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PARTHENIUM HYSTEROPHORUS L. A POTENTIAL SOURCE OF BIOHERBICIDE

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Abstract

Parthenium hysterophorus L., being a declared invasive weed is threatening the biodiversity and human health in several areas of Pakistan. Several researchers have documented the alleloapthic effect of this weed. In the present studies the allelopathic potential was studied in laboratory and in field conditions with the objectives to explore the potential of *P. hysterophorus* as bioherbicide in future. Laboratory based experiment showed that with the increasing concentration of *P. hysterophorus,* the germination percentage, seedling length and seedling weight of all the three species tested were significantly decreased. The tolerance order of the species against the extract concentration of *P. hysterophorus* were *Triticum aestivum* > *Avena fatua* > *Lepidium* sp. While field experiment showed that there was no effect of any concentration either pre or post-emergence on weed density 25 days after sowing, fresh biomass and dry biomass. However different concentration had significant effect on the weed density 50 DAS in post-emergence as well as pre-emergence. This might be due to delayed germination of weeds due to allelochemicals. Hence the present study suggest that *P. hysterophorus* can be used as a bioherbicide but still needs extensive study to fully explore its potential against different summer and winter weeds.

Introduction

There is an increasing interest in allelopathic studies and recently many researchers have focus on the exploration of plant allelopahty. The development of weed management strategies that make use of allelopathic crop plants is receiving increased national and international attention (Weston, 1996). There are many weed species that are allelopathic in nature. It is viable weed management strategy but needs to be extensively studied under laboratory as well as in the field conditions. It is a natural and environment friendly technique which may prove an effective strategy for weed management and thereby increase crop yields. Among weeds *Parthenium hysterophorus* L., is an aggressive weed native to Southern North America, Central America, the West Indies and Central South America (Picman & Picman, 1984), having allelopathic effect and drastically retards the growth of many species (Tefera, 2002). The idea that plants affect neighboring plants by releasing chemicals in the environment has been known since 370 BC (Willis, 1985; 1997). Allelopathy may prove harmful as plants containing allelochemicals release these chemicals to the soil, water bodies, plant environment and thus cause tremendous hazards. Apart from its competitive ability, the invasiveness of the noxious weed *P. hysterophorus* L., is thought to be due to an ability to displace other species by means of allelopathy. The sesquiterpene lactone parthenin that is biosynthesized by this species is thought to play a role in its allelopathic interference with surrounding plants (Regina *et al.*, 2007). *Parthenium* root extracts decreased the germination and growth of maize and barley (Rashid *et al.*, 2008).

Allelochemicals or plant derived chemicals offers a great potential for the pesticides because they are comparatively safer for the environment. In the past two decades, much more work has been done on plant derived compounds as environmentally safe alternatives to herbicides for the weed control (An *et al.,* 1998; Duke *et al.*, 2002). These chemicals could be used for weed management directly or their chemistry could be used to develop new herbicides. Some trees have negative effect on the seed germination and thus these trees can contribute to the pesticide industry if explored fully (Khan *et al.*, 2005). The inhibitory effects of *P. hysterophorus* L., on germination of many crops have been reported (Narwal, 1994). With the increasing concentration of *Parthenium* extracts the seed germination and growth of *Eragrostis* decreased significantly (Tefera, 2002). The inhibitory effects of the husk extracts of 7 rice varieties on growth of barnyard grass [*Ehinochola curssgali* (L.) Beauv.] was meaningful (Ko *et al.*, 2005). Adverse effects of water extracts of different *Brassica* sp. against germination and growth of cutleaf ground cherry weed (*Physlis anagulata* L.) have also been reported (Uremis *et al.*, 2005). *Prosopis juliflora* significantly inhibited the seed germination in Pearl millet (Sundramoothy *et al.*, 1995) while Ibrahim *et al.*, (1999) reported allelopathic effects of *Euclayptus camaldulensis* on crops. Release of parthenin by aqueous extraction of fresh leaf material of *P. hysterophorus* under laboratory conditions proved to be sufficient to provide significant phytotoxicity, and the relative role of parthenin to overall phytotoxic effects of the crude extract could be estimated to 16–100% (Regina *et al*., 2007).

Parthenium is rapidly spreading in Pakistan for the last 20-30 years and is now a serious weed of wastelands and grazing lands, especially in rainfed areas (Javaid & Anjum, 2006). Presently *Parthenium* can be found along the roadsides and even in agricultural crops like maize etc., in North West Pakistan, therefore detailed study of this weed will lead towards a better management approach. Keeping in view the importance of the allelopathic potential of *P. hysterophorus* L., this experiment was conducted under the laboratory as well as under field conditions with the objectives: a) to investigate the allelopathic status of *P. hysterophorus* L., b) to quantify the response of *Triticum aestivum,* maize and its associated weeds to the water extracts of *P. hysterophorus* at various levels and c) to decipher the efficacy of *P. hysterophorus* as pre-emergence and post-emergence and its effect on different weeds.

Materials and Methods

Laboratory based and field experiments were conducted during January to September 2007 in NWFP Agricultural University, Peshawar, Pakistan to investigate the allelopathic effects of *Parthenium hysterophorus* L. In Lab experiment, *P. hysterophorus* plants were collected at flowering stage in November 2006 and dried in shade. Leaves from the plants were separated, ground and then ground dry powder was soaked for 24 hrs in tap water at room temperature. Lab experiment was conducted during January 2007. After filtration, the concentrations @ 10, 50 and 100g L^{-1} were prepared by soaking 10, 50 and 100 g in one liter of water separately. Experiment was laid out in Completely Randomized Design at room temperature 20 ± 2^{o}C. Ten seeds of each, crop (wheat) and weeds (*Avena fatua* and *Lepidium pinnatifidum*) were placed in Petri-dishes containing blotting paper at bottom. The seeds of crop and weed species were collected in April 2006 and stored at room temperature until use. There were four treatments i.e. 0% (control), 10 g L^{-1}, 50 g L^{-1} and 100 g L^{-1}, replicated three times. The concentrations of extracts were applied at equal quantity to Petri dishes when needed. After ten days, the data on seed germination percentage, seedling length plant^{-1} (cm) and seedling weight (average of 10 seedlings) plant^{-1} (mg) were recorded. A second run of experiment was also completed in the same protocol and the data was averaged to make the results more meaningful.

Field experiment was conducted at Agricultural Research Farm, NWFP Agricultural University, Peshawar, Pakistan during June 2007. Peshawar lies between 71-27 and 72-47 east longitude and 33-40' and 34-31' north latitude and 359 m altitude. The experimental site has mean soil pH of 7.47 with 22.8, 55.7 and 21.5% clay, silt and sand, respectively. The field was irrigated and ploughed thrice at proper moisture condition to make a fine seedbed followed by planking. The experiment was laid out in Randomized Complete Block (RCB) design with split plot arrangement having three replications. There were two main plots and seven subplots. Time of application of *Parthenium* extracts i.e., soil application and foliar application were assigned to main plots while different concentrations of *Parthenium* extracts e.g., 50, 100, 150, 200, and 250 g L^{-1} were assigned to sub-plots. In pre-emergences treatments the extracts were applied soon after sowing maize while in post-emergence, extracts were applied 15 days after sowing when many weeds emerged. Extracts were applied by using knapsack sprayer. Weedy check was also included for comparison. The size of each treatment was 5 x 2.25 m^2. Numbers of rows per treatment were three and row to row distance was maintained at 75 cm. Maize seed were sown by drill method. Maize variety "Azam" was used in the experiment. Urea as a source of nitrogen (N) and Single Super Phosphate was used as a source of phosphorus (P) and were applied at 120 and 60 kg ha^{-1}, respectively.

Data collected for each experiment was analyzed separately as per design by using analysis of variance using MSTATC computer software and means were separated using least significance difference test at $P \leq 5\%$ (Steel & Torrie, 1980).

Results and Discussion

Lab experiment

Germination percentage: Statistical analysis of the data revealed that various concentrations of *Parthenium* significantly ($p \leq 0.05$) affected the germination of all the test species (Fig. 1). There was slightly inhibitory effect of *Parthenum* extract concentration on the wheat seed germination while inhibitory effect on the rest of the species was comparatively greater. With the increasing concentration of *Parthenium*, the seed germination of *Avena fatua* and *Lepidium pinnatifidum* decreased significantly. The present findings suggest that weed seeds are more sensitive to *Parthenium* extracts as compared to wheat seed. Hence the *Parthenium* can be used as a tool of weed management in the future research programs. *P. hysterophorus* extracts significantly inhibited the seed germination of *Eragrostis tef* (Tefera, 2002) due to released of phytotoxins from *Parthenium* leaves (Stephen & Sowerby, 1996). Seed germination of *Lepidium* pinnatifidum was more prone to higher concentration of *Parthenium* extracts where there was no germination at *Parthenium* concentration of 30g L^{-1}. Species-specific differences in the sensitivity to aqueous extracts of fresh or dry leaf material of *P. hysterophorus* were reported in previous studies (Kohli *et al.*, 1996; Mersie & Singh, 1987; Srivastava *et al.*, 1985). However, the results indicated that areas seriously infested with *Parthenium* can release greater amount of allelochemicals and thus can be proved harmful for the wheat and other crops as well because at higher concentrations *Parthenium* extracts negatively affected wheat germination. The most encouraging aspect of the present studies is the selective inhibition of weeds (*Lepidium pinnatifidum* and *A. fatua*). This might be due to the fact that broadleaf are more susceptible to parthenium extracts as compared to grasses. However this needs to be confirmed. In a similar study it was noted that species varied considerably in their sensitivity to aqueous extracts of *P. hysterophorus* for both root growth and germination (Regina *et al.,* 2007; Rashid *et al.,* 2008).

Seedling length (cm) plant^{-1}: Fig. 2 shows that different concentrations had significant ($p \leq 0.05$) effects on seedling length of wheat and associated weeds. Seedling length of all the test species (wheat, *A. fatua* and *Lepidium pinnatifidum*) were significantly decreased with the increase in concentration of *Parthenium* extracts. The release of parthenin during decomposition of leaf material has a potential to play a leading role for allelopathy in *P. hysterophorus*; however, its significance in a natural setting will very much rely on the amount of leaf material accumulated on soil surfaces and the concentration of parthenin in residues (Regina *et al.,* 2007). The present findings would suggest that heavy infestation of *Parthenium* in an area can cause accumulation of allelochemicals in greater amount in the soil which will adversely affect the subsequent crops and weeds. However, further studies of this *Parthenium* can enable us to use *Parthenium* extracts for weed management in agricultural crops because some crop seeds are tolerant while others are not. Results suggested that higher concentration of *Parthenium* retard the growth of plants which might be due to inhibition of cell division as allelopathic chemicals have been found to inhibit gibberellin and indoleacetic acid function (Tomaszewski & Thimann, 1966). Parthenin is among other inhibitors relevant for residue allelopathy as simulated under laboratory conditions by delaying germination and reducing plant growth (Regina *et al.,* 2007).

Seedling weight (mg) plant^{-1}: Statistical analysis of the data depicted that various concentrations had significant ($p \leq 0.05$) effects on the seedling weight of the test species (Fig. 3). Data indicated that seedling weight of all the species tested were significantly affected by the increasing concentration of *Parthenium*. However, *Lepidium* was more sensitive to the inhibitory property of *Parthenium*. Interestingly, the seedling weight (biomass) of *A. fatua* and *Lepidium* sp., were more affected by concentrations as compared to wheat. Similarly at lower concentrations, the biomass of wheat was increased (Fig. 3.) From the present findings it can be concluded that there are compounds in the tissues of *Parthenium* which may cause phytotoxic effects on agricultural crop seeds. Thus *Parthenium* plant should be prevented to establish and spread to the un-infested areas. A slight promotion in root length at low parthenin concentrations was previously found for *Triticum aestivum* L., (Batish *et al.*, 1997). Dry powder of an epiphytic plant *Cassytha* sp., at 1–2% w/v completely killed the leaves and drastically reduced the biomass of *Eichhornia crassipes* within 15 days (Kauraw & Bhan, 1994). Biomass reduction of plants by allelochemicals were noted (Kathiresan, 2000) and it has been concluded that using allelopathic plants and their products, offers promising scope for the biological control of water hyacinth, the biomass of which is drastically reduced by treatment with dry powder of *Coleus amboinicus*. As biomass reduction by allelochemicals is an important indication for controlling weeds therefore the focus should be as how to use the allelochemicals against the weeds selectively in the presence of the crops.

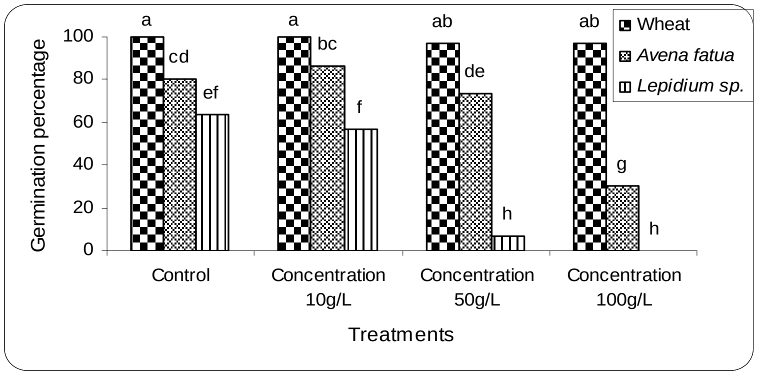

Fig. 1. Phytotoxic effect of *Parthenium* extracts on germination of different species. 9 Different letters over the bar are significantly different from each other at p≤0.05 according to LSD test.

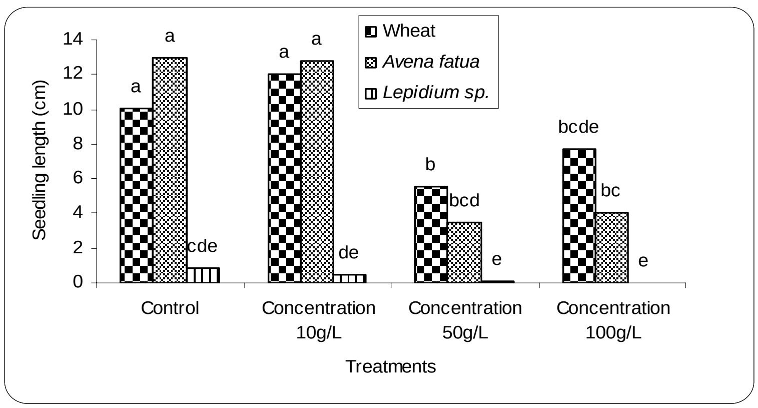

Fig. 2. Phytotoxic effect of *Parthenium* extracts on seedling length of different species. 9 Different letters over the bar are significantly different from each other at p≤0.05 according to LSD test.

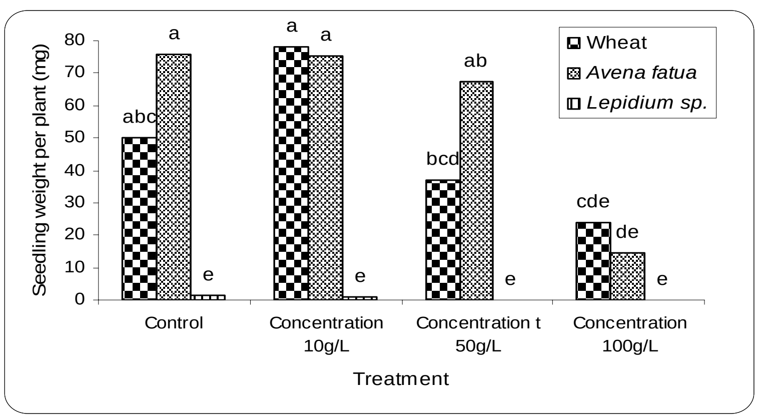

Fig. 3. Phytotoxic effect of *Parthenium* extracts on seedling weight of different species.
Different letters over the bar are significantly different from each other at p≤0.05 according to LSD test.

Field experiment

Weed density (m^{-2}) 25 days after sowing (DAS): Statistical analysis (Fig. 4) depicted that although there was non-significant affect on the weed density, yet the data indicated that *Parthenium* extracts application was more effective in affecting the numerical values of weed germination at post emergence as compared to control. It can be inferred from the data that with the increasing concentrations of *Parthenium* the weed density was decreased which shows the alellopathic property of *Parthenium*. The major weed species infesting the experimental field were; *Cynodon dactylon, Cyperus rotundus, Digiteria sanguinalis, Portulaca oleracea, Echinochloa curus-galli, Ehphorbia prostrata,* and *Xanthium strumariam.* The present findings show that *Parthenium* extract application at any stage (pre-emergence or post-emergence) slightly affected the seed germination of the above mentioned weeds at the same or higher concentrations. *Parthenium* extracts may prove effective in affecting the germination of other weeds as already reported by many researchers. It was noted that species differed considerably in their sensitivity to leaf extracts, whereby *A. conyzoides* was most sensitive (Regina *et al.*, 2007). They further stated that apart from its competitive ability, the invasiveness of the noxious weed (*Parthenium hysterophorus* L.) is thought to be due to an ability to displace other species by means of allelopathy and the sesquiterpene lactone parthenin that is biosynthesized by this species is thought to play a role in its allelopathic interference with surrounding plants. Thus, the present findings revealed that under laboratory conditions, there are convincing evidences of allelopathy but under field conditions there are weaker evidences to conclude, which may be due to irrigation and soil texture etc. Plant interference is very complicated in nature, and includes both allelopathy and competition and thus separation of allelopathy from competition is a real challenge to both biologist and ecologist (Qasem & Hill, 1989). All factors that favour enhanced extraction of parthenin from plant materials will enhance the importance of this phytotoxin with respect to allelopathic potential in *P. hysterophorus* (Regina *et al.,* 2007).

Weed density (m^{-2}) 50 days after sowing (DAS): Fig. 5 revealed that weed density (m^{-2}) 50 DAS was significantly ($p \leq 0.05$) affected by different concentrations. The data indicated that pre-emergence application of *Parthenium* extracts was more effective in inhibiting the weed germination as compared to post-emergence. The present results confirm the inhibition of weed seed germination by the extracts of *Parthenium*, though less affective than 25 DAS. Seed germination of sorghum was effected by *Parthenium* extracts (Ayala *et al.,* 1994). Time of application and different concentrations showed that time of application responded differently to the different concentrations in term of weed germination. After 50 days of extract application, there was a significant effect of different concentrations on the weed density. Fig. 5 shows that in pre-emergence application, maximum weed density was recorded in the control plots and there was decreasing trend in weed density with the increasing concentration of the *Parthenium*. In a similar way, in post-emergence application, maximum weed density was recorded in control. However the weed density in control was statistically at par with the concentration applied @50 and 100 g L^{-1}. The instant results indicate that there was a phototoxic effect of *Parthenium* on the weed density. However this phytotoxic effect appeared in the later stage after 50 DAS. Species varied considerably in their sensitivity to aqueous extracts of *P. hysterophorus* for both root growth and germination (Regina *et al.,* 2007). Species-specific differences in the sensitivity to aqueous extracts of fresh or dry leaf material of *P. hysterophorus* were reported in previous studies (Kohli *et al.,* 1996; Mersie & Singh, 1987). These results suggest that pre-emergence application proved more effective as compared to post-emergence.

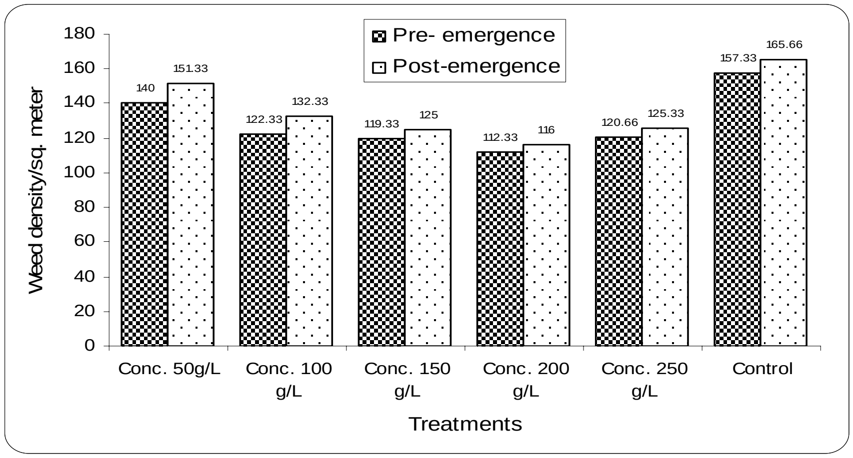

Fig. 4. Phytotoxic effect of *Parthenium* extract concentration at different time of application on weed density 25 DAS.

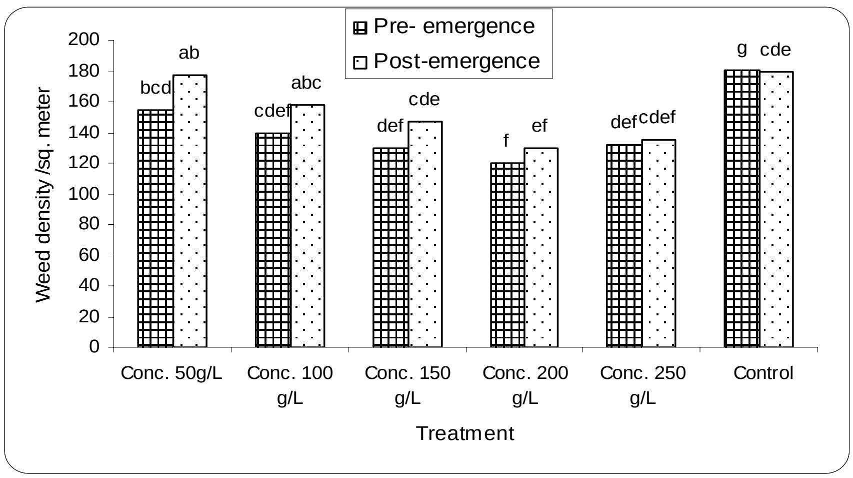

Fig. 5. Phytotoxic effect of *Parthenium* extract concentration at different time of application on weed density 50 DAS. Different letters over the bar are significantly different from each other at [$\leq$0.05 according to LSD test.

Fresh biomass (g m^{-2}) of weeds 50 days after sowing (DAS): The perusal of the data indicates that fresh biomass of weed 50 DAS was decreased by increasing concentration of *Parthenium* extracts (Fig. 6). However, statistically the values were at par with each other. *Parthenium* leaf residues inhibit *Salvania molesta* biomass (Pandey, 1994). Pre-emergence as well as post-emergence applications of parthenium extracts at higher concentrations proved more effective in decreasing the fresh biomass of weeds. Several researchers (Tefera, 2002; Stephen & Sowerby, 1996) have documented the importance of *Parthenium* as a potential source of herbicide. Delayed germination was also reported from investigations with infested soils (Kohli & Batish, 1994) and such germinationimpacting effects on receiver species can provide an important competitive advantage for the allelopathic donor and could be as important as allelopathic growth inhibition itself. Keeping in view the decreasing trend of weeds germination, density and biomass with the increasing concentration of *Parthenium*, there are strong evidences of parthenium extracts to be used as potential herbicide. However, many factors have yet to be studied.

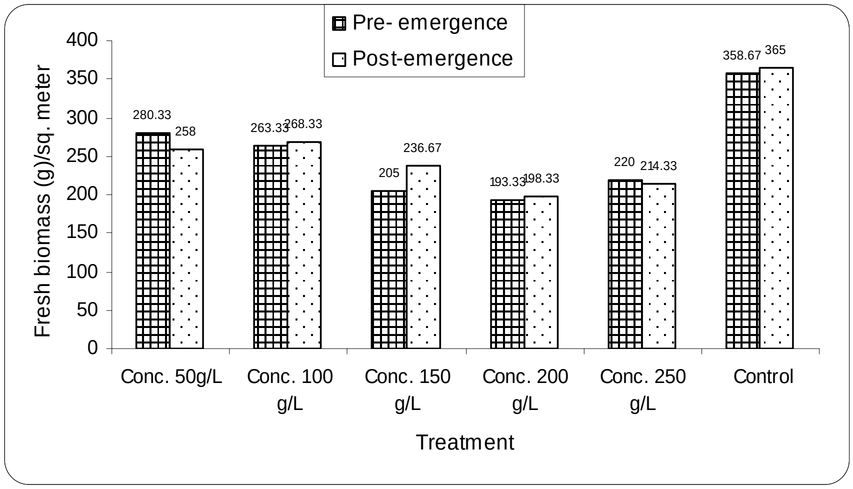

Fig. 6. Phytotoxic effect of *Parthenium* extract concentration at different time of application on fresh biomass of weeds 50 DAS.

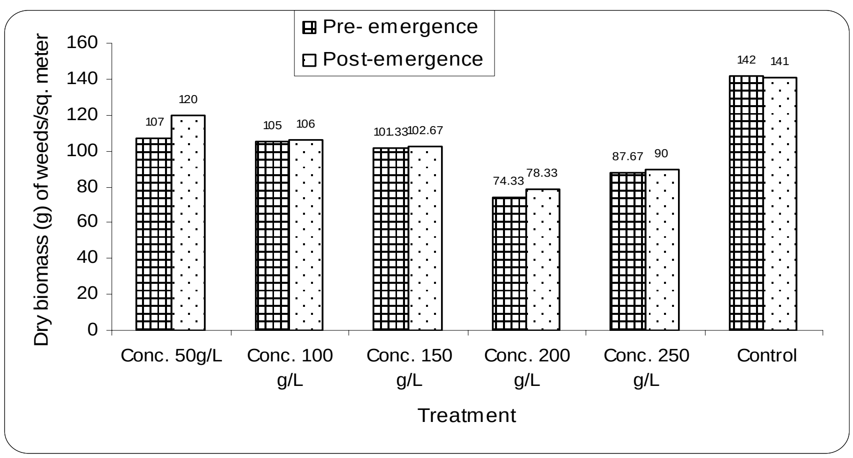

Fig. 7. Phytotoxic effect of *Parthenium* extract concentration at different time of application on dry biomass of weeds 50 DAS

Dry biomass (g m^{-2}) of weeds 50 days after sowing (DAS): Fig. 7 indicated that different concentrations had non-significant effect on dry biomass of weeds 50 DAS. Overall data indicated that higher concentrations were more effective in decreasing the dry biomass of weeds as compared to lower concentrations. Release of parthenin and some other chemicals during decomposition of leaf material has a potential to play a leading role for allelopathy in *P. hysterophorus*; however, its significance in a natural setting will very much rely on the amount of leaf material accumulated on soil surfaces and the concentration of parthenin in residues (Regina *et al.*, 2007). This reduction may be due to the fact that vegetative growth of the weeds was inhibited by the *Parthenium* extracts and ultimately the dry biomass was decreased. It was observed that with the increasing concentration of *Parthenium*, the dry biomass of weed decreased whether applied pre-emergence or post-emergence. Extracts prepared from *Parthenium* residues were found to be phytotoxic to both the test crops and were also rich in phenolics (Daizy *et al.*, 2002). The presence of phenolics in *Parthenium* residues and their interference with soil chemistry upon release may be responsible for a decrease in the growth of radish and chickpea. Unlike the laboratory results, field results seem to be unrealistic and thus many factors should be studied. The accuracy obtained using different techniques were evaluated which show that some of the techniques could be a misleading tool (Qasem & Hill, 1989). Comparing the values in control, with the treated plots, there are convincing evidences of the presence of allelochemicals in *Parthenium* extracts that can selectively affect weeds. However, more meaningful results could be obtained if plant allelopathy was studied under various conditions.

Conclusions

In these studies the pre-emergence application of *Parthenium* extracts was more effective compared to post-emergence application. Therefore in future studies this aspect should be exploited accordingly. Since *Parthenium* has become a major invasive in different parts of the country therefore all efforts should be made to restrict its further spread and eliminate it in a planned way by declaring it as noxious weed under the Seed Act.

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