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EXPLORING BIO-HERBICIDAL POTENTIAL OF NATURAL FLORA AGAINST GERMINATION AND SEEDLING GROWTH OF *SORGHUM HALEPENSE*

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Abstract

Sorghum halepense (L.) Pers. is a noxious weed of crop lands in Pakistan due to its persistent, competitive and allelopathic behavior. To find out allelopathic means of controlling this weed, repeated Petri -plate based germination bioassay and soil-filled pot-based seedling bioassay studies were conducted under laboratory and wire house conditions, respectively. The outcomes of studies showed that plant extracts caused significant reduction in *S. halepense* growth and germination. Among the extracts used, 10% (w/v) water extracts of *C. album* and *C. procera* in combination with *D. stramonium* had the strongest pre-emergence herbicidal potential by producing maximum reductions in germination percentage (up to 96%), shoot length (up to 85%), root length (up to 74%) and seedling vigor index (up to 99%) of *S. halepense*. However, in seedling bioassay, foliar spray of 10% water extracts of *A. aspera* and *C. procera* along with *D. stramonium* were proved to have the highest post-emergence phytotoxic ability by causing the highest decrease in shoot length (67%), root length (61%), and shoot fresh weight (90%) of *S. halepense*. According to HPLC analysis of plant water extracts, the greater phytotoxicity of *D. stramonium*, *C. procera*, *A. aspera* and *C. album* extracts might be attributed to their higher allelochemicals' composition including quercertin (0.66, 0.64, 0.32 and 0), gallic acid (0, 3.20, 0 and 16.85), chlorogenic acid (9.44, 8.12, 4.52 and 0), *p*-coumaric acid (0, 1.78, 0 and 0), sinapinic acid (2.03, 5.54, 0 and 0), *m*-coumaric acid (0, 0, 0.93 and 3.13), caffeic acid (6.67, 0, 0 and 7.41), benzoic acid (10.16, 0, 0 and 24.7) and syringic acid (0, 0, 1.92 and 9.21 mg L^{-1}), respectively.

Key words: Allelochemicals; Calotropis procera; Germination; Seedling growth; Sorghum halepense

Introduction

Sorghum halepense (L.) Pers. commonly called Johnson grass is a poaceous weed of Mediterranean origin. The highly persistent and competitive growth behavior, potential toxicity towards grazing livestock and allelopathicity render it to be very problematic weed of agricultural lands. The persistent behavior of this weed is due to its reproduction ability both through rhizomes and shoot system as well as seeds that are produced in much abundance and are long-lived (more than 7 years) in soil (Uremis & Uygur, 2005). Despite the fact that *S. halepense* has an extensive use as fodder, its high cyanic concentration sometimes makes it poisonous for cattle (Henderson, 2001). This is especially true under situation when plant is undergoing through rapid growth phase, or subjected to drought or frost stress. For instance, it has been observed that livestock fed with this plant was suffered from prussic acid poisoning USA and Australia (Parsons & Cuthbertson, 1992). This weed has been found to be highly allelopathic in nature being rich in variety of allelopathic compounds i.e. dhurrin, taxiphyllin, prunasin, sorgoleone, phenolic compounds (*p*-hydroxybenzoic acid, chlorogenic acid, *p*-coumaric acid, *p*-hydroxybenzaldehyde, p-hydroxybenzyl alcohol and ethyl *p*-hydroxybenzoate), flavonoids (diosmetin and tricin), phloroglucinol, and aliphatic acids (Czarnota *et al.*, 2003; Huang *et al.*, 2015). Being a C_4 plant, this weed is highly competitive in its growth habit, it continues to growth even under very hot and dry conditions. The areas that are sub-tropical and receive more rainfall in summer are best suited to *S. halepense* rather than areas that are purely tropical. That is why; climatic conditions prevailing in most parts of Pakistan favor the abundant proliferation of this weed in the country. Torma & Bereczki-Kovács (2004) proved that root exudates and decomposing plant products of *S. halepense* are rich in allelopathic compounds that suppressed emergence and growth of crops infested with this weed. The phytotoxic inhibition by allelopathic extracts of *S. halepense* have been noted in different crops (Nouri *et al.*, 2012; Vasilakoglou *et al.*, 2005; Golubinova & Ilieva, 2014).

There is a variety of methods used for controlling weeds which include manual, chemical, mechanical and biological methods. Each of them has its own pros and cons. Mechanical and manual weed control is considered most eco-friendly, however due to its higher cost is not feasible. That is why; most of the farmers rely upon herbicides as chemical method gives promising weed control (Moss, 2019). Despite the popularity of chemical weed control, sole dependence upon synthetic herbicides involves enormous ecological and health-related issues that include development of herbicide resistant weeds and herbicide contaminated crop produce. That is why; in one or the other way, research emphasis is always brought on alternative weed control tactics (Bhowmik, 2003). The use of allelopathic compounds extracted from plants as bio-

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herbicides is a potential biological approach for controlling weeds (Anjum *et al.*, 2005). Allelochemicals are natural plant products synthesized within plants and are released from their living or dead to suppress the nearby vegetation (Rice, 1984).

Some of the herbaceous plants including *Achyranthes aspera* (prickly chaff flower), *Datura stramonium* (thorn apple), *Calotropis procera* (rubber bush), *Melilotus indica* (yellow sweet clover) *Chenopodium album* (lambsquarters) and *C. murale* (nettleleaf goosefoot) are allelochemical-rich plants, well-adapted and widely distributed in agro-ecosystems of Punjab (Safdar *et al.*, 2021; Batool *et al*., 2020; Iqbal *et al.*, 2023; Usman *et al.*, 2020; Ali *et al.*, 2022). The previous studies had shown the phytotoxic inhibition of other plants by aforementioned plants (Blackshaw *et al.*, 2001; Oudhia & Tripathi, 2001; Al-Zahrani & Al-Robai, 2007; Batish *et al.*, 2007; Rezaie & Yarnia, 2009; Ghareib *et al.*, 2010; Butnariu, 2012; Ghasemi *et al.*, 2012; Majeed *et al.*, 2012; Tanveer *et al*., 2014; Safdar *et al.*, 2016). Germination and growth suppression of *S. halepense* by application of aqueous extracts of *D. stramonium* has already been proved by Butnariu (2012). The germination and growth inhibition by the application of extracts derived from allelopathic plants is due to retardation or blockage of some of the underlying physiological process (Einhellig, 1996). Moreover, there is a complex interaction exists among allelopathic compounds that produce an overall effect (Einhellig, 1996). There is a greater opportunity of utilizing phytotoxic plant extracts by their tank-mixed application along with little bit reduced dose of synthetic herbicides (Cheema *et al.*, 2005). By this we can make our weed management program more sustained and eco-friendly. The current investigation was therefore planned to ascertain the bio-herbicidal potential of aqueous extracts derived from five eminent phytotoxic plant species viz., *A. aspera*, *D. stramonium*, *C. procera*, *C. album*, *M. indica* and *C. murale* at their variable concentrations and blending against seed germination and initial seedling growth of *S. halepense* under lab and wire-house environments.

Materials and Methods

The studies were accomplished in Lab of Agronomy Department and in the wire house in winter season 2016-17. The study site (College of Agriculture, University of Sargodha, Sargodha, Punjab-Pakistan) was location at 31.41°N latitude, 74.17°E longitude and altitude of 194.4 m. One year before the execution of experiments, *S. halepense* seeds were collected through shaking the matured spikes from *S. halepense* plants growing around and inside crop fields at the same location. Collected seeds were preserved in in kraft paper bags after shade drying.

Preparation of water extracts*:* The plants of *A. aspera*, *D. stramonium*, *C. murale*, *C. procera*, *C. album*, and *M. indica* at their active growth stages were uprooted from the experimental fields at Department of Agronomy research areas, College of Agriculture. Collected plants were shade-dried at the room temperature (25°C) and water extracts were prepared. Dried plants were cut into small segments (2-3 cm) by using scissors. The chaffed pieces of plants were independently dipped in distilled water with 1:10 (w/v) ratio at room temperature for 24 h period. Each plant's extract was gained through the process of filtration via mesh sieves. The extraction assembly was used for the fine filtration of the extracts. From 10% concentrated plants' water extracts, their 2.5 and 5% concentrations were obtained by using parallel dilution formula ($C_1V_1=C_2V_2$). As per treatment plan, each of the 2.5, 5 and 10% water extracts of each plant species were mixed with one another in equal proportions to make their combinations. Those were then kept in properly labelled plastic bottles for further use.

Identification of phytotoxic chemicals in plants' water extracts: Plant water extracts of different plants were analyzed for determination of their suspected allelochemicals by using Shimadzu HPLC system equipped with a UV detector (Model SCL-10A, Tokyo, Japan) and Shim-pack CLC-octadecyl silicate C-18 column (25 cm length × 4.6 mm diameter of 5 µm particle size). In isocratic mobile phase, solvent was 100% methanol with injection volume of 50 µL. The UV detector wavelength was set at 280 nm and flow rate was 0.25 mL min^{-1}. The peaks of phytotoxins were quantified through the setting of their standards (Aldrich, St Louis, USA). By using the following equation, isolated compound's concentration was obtained:

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

Different concentrations of phytotoxic chemicals in water extracts of plants used in these experiments are presented in Fig. 1.

Petri plate and pot bioassays*:* To ascertain the bio herbicidal potential of each extracts' combination, two separate set of experiments were conducted, one named as Petri plate based germination bioassay and other as pot based seedling growth bioassay. In first set of experiments, Petri plates (diameter = 9 cm) lined with bilayer of Whatman No.42 filter paper were used for sowing 10 seeds of *S. halepense*. After preliminary testing, 5 mL quantity of extract per Petri plate and per pot was found sufficient for germinating seeds and seedlings of *S. halepense*. The 5 ml plant's water extract of 2.5, 5 and 10% concentrations were used to wet filter paper in each Petri plate. The control treatment utilizes the same quantity of distilled water instead of plant extract. Completely randomized design with factorial pre-arrangement was used with four replications. Petri plates were properly sealed with parafilm to avoid the drying throughout the incubation time before placing these on lab shelves. The 24 h temperatures (minimum and maximum) were recorded on daily basis over the entire study period of three weeks. The minimum temperature ranged between 25-28ºC while maximum temperature lies between 30-32ºC. The experiment was repeated in the same fashion after the completion of first one.

In the second set of experiments, 350 g of soil was filled in the plastic pots with 9 cm × 9 cm dimension. The soil was collected from agronomic field and dried, mixed

and sieved before putting into the pots. Five *S. halepense* seedlings growing in agronomy field at their 4-leaf stage were pulled out and shifted to each pot after the saturation of the soil with distilled water. The pots were placed in wire house. After two weeks of transplanting, when plants were properly established, 5 ml of each plant's water extract combination of each of the 2.5, 5 and 10% concentration was sprayed over *S. halepense* seedlings per pot. In control treatment, *S. halepense* seedlings were sprayed with same quantity of distilled water instead of water extracts. Completely randomized design with factorial arrangement was used and four replications were kept. Pots were supplied with equal amount of water as and when required throughout the course of experiment to avoid the seedlings' drying. The 24 h temperatures (minimum and maximum) were recorded on daily basis throughout the experimental period. Minimum temperature was 22.5ºC and maximum temperature 34ºC. These temperature ranges are optimum for germination and growth of *S. halepense*, a factor that excludes its temperature-induced germination and growth inhibition. After a period of three weeks, seedlings of *S. halepense* were uprooted and washed with tap water. The studies were performed twice.

Observations: Counting of germinated seeds was done on daily basis for both of the experiments. The ratio of total seeds germinated to total seeds sown was used to get germination percentage. Root length and shoot length were measured by a scale. Fresh weight and dry weight of the seedlings were obtained with the help of an electronic balance before and after drying at 70ºC in an electric oven till the attainment of constant weight.

Statistical analysis: Recorded data was analysed by using Fisher's analysis of variance technique (Steel *et al.*, 1997) and treatments' means were separated by applying least significant difference (LSD) at 0.05 probability level through computer statistical software (Anon., 1986). The individual as well as pooled data of repeated experiment-I and experiment–II were analysed and presented.

Results:

Allelochemical composition of plants' water extracts: The comparison of allelochemicals' composition of extracts used in studies is presented in Fig. 1. Data revealed that a total of 11 phytotoxic compounds have been detected in those extracts. In addition to 8 phenolics, there was one flavonoid (quercetin) found in water extract of *C. album, C. murale, D stramonium and C. procera*. The overall 10 different phenolics i.e., chlorogenic, gallic, *p*-coumaric, sinapinic, *m*-coumaric, caffeic, ferulic, syringic, benzoic and vanillic acids were identified in water extracts of plants with differential amounts. The highest total allelopathic compounds' concentration (61.37 mg L^{-1} of extract) was detected in water extract of *A. aspera*. However, *M. indica*, *C. murale* and *D. stramonium* can be placed at second, third and fourth position along with total allelochemicals concentrations of 55.19, 41.05 and 28.96 mg L^{-1} of extract, respectively. However, *C. album* water extract has the lowermost allelochemicals' content i.e. 7.69 mg L^{-1} of extract (Fig. 1).

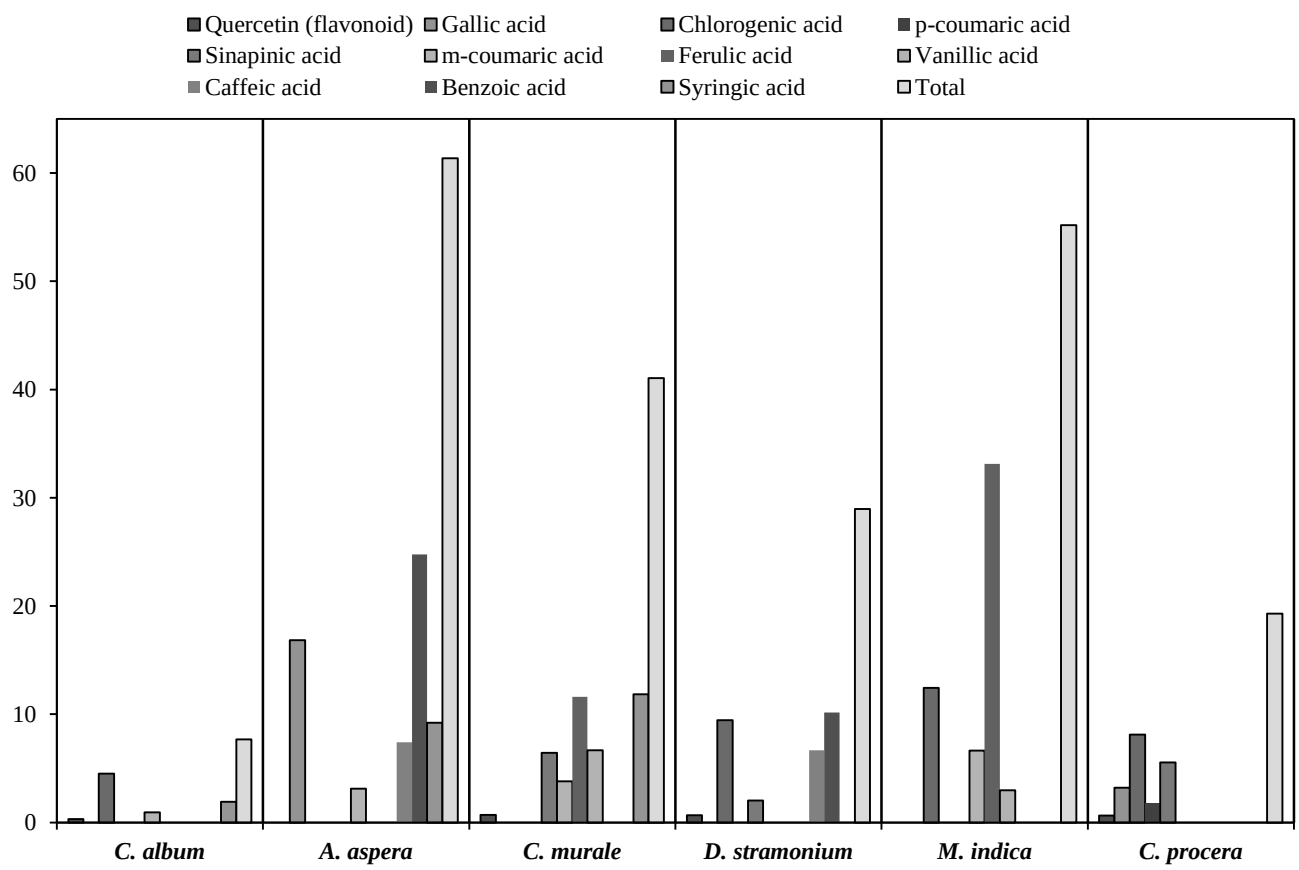

Fig 1. Comparison of allelopathic composition of plants' extract as detected through HPLC

Table 1. Germination percentage and shoot length of *S. halepense* as influenced by plants' extracts (Petri dish trials).

Plant extracts	Experiment-I			Experiment-II		
	Concentration			Concentration		
	2.5%	5%	10%	2.5%	5%	10%
Germination percentage						
Control (Distilled water)	95.0 a	90.6 a	89.6 a	91.2 a	88.9 a	91.2 a
Datura stramonium	54.3 b	38.3 cd	14.6 h	66.8 b	38.2 c	23.1 fg
Datura stramonium + Achyranthes aspera	34.0 def	25.6 g	9.6 hi	31.3 de	23.6 fg	15.2 hi
Datura stramonium + Chenopodium album	35.6 cde	23.6 g	7.0 ij	40.2 c	24.8 fg	9.5 ij
Datura stramonium + Calotropis procera	29.3 efg	14.0 h	3.0 j	20.1 gh	13.5 i	5.2 j
Datura stramonium + Chenopodium murale	41.0 c	28.0 fg	11.3 hi	24.7 fg	27.3 ef	21.3 g
Datura stramonium + Melilotus indica	37.0 cd	30.0 efg	12.0 hi	21.6 fg	34.9 cd	12.1 i
LSD value at 5%	**6.512**			**5.892**		
Shoot length (cm)						
Control (Distilled water)	18.7 a	19.4 a	20.7 a	26.9 a	20.7 ab	17.0 bc
Datura stramonium	14.9 b	11.8 cd	11.2 cde	15.5 bcd	13.2 c-g	9.2 d-j
Datura stramonium + Achyranthes aspera	11.9 cd	10.6 c-f	6.6 gh	15.3 bcd	12.5 c-g	7.2 g-j
Datura stramonium + Chenopodium album	11.6 cd	10.3 c-f	8.2 fgh	14.7 b-e	11.9 c-h	8.1 e-j
Datura stramonium + Calotropis procera	12.5 bc	9.8 def	5.8 h	13.8 b-g	10.6 f-h	3.8 j
Datura stramonium + Chenopodium murale	12.8 bc	10.7 c-f	8.1 fgh	16.2 bc	11.3 c-i	4.8 ij
Datura stramonium + Melilotus indica	11.907 cd	10.7 c-f	8.6 efg	11.2 c-i	14.2 b-f	5.3 hij
LSD value at 5%	**2.646**			**6.846**		

Mean values with dissimilar lettering vary significantly ($p<0.05$) from one another as per least significant difference (LSD) test

Table 2. Root length and seedling vigor index of *S. halepense* as influenced by different plants' extracts (Petri dish trials).

Plant extracts	Experiment-I			Experiment-II		
	Concentration			Concentration		
	2.5%	5%	10%	2.5%	5%	10%
Root length (cm)						
Control (Distilled water)	10.2 a	9.4 a	8.5 ab	8.5 ab	9.8 a	7.7 abc
Datura stramonium	6.9 bcd	5.5 cdef	5.2 cdef	6.5 bcde	4.5 defg	4.8 defg
Datura stramonium + Achyranthes aspera	5.5 cdef	5.8 cdef	4.5 ef	5.1 c-g	4.8 c-g	3.4 fg
Datura stramonium + Chenopodium album	6.4 cde	5.2 cdef	4.3 ef	5.8 b-f	5.2 c-g	3.1 fg
Datura stramonium + Calotropis procera	5.9 cdef	5.1 cdef	4.1 f	3.5 fg	3.3 fg	2.5 g
Datura stramonium + Chenopodium murale	6.0 cdef	5.4 cdef	4.8 def	3.7 efg	5.1 c-g	3.6 fg
Datura stramonium + Melilotus indica	7.1 bc	5.4 cdef	4.7 ef	6.8 bcd	4.1 defg	3.7 efg
LSD value at 5%	**2.066**			**2.911**		
Seedling vigor index						
Control (Distilled water)	2754.1 a	2716.8 a	2724.0 a	3244.3 a	3032.4 a	3031.1 a
Datura stramonium	1186.1 c	664.6 de	241.1 j	1694.1 d	968.3 e	326.7 jklm
Datura stramonium + Achyranthes aspera	593.4 efg	427.4 hi	108.0 klm	654.4 ghi	405.6 ijkl	162.1 lmn
Datura stramonium + Chenopodium album	647.1 ef	369.5 i	88.6 lm	913.3 ef	424.9 ijk	108.1 mn
Datura stramonium + Calotropis procera	543.8 fgh	209.7 jk	30.0 m	554.5 ghij	162.0 jkl	32.9 n
Datura stramonium + Chenopodium murale	774.7 d	455.2 hi	146.5 jklm	779.5 efg	520.3 hij	177.7 klmn
Datura stramonium + Melilotus indica	706.3 de	483.9 ghi	254.6 klmn	709.6 fgh	574.0 ghij	175.3 klmn
LSD value at 5%	**117.46**			**252.82**		

Mean values with dissimilar lettering vary significantly ($p<0.05$) from one another as per least significant difference (LSD) test

Petri plate based germination bioassay: In comparison to distilled water control, all plants' extracts at their all the three concentrations significantly reduced the germination percentage and shoot length of *S. halepense* to variable degree (Table 1, Fig. 2). In both the trial-I and trial-II, 10% concentrated *D. stramonium* extract with *C. procera* and *C. album* resulted in maximum reduction (up to 96% by *D. stramonium + C. procera*) in germination percentage of *S. halepense*. Regarding shoot length of *S. halepense*, maximum reduction was caused by 10% *D. stramonium* extract with *C. procera* (71%), *A. aspera* (68%), *C. murale* (61%) and *C. album* (60%) compared to control in trial-I. However, in trial-II, 10% *D. stramonium* alone and with all other 10% extracts yielded significantly lower shoot lengths (maximum up to 85% by *D. stramonium + C. procera*) of *S. halepense*. Compared to control, significant decline in root length and seedling vigor index (SVI) of *S. halepense* was caused by 2.5%, 5% and 10% concentrated water extracts of all plants used in the study as revealed by individual and pooled data of both the trials I and II (Table 2, Fig. 3). The 10% concentrated *D. stramonium* extract with *C. procera* brought about the highest reduction (60 and 74%) in root length of *S. halepense* in trial-I and trial-II, respectively. However, in both trials, this treatment remained statistically at par with all other plant extracts of 5 and 10% concentrations and 2.5% concentrated *D. stramonium* extract with those of *A. aspera, C. procera and C. murale* regarding root length. Significantly the lower values of SVI of *S. halepense* were noted by application of 10% *D. stramonium* extract in combination with all other extracts with 10% concentration. Among all, 10% extract of *D. stramonium + C. procera* caused the highest reductions (98 and 99%) in SVI of *S. halepense* as observed in trial-I and trial-II, respectively (Table 2).

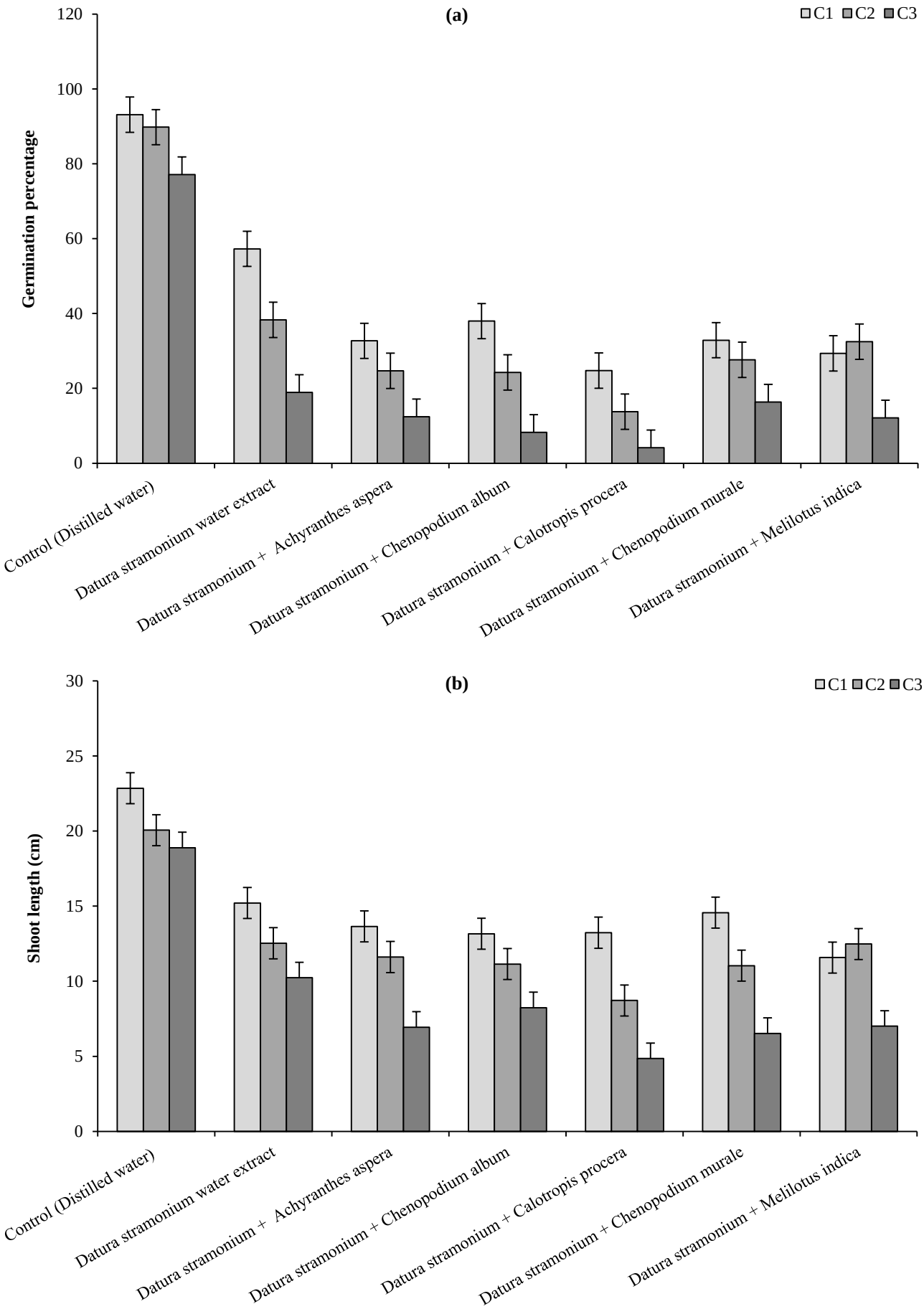

Fig. 2. Two trials' pooled means of (a) germination percentage and (b) shoot length of *S. halepense* as affected by different plant extracts (Petri dish trials).

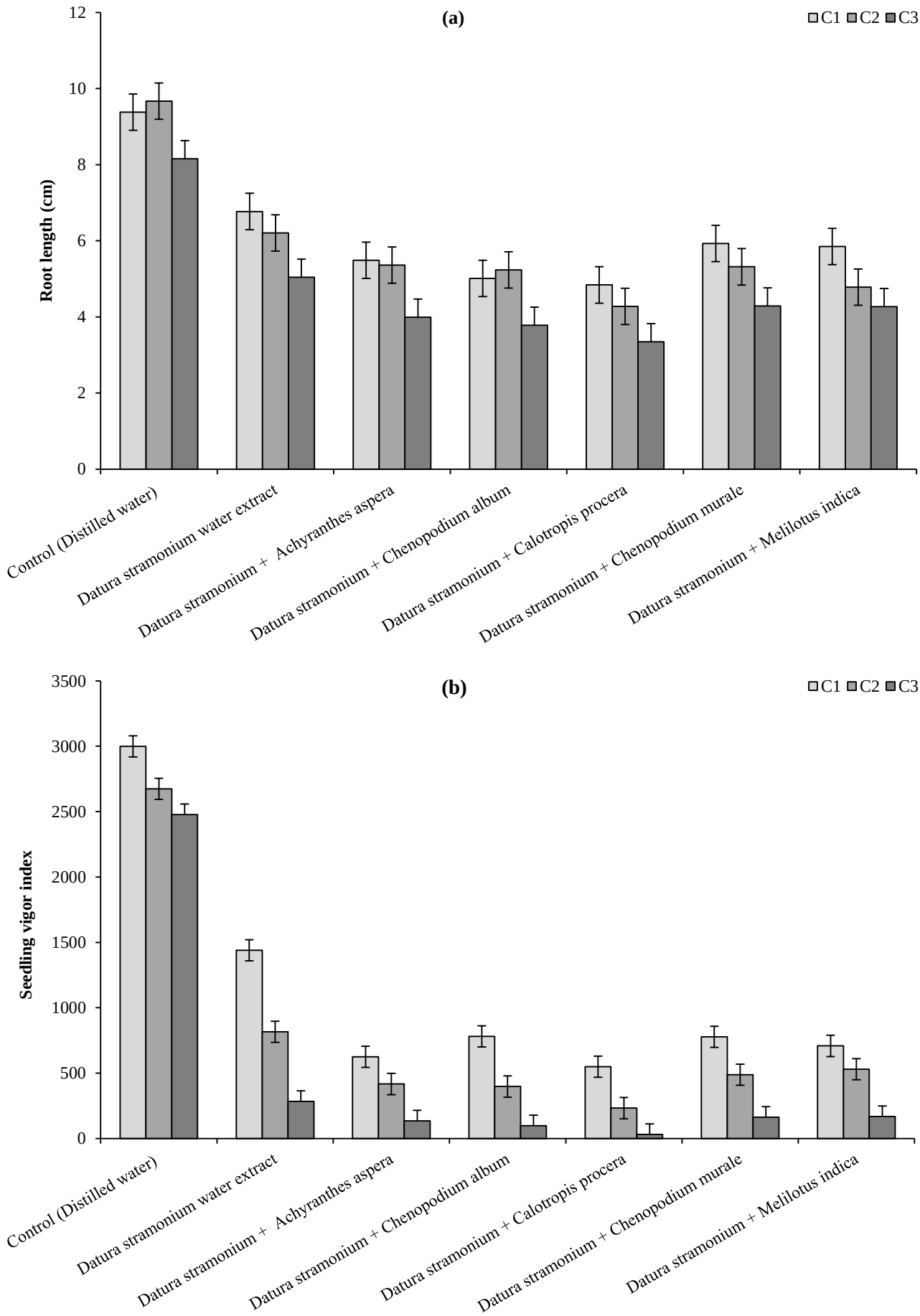

Fig. 3. Two trials' pooled means of (a) root length (b) seedling vigor index of *S. halepense* as affected by different plant extracts (Petri dish trials).

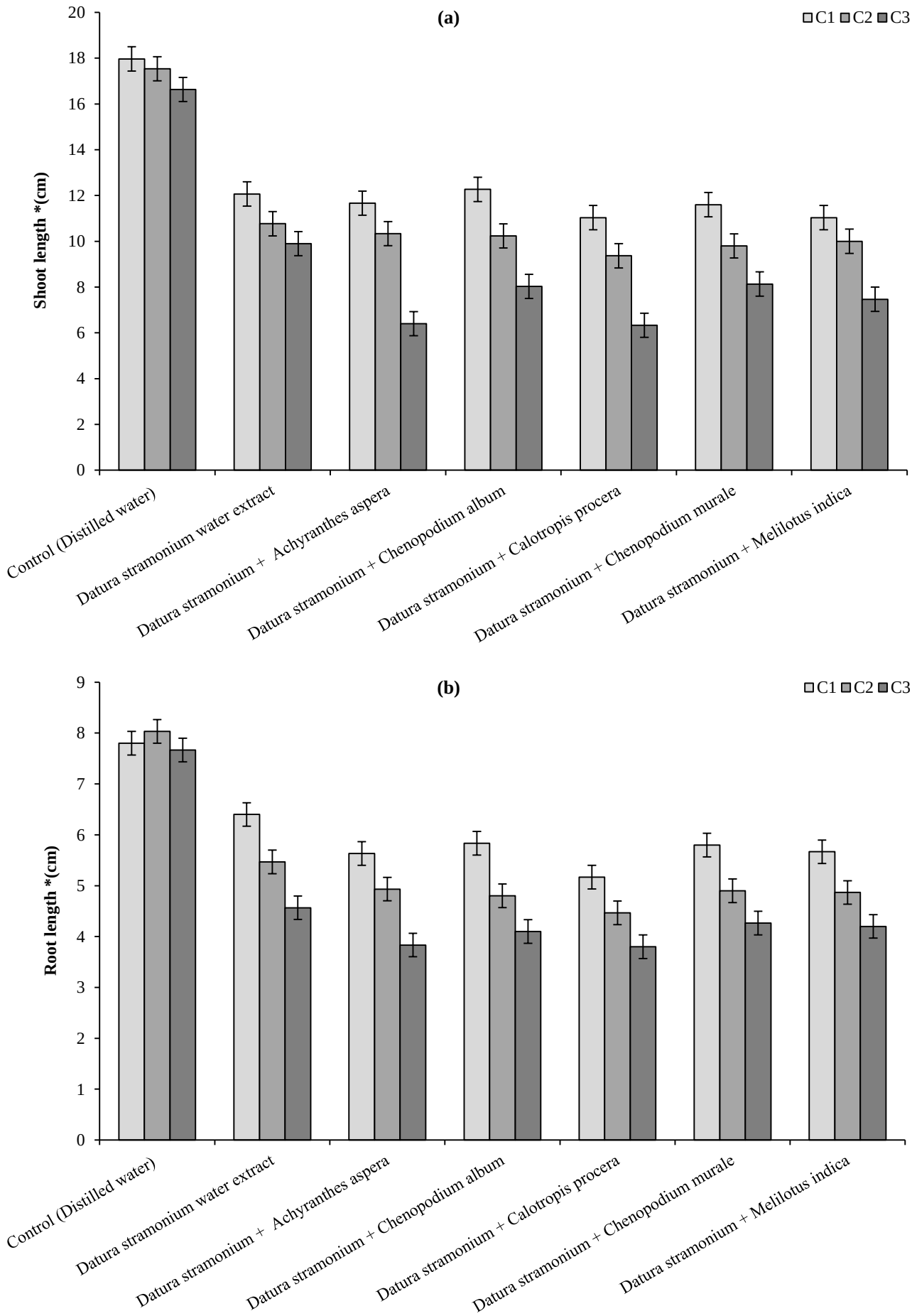

Fig. 4. Two trials' pooled means of (a) shoot length (b) root length of *S. halepense* as affected by different plant extracts (Pot trials).

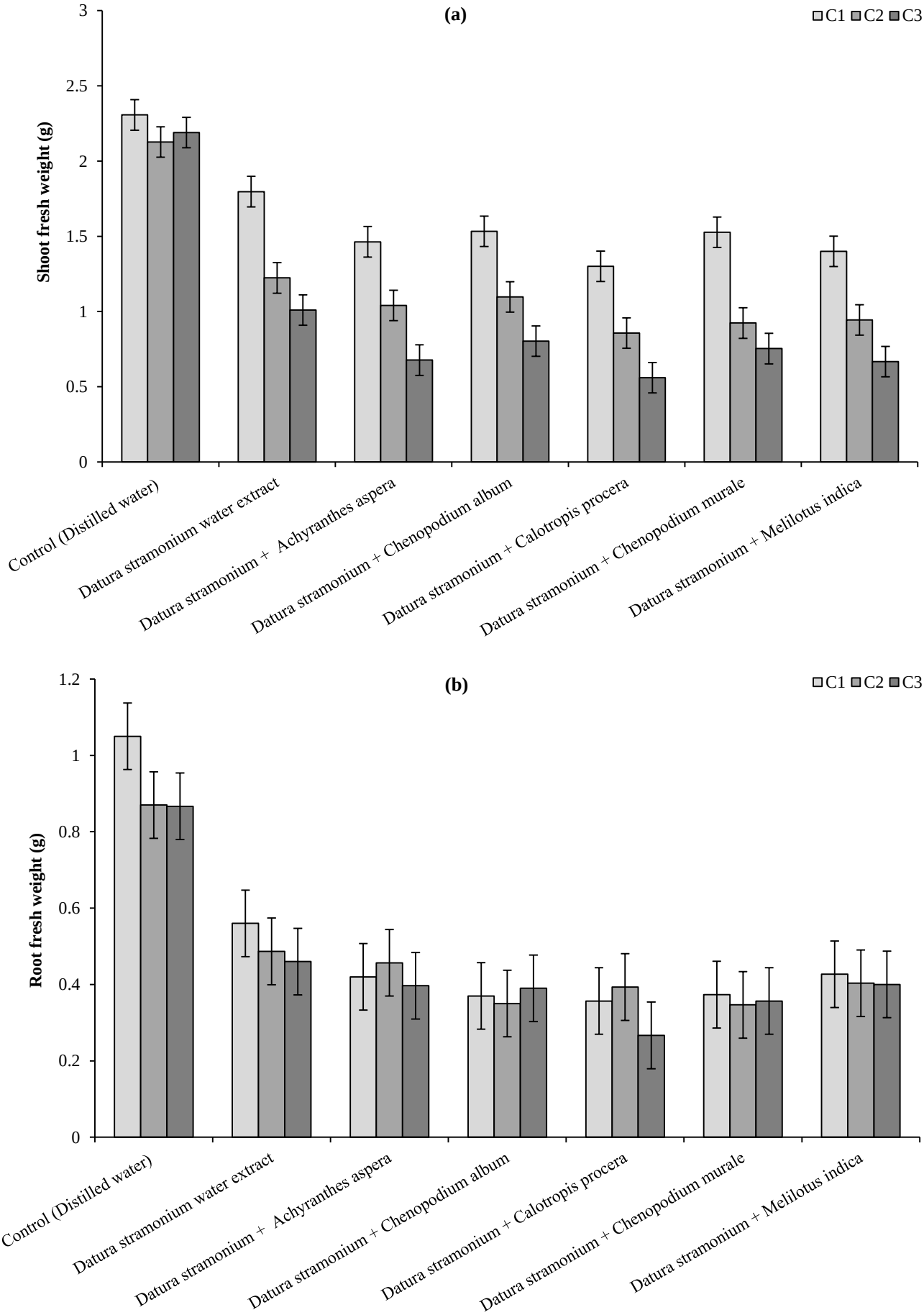

Fig. 5. Two trials' pooled means of (a) shoot fresh weight (b) root fresh weight of *S. halepense* as affected by different plant extracts (Pot trials).

Table 3. Shoot and root lengths of *S. halepense* under the influence of different plants' extracts (Pot trials).

Plant extracts	Experiment-I			Experiment-II		
	Concentration			Concentration		
	2.5%	5%	10%	2.5%	5%	10%
Shoot length (cm)						
Control (Distilled water)	19.2 a	18.2 ab	16.8 b	16.7 a	16.7 a	16.4 a
Datura stramonium	13.7 c	11.0 ef	10.6 fgv	10.4 bcd	10.4 bcd	9.0 cde
Datura stramonium + Achyranthes aspera	11.8 def	11.4 ef	7.0 i	11.4 b	9.1 cde	5.8 g
Datura stramonium + Chenopodium album	13.0 cd	11.2 ef	8.6 h	11.5 b	9.1 cde	7.4 efg
Datura stramonium + Calotropis procera	11.4 ef	10.5 fg	7.1 i	10.6 bcd	8.1 ef	5.5 g
Datura stramonium + Chenopodium murale	12.3 cde	10.7 fg	9.4 gh	10.8 bc	8.9 de	6.7 fg
Datura stramonium + Melilotus indica	11.1 ef	10.6 fg	8.6 h	10.8 bc	9.2 cde	6.3 fg
LSD value at 5%	**1.404**			**1.893**		
Root length (cm)						
Control (Distilled water)	8.2 a	8.5 a	8.1 a	7.3 ab	7.4 a	7.1 abc
Datura stramonium	6.3 b	5.7 cd	5.0 efg	6.4 cde	5.1 f-i	4.7 h-l
Datura stramonium + Achyranthes aspera	4.8 gh	4.1 i	3.5 j	6.4 de	5.7 ef	4.0 klm
Datura stramonium + Chenopodium album	5.0 efg	4.3 i	3.4 j	6.6 bcd	5.2 fgh	4.0 lm
Datura stramonium + Calotropis procera	4.5 hi	4.1 i	3.3 j	5.7 ef	4.7 g-k	4.1 klm
Datura stramonium + Chenopodium murale	6.5 b	5.3 de	4.9 e-h	5.0 f-j	4.4 i-l	3.6 m
Datura stramonium + Melilotus indica	5.8 c	5.2 ef	4.8 fgh	5.4 fg	4.4 jkl	3.5 m
LSD value at 5%	**0.442**			**0.728**		

Mean values with dissimilar lettering vary significantly ($p<0.05$) from one another as per least significant difference (LSD) test

Table 4. Shoot and root fresh weights of *S. halepense* under the influence of different plants' extracts (Pot trials).

Plant extracts	Experiment-I			Experiment-II		
	Concentration			Concentration		
	2.5%	5%	10%	2.5%	5%	10%
Shoot fresh weight (g)						
Control (Distilled water)	2.5 a	2.5 a	2.3 a	2.0 a	1.6 a-d	1.9 ab
Datura stramonium	1.6 bc	1.6 bc	1.1 e-h	1.9 abc	0.8 f-i	0.8 efgh
Datura stramonium + Achyranthes aspera	1.6 bcd	1.2 b-h	0.8 gh	1.3 def	0.7 f-i	0.5 hi
Datura stramonium + Chenopodium album	1.7 b	1.3 b-g	1.0 e-h	1.3 c-f	0.9 e-h	0.5 hi
Datura stramonium + Calotropis procera	1.4 b-e	1.1 d-h	0.8 gh	1.1 d-g	0.5 hi	0.2 i
Datura stramonium + Chenopodium murale	1.5 bcd	1.2 c-h	1.1 e-h	1.4 b-e	0.5 ghi	0.3 hi
Datura stramonium + Melilotus indica	1.4 b-e	1.3 b-f	0.9 f-h	1.2 def	0.5 hi	0.4 hi
LSD value at 5%	**0.442**			**0.589**		
Root fresh weight (g)						
Control (Distilled water)	1.19 a	1.07 ab	0.96 abc	0.90 a	0.66 abc	0.76 ab
Datura stramonium	0.70 bcd	0.63 bcd	0.46 d	0.39 cd	0.34 cd	0.45 bcd
Datura stramonium + Achyranthes aspera	0.51 cd	0.48 cd	0.44 d	0.32 d	0.42 cd	0.34 cd
Datura stramonium + Chenopodium album	0.44 d	0.44 d	0.49 cd	0.30 d	0.26 d	0.28 d
Datura stramonium + Calotropis procera	0.43 d	0.39 d	0.33 d	0.28 d	0.39 cd	0.20 d
Datura stramonium + Chenopodium murale	0.41 d	0.43 d	0.38 d	0.32 cd	0.26 d	0.32 d
Datura stramonium + Melilotus indica	0.49 cd	0.48 cd	0.48 cd	0.36 cd	0.33 cd	0.32 d
LSD value at 5%	**0.486**			**0.335**		

Mean values with dissimilar lettering vary significantly ($p<0.05$) from one another as per least significant difference (LSD) test

Pot based seedling growth bioassay: The individual and pooled data of trial-I, trail-II regarding shoot and root lengths of *S. halepense* as affected by different plant extract treatments have been presented in Table 3 and Fig. 4, respectively. It can be inferred from data that in comparison to distilled water control, all extracts brought about significant decline in shoot and root lengths of *S. halepense* in both trials. Among all extracts, 10% *D. stramonium* plant extract in mixture with those of *A. aspera* and *C. procera* resulted in the highest (maximum up to 67%) decrease in shoot length of *S. halepense* consistently in both trials (Table 3). The root length of *S. halepense* also followed same trend (Table 3, Fig. 4). The same extract combinations i.e. each of the 10% exacts of *D. stramonium* along with *A. aspera*, *C. procera* and *C. album* also expressed the highest phytotoxic action (up to 61%) against root length of *S. halepense* consistently in both trials. In addition, however 10% *D. stramonium* extract along with each of the *C. murale* and *M. indica* extracts also performed better in trial-II by producing 49% and 50% reduction in root length, respectively compared to control of *S. halepense*. Compared to control, the combination of all plants' extracts significantly reduced shoot fresh weight and root fresh weight of *S. halepense* as shown by the individual trial-I and trial-II data as well as their pooled data (Table 4, Fig. 5). In both trials, 10% water extract combinations (*D. stramonium* along with each of the *A. aspera* and *C. procera*) produced the highest reductions (65% and 73%) in shoot fresh weights respectively compared to control of *S. halepense*. However, these treatments do not differ significantly from 10% concentrated extract of *D. stramonium* with *C. murale*, *C. album* and *M. indica*, and 5% concentrated *D. stramonium* water extracts along with each of the *C. procera*, *C. murale* and *A. aspera* regarding shoot fresh weight of *S. halepense* consistently in

both the trials. It is obvious from the data (Table 4) that fresh weight of *S. halepense* root suffered from significant reduction (up to 72%) compared to control in both the trials by foliar application of all extracts used. In trial-I, significant decline in shoot and root dry weight of *S. halepense* was noted by application of all extracts (Table 5). Among these, foliar application of 5 and 10% *D. stramonium* extracts along with each of the *C. procera, C. album, A. aspera* and *C. murale* resulted in significantly the least dry weight of *S. halepense* shoot that was up to 78% lower than control. However, in trial-II, there was no significant difference that exists among treatments regarding dry weight of *S. halepense* shoot. Regarding dry weight *S. halepense* root, all extract combinations caused significantly the lowest (up to 93% lower than control) root dry weight of *S. halepense* in both pot trials.

Table 5. Shoot and root dry weights of *S. halepense* as affected by different plants' extracts (Pot trials).

Plant extracts	Experiment-I			Experiment-II		
	Concentration			Concentration		
	2.5%	5%	10%	2.5%	5%	10%
Shoot dry weight (g)						
Control (Distilled water)	0.51 a	0.45 a	0.44 a	0.55	0.49	0.41
Datura stramonium	0.28 c	0.20 def	0.20 def	0.41	0.38	0.78
Datura stramonium + Achyranthes aspera	0.21 cdef	0.14 fgh	0.13 gh	0.35	0.41	1.01
Datura stramonium + Chenopodium album	0.22 cde	0.15 e-h	0.14 fgh	0.31	0.25	0.36
Datura stramonium + Calotropis procera	0.21 def	0.13 gh	0.11 h	0.11	0.14	0.08
Datura stramonium + Chenopodium murale	0.22 cde	0.16 e-h	0.15 e-h	0.24	0.21	0.68
Datura stramonium + Melilotus indica	0.24 cd	0.18 d-g	0.18 d-g	0.22	7.18	0.22
LSD value at 5%	**0.070**			**Non-significant**		
Root dry weight (g)						
Control (Distilled water)	0.23 a	0.21 a	0.19 ab	0.31 a	0.32 a	0.31 a
Datura stramonium	0.16 abc	0.13 bc	0.14 bc	0.11 b	0.12 b	0.12 b
Datura stramonium + Achyranthes aspera	0.12 bc	0.12 c	0.11 c	0.06 bc	0.06 bc	0.05 bc
Datura stramonium + Chenopodium album	0.11 c	0.11 c	0.10 c	0.06 bc	0.05 bc	0.06 bc
Datura stramonium + Calotropis procera	0.11 c	0.11 c	0.10 c	0.04 c	0.03 c	0.02 c
Datura stramonium + Chenopodium murale	0.14 bc	0.13 bc	0.12 bc	0.07 bc	0.06 bc	0.04 c
Datura stramonium + Melilotus indica	0.12 bc	0.11 c	0.12 c	0.06 bc	0.06 bc	0.04 c
LSD value at 5%	**0.073**			**0.074**		

Mean values with dissimilar lettering vary significantly ($p<0.05$) from one another as per least significant difference (LSD) test

Discussion

An overlook of data indicated a differential phyto-inhibitory influence as their pre- and post-emergence application towards *S. halepense*. It is also clear that in response to increasing concentrations (2.5 to 10%) of all extracts, their suppressive action was augmented. Among all extracts used, *D. stramonium* extract in mixture with each of the *C. procera, A. aspera* and *C. album* extracts was verified to the most effective in inhibiting the seed germination and growth of *S. halepense* seedlings in both the Petri plate- and pot-based bioassays. A more critical examination of the results revealed that among *C. album and A. aspera,* shoot growth of *S. halepense* was more strongly suppressed by *A. aspera* whereas its root growth by *C. album*. The greater phytotoxicity of *D. stramonium, C. procera, C. album and A. aspera* plant water extracts seem to be due to their higher allelopathic compounds composition including quercertin (0.66, 0.64, 0.32 and 0), gallic acid (0, 3.20, 0 and 16.85), chlorogenic acid (9.44, 8.12, 4.52 and 0), *p*-coumaric acid (0, 1.78, 0 and 0), sinapinic acid (2.03, 5.54, 0 and 0), *m*-coumaric acid (0, 0, 0.93 and 3.13), caffeic acid (6.67, 0, 0 and 7.41), benzoic acid (10.16, 0, 0 and 24.7) and syringic acid (0, 0, 1.92 and 9.21 mg L^{-1}), respectively as identified by their HPLC analysis (Fig. 1).

In Petri plate germination bioassay, *C. procera* and *C. album* plant extracts in mixture with *D. stramonium* were exhibited to be more phyto-toxic against germination, root growth and seedling vigor index of *S. halepense* as compared to *A. aspera* extract combination with it that exhibited more phytotoxicity against shoot growth of *S. halepense*. This was an indication that pre-emergence herbicidal potential of *C. procera* and *C. album* was more than that of *A. aspera*. However, in pot-based seedling growth bioassay, there was greater phyto-inhibitory effect of extracts on root growth as compared to shoot growth. *Datura stramonium* sole application did not reduce significantly the shoot fresh weight but root fresh weight was significantly reduced. But in case of dry weights, the same effect was further diluted resulting in non-significant effect by all extracts on shoot dry weight in Experiment-II. Uremis *et al*., (2009) screened out some crops to have strong herbicidal potential against *S. halepense*. They found that shoot extracts of rapeseed (*Brassica napus* L.), turnip (*Brassica campestris* L.), and radish (*Raphanus sativus* L.), reduced the germination of *S. halepense* up to 40-50%. However, previous studies confirmed that growth and germination of *S. halepense* was suppressed by exogenous application of aqueous extract derived from *D. stramonium* due to presence of tropane alkaloids in it (Butnariu, 2012). The bio-herbicidal potential of *D. stramonium* against some weeds (*Cenchrus ciliaris* and *Neonotonia wightii*) and crops (*Helianthus annuus* L.) in terms of reduced seedling growth and germination has also been demonstrated by Elisante *et al*., (2013) and Pacanoski *et al*., (2014), respectively. The *C. procera* has been extensively

reported as highly phytotoxic plant against germination and seedling growth of barley (*Hordeum vulgare* L.), cucumber (*Cucumis satirus* L.), wheat (*Triticum aestivum* L.), alssana (*Senna occidentalis* L. Link) and fenugreek (*Trigonella foenum graecum* L.), (Al-Zahrani & Al-Robai, 2007; Yasin *et al.*, 2012); tomato (*Lycopersicon esculentum*), and eggplant (*Solanum melongena*) (Ghasemi *et al.*, 2012); radish (*Raphanus sativus*) and canola (*Brassica napus*) (Abdel-Farid *et al.*, (2013); *Brassica oleracea* (Gulzar & Siddiqui, 2017). Rezaie & Yarnia (2009) and Majeed *et al*., (2012) in Petri plate and soil-filled pot studies, demonstrated that 10% and 50% aqueous shoot extracts of *C. album* applied to germinating seeds and plants of safflower (*Carthamus tinctorius* L.) and wheat resulted in significant decline in germination, seedling and plant growth of these plants, respectively. The *A. aspera*, a well-known allelopathic plant has been proved to have strong phytotoxic potential in its aqueous shoot and root extracts against wheat and four weeds namely *Chloris barbata Cenchrus setigerus, C. pennisetiformis*, and *Peristrophe bicalyculata* as revealed by Khan & Shaukat (2006). Tanveer *et al.*, (2014) showed that *A. aspera* aqueous extracts derived from root, stem, leaf and fruit significantly reduced the germination percentage, germination speed and seedling growth of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), pearl millet (*Pennisetum americanum* L.) and guar (*Cyamopsis tetragonolobus* L.). However, seed germination and seedling growth bio herbicidal potential of *A. aspera* plant water leachates against *Parthenium hysterophorus* L. in Petri plate germination bioassay and pot-based seedling growth bioassay was also verified by Safdar *et al*., (2016). Srivastav *et al*., (2011) isolated variety of phytotoxic compounds i.e. alkaloids, oleonolic acid, phenolics, dihydroxy ketones, saponins and long chain compounds from *A. aspera* plant. Later on, six more phenolic compounds viz., caffeic acid, gallic acid, chromatotropic acid, syringic acid, *m*-coumaric acid and 4-hydroxy-3-methoxy benzoic acid) in considerably higher amounts were found to be present in aqueous extract derived from *A. aspera* plant (Safdar *et al.*, 2016).

Conclusion

The 10% water extracts of *C. album* and *C. procera* in mixture with that of *D. stramonium* was proved to have higher pre-emergence herbicidal potential against *S. halepense* as these performed better in Petri plate-based germination bioassay. Whereas 10% water extract of *A. aspera* and *C. procera* in combination with *D. stramonium* water extract of same concentration exhibited strong post-emergence herbicidal potential against *S. halepense* due to its higher phytotoxicity as foliar application in pot-based seedling growth bioassay. The phytotoxic properties of these extracts were ascertained due to presence of quercetin, *p*-coumaric acid, chlorogenic acid, caffeic acid, benzoin acid and sinapinic acid as shown by their HPLC analysis. This suggests strong potential of these extracts for the development of bioherbicides.

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