INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–1021/2019/21–1–215–222
DOI: 10.17957/IJAB/15.0884
http://www.fspublishers.org

*Full Length Article*

# Management Tactics for Handling *Parthenium hysterophorus* in Non-Native Environment through Phytotoxic Compounds of Local Species

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## Abstract

*Parthenium hysterophorus* L. is an invasive, ubiquitous and infamous herbaceous weed causing suppression of natural vegetation and crop plants. The identification of phytotoxins in local weed species (*Datura stramonium*, *Achyranthes aspera*, *Chenopodium album*, *Calotropis procera*, *C. murale* and *Melilotus indica*) was done through high performance liquid chromatography (HPLC) to check their herbicidal potential against *P. hysterophorus*. Additionally, filter paper petri plate based and soil filled pot experiments were conducted in laboratory and wire house to evaluate the pre- and post-emergence herbicidal potential of plant water extract of *D. stramonium* alone and in combination with *A. aspera*, *C. album*, *C. procera*, *C. murale* and *M. indica* at 2.5, 5 and 10% (w/v) concentrations against germination and seedling growth of *P. hysterophorus*. The phytotoxins detected in extracts of these plant species were quercetin, gallic, chlorogenic, *p*-coumaric, *m*-coumaric, sinapinic, caffeic, benzoic and syringic acids with variable concentrations. Total highest concentration of phytotoxins (61.37 mg $L^{-1}$) was found in *A. aspera* while the lowest concentration (7.69 mg $L^{-1}$) was found in *C. album* aqueous extract. Significant reduction in germination and seedling growth of *P. hysterophorus* was shown by all extract combinations that increased in direct proportion to their concentrations. The 10% water extract of *D. stramonium* in combination with *C. procera* and *A. aspera* proved to be the best as they resulted maximum reductions in germination percentage (100 and 95%), shoot length (67 and 62%), and shoot dry weight (67 and 78%) of *P. hysterophorus*, respectively. © 2019 Friends Science Publishers

**Keywords:** Allelopathy; Weed-plant water extracts; Allelopathic mixtures; Bio-herbicide; Phenolics

## Introduction

*Parthenium hysterophorus* is an aggressive, ubiquitous, annual and herbaceous weed which belongs to family Asteraceae (Navie *et al.*, 1996). This weed is native to the American tropics but now it is spreading in Asia, Africa and Australia. Its common names include parthenium weed, false ragweed, carrot weed, bitter weed, Santa Maria fever few, Congress weed or grass and white top (Warshaw and Zug, 1996; Evans, 1997). This weed has spread out to about 30 countries including Pakistan where it has become harmful to agro-ecosystems (Riaz and Javaid, 2010; EPPO, 2014). Its allelopathic ability is the main reason of its high invasiveness that is caused by the presence of sesquiterpene lectones including parthenin, hysterin, hymenin, amobrosin and coronopilin. All these compounds facilitate it to compete well with pastures and different crops (Gunaseelan, 1998; Belz *et al.*, 2007). Additionally, there are other phytotoxic phenolics as well that are segregated by different parts of this weed; these phenolic compounds are caffeic, vanillic, ferulic, chlorogenic and anisic acids. These chemicals at different concentrations are known to suppress or stimulate the growth of other plants (Batish *et al.*, 2007; Reinhardt *et al.*, 2009). *P. hysterophorus* affects nodulation in leguminous crops by inhibiting the action of nitrogen fixing and nitrifying bacteria like *Rhizobium*, *Actinomycetes* and *Azotobacter* (Kanchan and Jayachandra, 1981). It is well reported that this weed can survive on different places like water courses, road sides, railway tracks and agricultural fields. It also has ability to adapt and persist in new environment by the reduction in the growth of indigenous species (Akter and Zuberi, 2009; Patel, 2011). Tamado *et al.* (2002) explained that 40-97% grain yield losses in sorghum (*Sorghum bicolor* L.) are caused by *P. hysterophorus* by left it uncontrolled during the season in Ethiopia. It was estimated to reduce the carrying capacity of affected farms on cracking clay soil with an annual rainfall of 600 and 800 mm (McConnachie *et al.,* 2011). In an agro ecosystem, weed management is one of the most important tasks (Yadollahi *et al.,* 2014).

Various methods including mechanical, chemical, biological and integrated weed management strategies are generally practiced managing weeds all over the world (Shahzad *et al.*, 2016; Farooq *et al*., 2017). Mechanical control is getting expensive but chemical control is the most effective method (Armstrong *et al.*, 1968). Continuous usage of synthetic herbicides has increased herbicide resistance which attracted the devotion of researchers to struggle for planning alternative weed management approaches (Bhowmik, 2003). Allelopathy is a process of using chemicals that are excreted from plant material dead or alive even decomposing litter, for the inhibition of related plants that are growing (Rice, 1984). This investigation about allelochemicals concentrates on separation, identification and specification of working allelopathic compounds. When the compounds are found and specified, they are ready to be utilized as a bio-herbicide (Anjum *et al.*, 2005).

Allelopathic weeds including *D. stramonium* (Butnariu, 2012), *A. aspera* (Dogra and Sood, 2012), *C. album* (Majeed *et al.,* 2012), *C. procera* (Al-Zahrani and Al-Robai, 2007; Ghasemi *et al.*, 2012), *C. murale* (Ghareib *et al.,* 2010) and *M. indica* (Blackshaw *et al.,* 2001) suppress the other plants. Using allelopathic plant extracts, weed management program against certain noxious weeds could be made more efficient and sustained through their tank mixed application with slightly lower herbicide doses (Cheema *et al.*, 2005).

Therefore, this study was planned to investigate the effects of water extracts derived from already well-known allelopathic plant species *D. stramonium*, *A. aspera*, *C. album*, *C. procera*, *C. murale* and *M. indica* in different combinations and concentrations on emergence and seedling growth of *P. hysterophorus* under laboratory and wire-house conditions.

## Materials and Methods

The experiments were conducted in the Agronomic laboratory and in wire house of College of Agriculture, University of Sargodha, Sargodha (32.07ºN, 72.68ºE), Punjab, Pakistan during 2016-2017. The seeds of *P. hysterophorus* were collected from different cropped and non-cropped fields around the district Sargodha one year before experiments. The seeds were properly saved in plastic jar for their protection from insects and diseases.

### Collection of Plant Materials

The actively growing plants of *D. stramonium*, *A. aspera*, *C. album*, *C. procera*, *C. murale* and *M. indica* were collected from the research area of the Department of Agronomy, College of Agriculture, University of Sargodha in October, 2016. The plants were dried under shade at room temperature (29 ± 4ºC) and were used in the preparation of water extracts according to procedure detailed blow.

### Plant Extracts Preparation

Whole plants of each weed were dried under shade and chaffed down into the pieces of 2-3 cm with the help of scissor and were separately soaked in distilled water for 24 h at room temperature with 1:10 (w/v) ratio. The 10% (w/v) extracts of each weed was then obtained by filtering through mesh sieves. A fine filtration was carried out further with the help of Soxhlet's extraction assembly. To prepare the concentrations of 2.5, 5 and 10%, plant water extracts were diluted by using parallel dilution techniques ($C_1V_1=C_2V_2$). Different plant water extract combinations with different concentrations were prepared with mixing them in equal proportions.

### Determination of Phytotoxins in Aqueous Extracts of Weeds

For identification and quantification of their suspected phytotoxins, aqueous extracts were chemically analysed using a Shimadzu HPLC system equipped with a UV detector (Model SCL-10A, Tokyo, Japan). Standards of suspected phytotoxins (Aldrich, St. Louis, USA) were run similarly for their identification and quantification. The concentration of each isolated compound (Table 1) was determined using the following equation:

$$\text{Concentration (ppm)} = \frac{\text{Area of the sample}}{\text{Area of the standard}} \times \text{Concentration of the standard} \times \text{Dilution factor}$$

Allelochemicals' concentrations of aqueous extracts of weeds used in study are presented in Table 1.

### Petri Dish based Pre-emergence Phytotoxic Bioassay Studies

Ten seeds of *P. hysterophorus* were sown in petri dishes with 9 cm diameter lined with double layer of filter paper (Whatman No. 42). The 4 mL of each plant extract combination was applied to each petri dish as per treatment plan. For comparison, same volume of distilled water was applied as control treatment. Experiment was laid out in completely randomized design and each treatment was replicated four times. Throughout the incubation period, seeds were avoided to dry by sealing petri dishes with parafilm. The petri dishes were placed on laboratory shelf. Minimum temperature and maximum temperature throughout the course of experiment was 25-28 and 30-32$^{o}$C, respectively. The germination count data were taken on daily basis throughout experiment period. The same experiment was repeated after the completion of first one.

### Pot based Post-emergence Phytotoxic Bioassay Studies

Plastic pots of 9 cm height and diameter were filled with soil at 350 g per pot collected from field of agronomic research area. Before putting into pots, soil was dried, crushed and thoroughly mixed. The soil was saturated with distilled water. The *P. hysterophorus* seedlings at 3-4 leaf stage were uprooted from field and were transplanted in pots. At proper establishment of plants after 15 days of transplanting, plant extract combinations at 4 mL per pot were foliarly sprayed on the *P. hysterophorus* seedlings. Same amount of distilled water was sprayed instead of plant extract on seedlings of control treatment for comparison. Completely randomized design with four replications was followed in this experiment. Throughout the experiment, water in equal quantity was applied to all pots to avoid the drying of seedlings. Minimum temperature and maximum temperature throughout the course of experiment was 22.5 and 34°C, respectively. Seedlings of *P. hysterophorus* were uprooted and washed with distilled water after the duration of 21 days. Fresh and dry weights of seedlings, root and shoot lengths were measured. The same experiment was repeated after the completion of first one.

### Observations and Data Recording

For laboratory and wire-house experiments, number of germinated/emerged seeds was recorded on daily basis. Emergence/germination percentage was calculated by taking ratio of emerged/germinated and total seeds in percent. By measuring tape, the root and shoot lengths of *P. hysterophorus* were measured in centimeter (cm). With the help of electric balance, fresh and dry weights of *P. hysterophorus* were measured in grams before and after drying in oven at 70ºC until constant weight.

### Statistical Analysis

All the data recorded were subjected to Fisher's analysis of variance technique (Steel *et al.*, 1997) and means were separated by using least significant difference was used at 0.05 probability with the help of "MSTATC" statistical package on the computer (Anonymous, 1986).

## Results

### Allelochemicals in Aqueous Extracts of Weeds

About 11 different allelopathic compounds including one flavonoid (quercetin), and ten phenolics *viz*., gallic, chlorogenic, *p*-coumaric, *m*-coumaric, sinapinic, ferulic, vanillic, caffeic, benzoic and syringic acids were detected in these extracts with variable concentrations (Table 1). Total highest concentration of allelochemicals (61.37 mg $L^{-1}$) was found in *A. aspera* that was followed by *M. indica* (55.19 mg $L^{-1}$), *C. murale* (41.05 mg $L^{-1}$) and *D. stramonium* (28.96 mg $L^{-1}$) extracts. However, the lowest concentration (7.69 mg $L^{-1}$) of allelopathic compounds was found in *C. album* aqueous extract (Table 1).

### Petri Dish based Pre-emergence Phytotoxic Bioassay Studies

Germination of *P. hysterophorus* was significantly inhibited to variable degree by all the plant extracts used as compared to control in both petri dish trials (Table 2). In trial-I, combined application of *D. stramonium* + *C. procera* extracts at their 10% concentration whereas in trial-II, all extract combinations at their 10% concentrations completely inhibited the germination of *P. hysterophorus*. These treatments are followed by *D. stramonium* extract at its 10% concentration and *D. stramonium* + *C. procera* extract combination at their 5% concentrations regarding the lowest germination percentages of 10 and 15% in both trials-I and trial-II.

All the plant extract used at their low (2.5%), medium (5%) and high (10%) concentrations significantly reduced the shoot length of *P. hysterophorus* (Table 2). In trial-I, significantly the lowest shoot lengths of germinated seedlings of *P. hysterophorus* were noted with the application of 10% plant extracts combination of *D. stramonium* + *C. procera* (0.03 cm) and *D. stramonium* + *A. aspera* (0.03 cm) that were followed by *D. stramonium* + *M. indica* extract combination at their 5% concentration by showing 0.13 cm shoot length. However, in trail-II, *D. stramonium* + *M. indica* extract combination at their 5% concentration result in significantly the lowest shoot length (0.05 cm) of *P. hysterophorus* which was followed by *D. stramonium* 10% extract and *D. stramonium* + *C. procera* extract combination of 5% concentration as these produced shoot lengths of 0.14 and 0.18 cm, respectively.

In both trials, the root length of *P. hysterophorus* was significantly inhibited by the interaction of plant water extract combinations and extract concentrations (Table 3). It was observed in trial-I that root length of germinated seedlings of *P. hysterophorus* was highly suppressed in response to *D. stramonium* + *A. aspera* and *D. stramonium* + *C. album* extract combinations at 10% concentration as these combinations produced the lowest (0.12 and 0.123 cm, respectively) root lengths of *P. hysterophorus* seedlings. These treatments were followed by *D. stramonium* + *M. indica* and *D. stramonium* + *C. murale* water extracts at 10% concentrations regarding root length (0.26 and 0.30 cm, respectively) suppression. In trial-II, significantly the lowest root length of *P. hysterophorus* was noted with *D. stramonium* + *M. indica* (0.23 cm) and *D. stramonium* + *C. procera* (0.26 cm) extracts of 10 and 5% concentrations, respectively. However, these treatments were followed by combinations of *D. stramonium*, *D. stramonium* + *A. aspera* and *D. stramonium* + *C. procera* at their 10%, 5% and 2.5% concentrations, respectively.

**Table 1:** Allelopathic contents of aqueous extracts of different weeds as determined by HPLC analysis

| Sr. No. | Allelo-chemicals | Concentration (mg $L^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *C. album* | *A. aspera* | *C. murale* | *D. stramonium* | *M. indica* | *C. procera* |
| 1 | Quercetin (flavonoid) | 0.32 | - | 0.69 | 0.66 | - | 0.64 |
| 2 | Gallic acid | - | 16.85 | - | - | - | 3.20 |
| 3 | Chlorogenic acid | 4.52 | - | - | 9.44 | 12.44 | 8.12 |
| 4 | *p*-coumaric acid | - | - | - | - | - | 1.78 |
| 5 | Sinapinic acid | - | - | 6.42 | 2.03 | - | 5.54 |
| 6 | *m*-coumaric acid | 0.93 | 3.13 | 3.81 | - | 6.63 | - |
| 7 | Ferulic acid | - | - | 11.61 | - | 33.14 | - |
| 8 | Vanillic acid | - | - | 6.68 | - | 2.98 | - |
| 9 | Caffeic acid | - | 7.41 | - | 6.67 | - | - |
| 10 | Benzoic acid | - | 24.77 | - | 10.16 | - | - |
| 11 | Syringic acid | 1.92 | 9.21 | 11.83 | - | - | - |
| Total | | 7.69 | 61.37 | 41.05 | 28.96 | 55.19 | 19.28 |

**Table 2:** Allelopathic effects of different combined plant water extracts on germination percentage and shoot length of *P. hysterophorus* (Petri dish trials)

| Extracts | Trial-I | | | Trial-II | | |
|---|---|---|---|---|---|---|
| | Concentrations | | | Concentrations | | |
| | 2.5% | 5% | 10% | 2.5% | 5% | 10% |
| Germination percentage | | | | | | |
| Control (Distilled water) | 95.00 a | 92.50 a | 90.00 a | 92.50 a | 90.00 a | 92.50 a |
| *Datura stramonium* | 55.00 b | 40.00 c | 15.00 f | 50.00 b | 40.00 cd | 10.00 ij |
| *D. stramonium + Achyranthes aspera* | 35.00 cd | 25.00 e | 10.00 f | 30.00 d-g | 25.00 fg | 0.00 k |
| *D. stramonium + Chenopodium album* | 37.50 cd | 25.00 e | 07.50 f | 35.00 d-f | 25.00 fg | 0.00 k |
| *D. stramonium + Calotropis precera* | 30.0 de | 15.00 f | 0.000 g | 20.00 gh | 15.00 hi | 0.00 k |
| *D. stramonium + C.henopodium murale* | 42.50 c | 30.00 de | 10.00 f | 35.00 d-f | 27.50 e-g | 0.00 k |
| *D. stramonium + Melilotus indica* | 37.50 cd | 30.00 de | 10.00 f | 47.50 bc | 37.50 de | 5.00 jk |
| LSD value at 5% | 0.954 | | | 11.828 | | |
| Shoot length (cm) | | | | | | |
| Control (Distilled water) | 1.85 a | 1.55 c | 1.67 b | 1.76 a | 1.60 b | 1.80 a |
| *D. stramonium* | 1.04 d | 0.50 h | 0.24 k | 0.89 c | 0.54 e | 0.14 k |
| *D. stramonium + A. aspera* | 0.77 g | 0.38 j | 0.06 no | 0.33 gh | 0.73 d | 0.00 l |
| *D. stramonium + C. album* | 0.96 e | 0.42 ij | 0.03 o | 0.79 d | 0.40 fg | 0.00 l |
| *D. stramonium + C. precera* | 0.72 g | 0.20 kl | 0.00 o | 0.34 gh | 0.18 jk | 0.00 l |
| *D. stramonium + C. murale* | 0.86 f | 0.48 hi | 0.14 lm | 0.46 f | 0.29 hi | 0.00 l |
| *D. stramonium + M. indica* | 0.91 ef | 0.43 hij | 0.13 mn | 0.46 f | 0.05 l | 0.24 ij |
| LSD value at 5% | 0.081 | | | 0.096 | | |

Means not sharing a letter in common, within a row or column, differ significantly at $p \leq 0.05$

Data revealed that all the extracts used imposed significant inhibitory effect on seedling vigor index (SVI), a parameter showing cumulative response of germination and seedling length, of *P. hysterophorus* compared to distilled water control (Table 3). In trial-I, all extracts with their 10% and 5% concentrations resulted in significantly the lowest SVI of *P. hysterophorus*. However, in trial-II among germinated treatments, significantly the lowest values of SVI were recorded with *D. stramonium + M. indica* (2.34) and *D. stramonium* (4.88) extracts with 10% concentration, and *D. stramonium + C. precera* (6.55) and *D. stramonium + A. aspera* (8.75) extracts with 5% concentrations.

**Pot based Post-emergence Phytotoxic Bioassay Studies**

All the plant extracts used in the study caused significant reduction in shoot length of *P. hysterophorus* compared to distilled water in both the soil filled pot trials (Table 4). Data of individual trial means (Table 4) indicated that in both trials, maximum inhibitory effect on shoot length of *P. hysterophorus* was shown by *D. stramonium + C. precera* and *D. stramonium + A. aspera* extract combinations at 10% concentrations results in the lowest shoot lengths (7.00 and 5.59, and 6.79 and 5.81 cm, respectively) of *P. hysterophorus.* Significant reduction from distilled water control in root length of *P. hysterophorus* occurred with application of all plant extracts (Table 4). In both trials, significantly the lowest root lengths of *P. hysterophorus* were recorded with the application of 10% extracts of *D. stramonium + A. aspera* (4.22 and 3.49 cm, respectively), *D. stramonium + C. album* (4.39 and 3.32 cm, respectively) and *D. stramonium + C. precera* (4.20 and 3.30 cm, respectively).

In both trials, the combination of all plant water extracts caused significant reduction in shoot fresh weight of *P. hysterophorus* (Table 5). In trial-I, the shoot fresh weight of *P. hysterophorus* suffered from significantly the highest reduction in pots watered with the water extract combinations of *D. stramonium + M. indica* (0.858 g), *D. stramonium + A. aspera* (0.863 g) and *D. stramonium + C. procera* (0.893 g) applied at higher concentration (10%).

**Table 3:** Allelopathic effects of different combined plant water extracts on root length and seedling vigor index of *P. hysterophorus* (Petri dish trials)

| Extracts | Trial-I | | | Trial-II | | |
|---|---|---|---|---|---|---|
| | Concentrations | | | Concentrations | | |
| | 2.5% | 5% | 10% | 2.5% | 5% | 10% |
| Root length (cm) | | | | | | |
| Control (Distilled water) | 3.27 a | 2.92 b | 3.00 b | 2.10 a | 1.87 c | 1.92 b |
| *Datura stramonium* | 1.83 c | 1.09 f | 0.43 i | 0.95 d | 0.61 f | 0.35 j |
| *D. stramonium* + *Achyranthes aspera* | 1.30 e | 0.71 h | 0.12 k | 0.46 h | 0.35 j | 0.00 l |
| *D. stramonium* + *Chenopodium album* | 1.52 d | 0.80 gh | 0.13 k | 0.87 e | 0.41 hi | 0.00 l |
| *D. stramonium* + *Calotropis precera* | 1.17 f | 0.40 i | 0.00 l | 0.38 ij | 0.26 k | 0.00 l |
| *D. stramonium* + *C.henopodium murale* | 1.50 d | 0.86 g | 0.30 j | 0.64 f | 0.36 j | 0.00 l |
| *D. stramonium* + *Melilotus indica* | 1.438 d | 0.817 g | 0.2675 j | 0.90 e | 0.55 g | 0.23 k |
| LSD value at 5% | 1.169 | | | 0.059 | | |
| Seedling vigor index | | | | | | |
| Control (Distilled water) | 486.35 a | 413.47 b | 420.29 b | 357.05 a | 312.29 c | 344.10 b |
| *D. stramonium* | 157.92 c | 72.42 e | 10.05 i | 92.01 d | 46.05 f | 4.88 ij |
| *D. stramonium* + *A. aspera* | 63.58 ef | 27.26 h | 1.79 i | 23.68 g | 8.75 hij | 0.00 j |
| *D. stramonium* + *C. album* | 92.97 d | 30.50 gh | 1.20 i | 58.09 e | 20.25 g | 0.00 j |
| *D. stramonium* + *C. precera* | 56.71 f | 9.03 i | 0.00 i | 14.38 ghi | 6.55 ij | 0.00 j |
| *D. stramonium* + *C. murale* | 100.30 d | 40.20 g | 4.37 i | 38.35 f | 17.87 gh | 0.00 j |
| *D. stramonium* + *M. indica* | 88.05 d | 37.38 gh | 4.00 i | 64.61 e | 22.53 g | 2.34 j |
| LSD value at 5% | 10.024 | | | 12.440 | | |

Means not sharing a letter in common, within a row or column, differ significantly at $p \leq 0.05$

**Table 4:** Allelopathic effects of different combined plant water extracts on shoot and root lengths of *P. hysterophorus* (Pot trials)

| Extracts | Trial-I | | | Trial-II | | |
|---|---|---|---|---|---|---|
| | Concentrations | | | Concentrations | | |
| | 2.5% | 5% | 10% | 2.5% | 5% | 10% |
| Shoot length (cm) | | | | | | |
| Control (Distilled water) | 18.01 a | 18.26 a | 17.33 b | 16.82 a | 17.06 a | 16.13 b |
| *D. stramonium* | 14.24 c | 11.66 f | 10.27 gh | 12.03 c | 10.46 f | 9.075 ij |
| *D. stramonium* + *A. aspera* | 12.27 d-f | 10.65 g | 7.008 j | 11.03 e | 9.45 gh | 5.81 m |
| *D. stramonium* + *C. album* | 12.3 de | 10.37 gh | 8.642 i | 11.10 e | 9.17 hi | 7.44 l |
| *D. stramonium* + *C. precera* | 11.77 ef | 10.00 h | 6.793 j | 10.57 f | 8.80 j | 5.59 m |
| *D. stramonium* + *C. murale* | 12.65 d | 10.81 g | 9.142 i | 11.11 de | 9.58 g | 7.83 k |
| *D. stramonium* + *M. indica* | 12.24 d-f | 10.77 g | 9.033 i | 11.45 d | 9.61 g | 7.94 k |
| LSD value at 5% | 0.751 | | | 0.744 | | |
| Root length (cm) | | | | | | |
| Control (Distilled water) | 8.33 ab | 8.550a | 8.08 b | 7.43 ab | 7.65 a | 7.18 b |
| *D. stramonium* | 6.62 c | 5.78 de | 5.02 f-h | 5.69 c | 4.88 d | 4.12 ef |
| *D. stramonium* + *A. aspera* | 5.85 de | 5.24 fg | 4.22 i | 4.95 d | 4.24 ef | 3.49 g |
| *D. stramonium* + *C. album* | 6.11 d | 5.20 f-h | 4.39 i | 4.85 d | 4.30 ef | 3.32 g |
| *D. stramonium* + *C. precera* | 5.75 e | 5.09 f-h | 4.20 i | 4.85 d | 4.19 ef | 3.30 g |
| *D. stramonium* + *C. murale* | 6.10 d | 5.34 f | 4.92 gh | 5.11 d | 4.40 e | 3.97 f |
| *D. stramonium* + *M. indica* | 6.01 de | 5.30 f | 4.87 h | 5.20 d | 4.40 e | 3.965 f |
| LSD value at 5% | 1.258 | | | 0.455 | | |

Means not sharing a letter in common, within a row or column, differ significantly at $p \leq 0.05$

In trial-II, the water extract combination of *D. stramonium* + *C. murale* at higher concentrations (10%) resulted in significantly the lowest shoot fresh weight (0.333 g) and it was followed by the water extracts of *D. stramonium* + *A. aspera* at their 10% concentration.

In both pot trials, significant reductions compared to control in shoot dry weight of *P. hysterophorus* were observed with all extracts applied (Table 5). In trial-I, all the extracts at their 10% concentration resulted in significantly the lowest shoot dry weight of *P. hysterophorus*. However, in trial-II, *D. stramonium* + *A. aspera* and *D. stramonium* + *C. precera* extracts at their 10% concentrations produced significantly the lowest shoot dry weights (0.070 and 0.043 g, respectively). In both trials, there was non-significant effect of plant extracts on root fresh and dry weights of *P. hysterophorus* (data not shown).

## Discussion

All the plant extracts and their combinations used in the study showed phyto-inhibitory effect to variable degree against germination and seedling growth of *P. hysterophorus* weed in petri plate studies. Moreover, by increasing the concentration of extracts from 2.5 to 10%, their allelopathicity was also enhanced.

**Table 5:** Allelopathic effects of different combined plant water extracts on shoot fresh and dry weights of *P. hysterophorus* (Pot trials)

| Extracts | Trial-I | | | Trial-II | | |
|---|---|---|---|---|---|---|
| | Concentrations | | | Concentrations | | |
| | 2.5% | 5% | 10% | 2.5% | 5% | 10% |
| Shoot fresh weight (g) | | | | | | |
| Control (Distilled water) | 2.384 b | 2.598 a | 2.418 b | 1.928 a | 1.935 a | 1.951 a |
| *D. stramonium* | 1.931 c | 1.580 ef | 1.136 hi | 1.362 b | 0.907 d | 0.666 hi |
| *D. stramonium* + *A. aspera* | 1.602 f | 1.283 g | 0.863 k | 1.075 c | 0.758 fg | 0.396 j |
| *D. stramonium* + *C. album* | 1.706 d | 1.287 g | 1.065 j | 1.128 c | 0.810 ef | 0.599 i |
| *D. stramonium* + *C. precera* | 1.618 e | 1.189 h | 0.893 k | 1.123 c | 0.722 gh | 0.613 i |
| *D. stramonium* + *C. murale* | 1.590 ef | 1.308 g | 1.101 ij | 1.076 c | 0.838 e | 0.333 k |
| *D. stramonium* + *M. indica* | 1.543 f | 1.309 g | 0.858 k | 1.144 c | 0.841 e | 0.635 i |
| LSD value at 5% | 0.081 | | | 0.085 | | |
| Shoot dry weight (g) | | | | | | |
| Control (Distilled water) | 0.409 a | 0.429 a | 0.431 a | 0.402 a | 0.403 a | 0.400 a |
| *D. stramonium* | 0.270 b | 0.213 c-e | 0.147 fg | 0.238 b | 0.175 c-f | 0.131 fg |
| *D. stramonium* + *A. aspera* | 0.206 c-e | 0.153 fg | 0.108 g | 0.189 c-e | 0.134 e-g | 0.070 hi |
| *D. stramonium* + *C. album* | 0.230 bc | 0.164 ef | 0.133 fg | 0.190 cd | 0.129 fg | 0.101 gh |
| *D. stramonium* + *C. precera* | 0.214 cd | 0.143 fg | 0.106 g | 0.169 c-f | 0.106 gh | 0.043 i |
| *D. stramonium* + *C. murale* | 0.235 bc | 0.167 d-f | 0.132 fg | 0.193 c | 0.142 d-g | 0.104 gh |
| *D. stramonium* + *M. indica* | 0.235 bc | 0.179 d-f | 0.142 fg | 0.184 c-e | 0.140 d-g | 0.108 gh |
| LSD value at 5% | 0.059 | | | 0.063 | | |

Means not sharing a letter in common, within a row or column, differ significantly at $p \leq 0.05$

Among all extracts used, aqueous extract combination of *D. stramonium* and *C. procera* was proved to the most potent as it showed the highest reduction in germination percentage, shoot length, root length, seedling length and seedling vigor index of *P. hysterophorus* consistently in all experiments at its all concentration levels. The highest phytotoxic potential of this extract combination could be related to their rich composition in allelopathic compounds *viz*., quercertin (0.66 and 0.64 mg $L^{-1}$), gallic acid (0 and 3.20 mg $L^{-1}$), chlorogenic acid (9.44 and 8.12 mg $L^{-1}$), *p*-coumaric acid (0 and 1.78 mg $L^{-1}$), sinapinic acid (2.03 and 5.54 mg $L^{-1}$), caffeic acid (6.67 and 0 mg $L^{-1}$) and benzoic acid (10.16 and 0 mg $L^{-1}$) in *D. stramonium* and *C. procera* extracts, respectively as revealed by their HPLC studies (Table 1).

The best performance of *D. stramonium* + *C. procera* plant extract combination in suppressing germinating seeds of parthenium seed in petri plate based bioassay studies proved it to had the highest pre-emergence herbicidal potential against this weed. Previously, liquid extracts of a number of herbaceous and grassy species have been tested which showed strong herbicidal potential against parthenium. Those included spiny amaranth (*Amaranthus spinosus* L.) (Swain *et al*., 2005), foetid cassia (*Cassia tora* L.) (Prasad *et al*., 2006), ban tulsi [*Croton bonplandianum* (Baill)] (Thapar and Singh, 2006), sessile joyweed (*Alternanthera polygonoides* L.) (Quazi and Khan, 2010), common cocklebur (*Xanthium strumarium* L.) (Sinha and Singh, 2004), cogongrass [*Imperata cylindrica* (L). P. Beauv.] (Anjum *et al.*, 2005), jilda [*Desmostachya bipinnata* (L.) Stap)] (Javaid *et al.*, 2005), ringed dichanthium [*Dicanthium annulatum* (Forssk.) Stapf], cloncurry (*Cenchrus pennisetiformis* Hochst. & Steud.**)** and Johnsongrass [*Sorghum halepense* (L.) Pers.] (Javaid and Anjum, 2006) and African marigold (*Tagetes erecta* L.) (Shafique *et al*., 2011). Safdar *et al.* (2016) calculated 82% decline in germination percentage and 31% in seedling length of parthenium by the application of 5% aqueous extract of *Datura metel* L. leaf. They isolated vanillic acid, benzoic acid and syringic acids in higher concentrations from its extract.

In soil filled pot based bioassay studies, foliar spray of all extracts used in study caused significant reduction in seedling growth of parthenium compared to that in case of control. Moreover, it has been observed that by increasing the concentration of all extracts from 2.5 to 10%, their phytotoxicity also increased. Among all extracts, aqueous plant extracts of *D. stramonium* + *C. procera* and *D. stramonium* + *A. aspera* combinations showed the highest phyto-inhibitory action against seedling growth of parthenium consistently over all their concentrations and experiments. The lowest shoot and root lengths, and fresh and dry weights of parthenium were recorded with these extract combinations that indicated their best post-emergence herbicidal potential against this weed. In addition to *D. stramonium* + *C. procera*, that also showed the best pre-emergence herbicidal potential, the higher post-emergence herbicidal potential of *D. stramonium* + *A. aspera* extract combination could be attributed to the rich phenolic composition of *A. aspera* extract in gallic acid (16.85 mg $L^{-1}$), *m*-coumaric acid (3.13 mg $L^{-1}$), caffeic acid (7.41 mg $L^{-1}$), benzoic acid (24.77 mg $L^{-1}$) and syringic acid (9.21 mg $L^{-1}$) detected through its HPLC analysis. Phytotoxins (phenolics) do have inhibitory effects on germination and growth of weeds and crop plants (Colpas *et al.*, 2003; Abbas *et al.*, 2014; Jabran, 2017; El-Sadek *et al.*, 2017). The increase in inhibitory effect on germination and seedling growth with increasing allelopathic extract concentration was also established (Abbas *et al.*, 2014).

The previous studies also proved *A. aspera* to be the highly allelopathic plant (Srivastav *et al.*, 2011; Safdar *et al.*, 2016). It has been proved that *A. aspera* was rich in allelopathic compounds like alkaloids, phenolics, oleonolic acid, dihydroxy ketones, saponins, and long chain compounds (Srivastav *et al.*, 2011). Its aqueous extract has been proved to be highly phytotoxic against parthenium weed. Safdar *et al.* (2016) carried out laboratory and green-house experiments to explore herbicidal potential of four herbaceous plants (*Achyranthes aspera*, *Alternanthera philoxeroides*, *Datura metel* and *Rumex dentatus*) against parthenium weed. Based upon their pre- and post-emergence inhibitory effects of their aqueous extracts against parthenium germination and seedling biomass, they concluded that *A. aspera* is the most persuasive allelopathic plant that could be used as bio-herbicide for controlling this weed. The foliar spray of *A. aspera* on 15 days old parthenium seedlings resulted in 68, 64, 96, 91, 66 and 96% reductions in shoot length, root length, shoot dry weight, root dry weight, seedling length, and seedling biomass of parthenium, respectively. They isolated about six phenolic compounds (gallic acid, caffeic acid, chromatotropic acid, 4-hydroxy-3-methoxy benzoic acid, syringic acid and *m*-coumaric acid) at higher concentrations from plant extract taken from actively growing plants of *A. aspera*.

## Conclusion

Use of *D. stramonium* water extract in combination with water extracts of *C. procera* and *A. aspera* showed the best phytotoxic potential against parthenium. Presence of considerable quantities of gallic, caffeic, syringic, chlorogenic, *m*-coumaric, *p*-coumaric, sinapinic and benzoic acids, and quercertin in their water extracts proved their potential to be used as pre- and post-emergence herbicides for this weed.

## Acknowledgements

The authors are highly thankful and greatly acknowledge to the support from Higher Education Commission (HEC), Islamabad, Pakistan under Start-Up Research Grant Project No. 21-1065/SRGP/R&D/HEC/2016.

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**(Received 21 July 2018; Accepted 03 September 2018)**