7	-9
PE metabolites (100%)	-37	-68	-88	-73	-75
PE metabolites (50%)	-24	-54	-74	-76	-73
Metabolites prepared in potato dextrose broth (PDB)					
PDB (100%)	-2	-7	+18	-3	+10
PDB (50%)	0	-3	+17	-6	+3
PE metabolites (100%)	-100	-100	-100	-100	-100
PE metabolites (50%)	-66	-93	-92	-94	-95

Effect of *P. expansum* metabolites on root length of Parthenium

In case of root length, both the growth media increased root length of seedlings. The effect of original concentrations of both the growth media was significant ($P \leq 0.05$) as compared to control. There was 17–18% and 17–28% increase in root length due to different concentrations of the growth media *viz.* PDB and MEB, respectively. By contrast, metabolites in PDB and MEB suppressed root length by 92–100% and 74–88%, respectively (Table 2, Fig. 1C & 2C). Inhibition in root length due to metabolites in MEB was more pronounced than corresponding inhibition in shoot length (Table 2). Similar variable effect on shoot and root lengths of parthenium and other weed species have also been found due to the application of metabolites of other fungal species such as *P. citrinum* and *Trichoderma* spp. (Javaid and Ali, 2011; Javaid *et al.,* 2021) as well as extracts of allelopathic plants such as *Coronopus didymus* (Javaid and Khan, 2020).

Effect of *P. expansum* metabolites on biomass of Parthenium

Both fresh and dry biomass of parthenium seedlings remained unaffected by application of original growth media. However, fungal metabolites in both the growth media significantly reduced biomass of seedlings. Fresh and dry weights of seedlings were reduced by 94–100% and 95–100% due to metabolites in PDB and 73–76% and 73–75% due to metabolites in MEB, respectively (Table 2, Fig. 12D-E & 2D-E). Reduction in seedling's biomass due to *P. expansum* metabolites could be attributed to the reduced shoot and root growth/length due to these metabolites as also reported in various previous studies due to metabolites of *P. citrinum* (Javaid *et al.*, 2021), *P. crustosum* (Khan *et al.*, 2022), *F. oxysporum*, *F. solani* (Javaid and Adrees, 2009), *Trichoderma* spp. (Javaid and Ali, 2011), and *A. niger* (Bashir *et al.*, 2018).

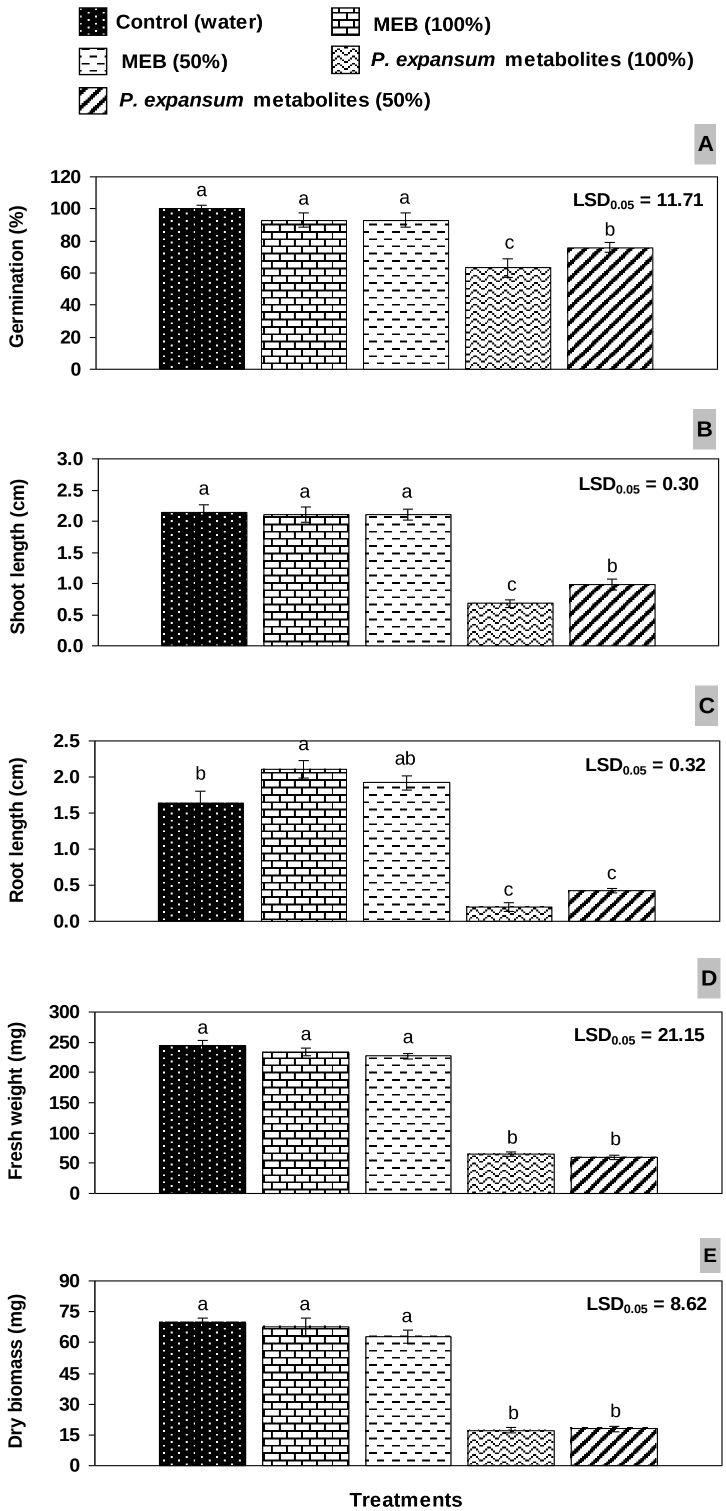

Fig. 1. Effect of metabolites of *Penicillium expansum*, prepared in malt extract broth, on germination and growth of parthenium. Vertical bars show standard errors and different letters show significant difference ($P \leq 0.05$) as determined by LSD Test.

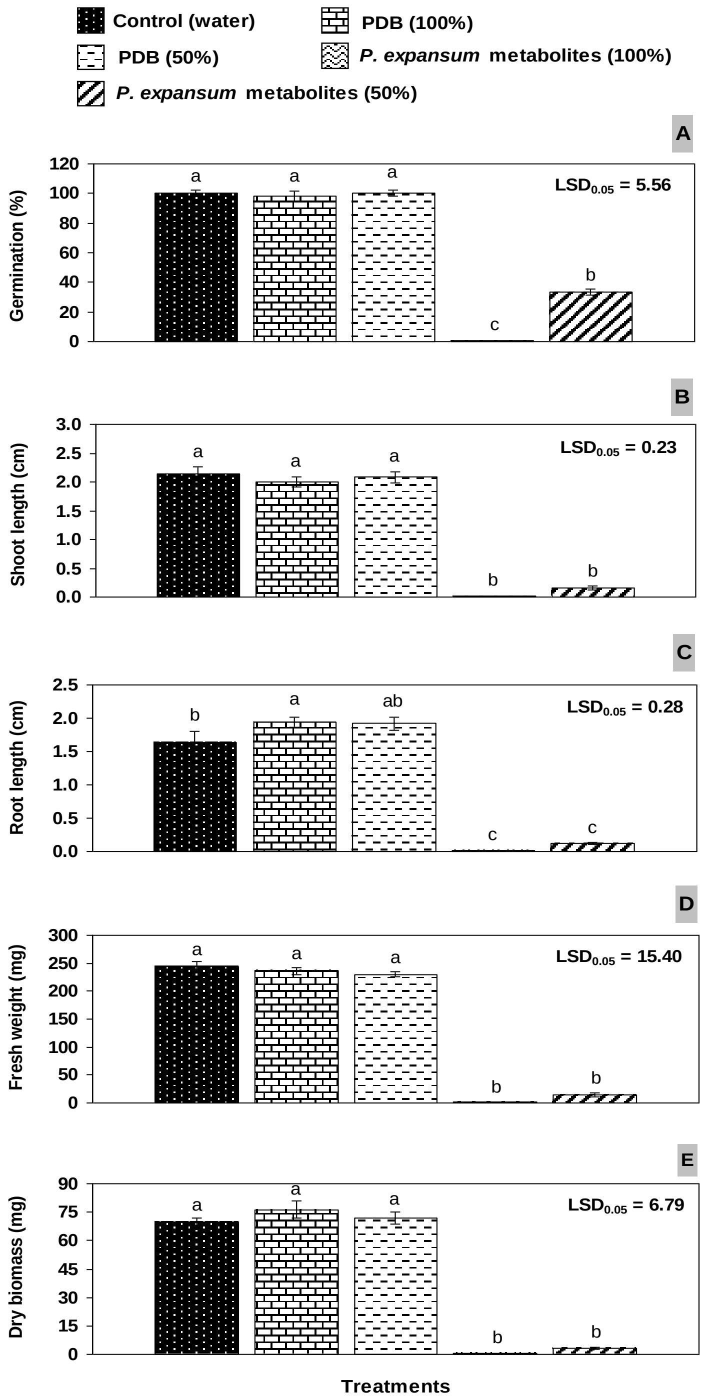

Fig. 2. Effect of metabolites of *Penicillium expansum*, prepared in potato dextrose broth, on germination and growth of parthenium. Vertical bars show standard errors and different letters show significant difference ($P \leq 0.05$) as determined by LSD Test.

Conclusion

P. expansum metabolites were found highly herbicidal in nature and significantly suppressed germination and growth of parthenium. Selection of a suitable growth media is very important with respect to herbicidal potential of fungal metabolites. In the present study, metabolites of *P. expansum* prepared in PDB showed a greater herbicidal activity against parthenium than those prepared in MEB. Further studies are suggested to identify and quantify herbicidal compounds present in the metabolites for their future use as analogues for preparation of natural product based herbicides against parthenium and other problematic weeds.

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