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Research Paper

Allelopathic evaluation of selected plants extract against broad and narrow leaves weeds and their associated crops

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ABSTRACT

Aqueous extract of *Salvia moorcroftiana*, *Verbascum thapsus* and *Chenopodium album* was used to study the effect on germination and growth of selected crops and weeds. Aqueous extract of *S. moorcroftiana* in soil had detrimental effect on weed species. While in crop species, plumule length of wheat was enhanced while radical length of sunflower was reduced. When extract was applied on seedlings in soil survival of weed seedling, their plumule and radical growth were negatively affected while in crops plumule length of sunflower was reduced. Extract of *V. thapsus* on seeds in soil had no affect on germination, plumule and radical length of crops but significant reduction occur in germination and radical length of weeds. No effect on plumule length of *Euphorbia helioscopia* was observed. When applied on the seedling, the survival of wheat and *E. helioscopia* was reduced. When the seeds were used in pre germinated experiment plumule length of *A. fatua* and radical length and percent survival of weeds were significantly reduced. In *C. album* extract, most promising result was the complete inhibition of *A. fatua* on filter paper. All treatments showed most promising results in soil. Even 100% extract of all treatments showed no significant effect on tested crop species, while, significantly affected weed species in seed and seedling stage.

Key words: Allelopathy, wheat, sunflower, *A. fatua, E. helioscopia,* suppression, enhancement.

INTRODUCTION

In crop production, weeds create a serious problem. In Pakistan, the average loss in different crops by weeds is 20-30%, while total monitory losses exceed Rs.120 billion. In major cereal crops, this loss is in the range of 30, 40, 4 and Rs.5 billion for wheat, rice, maize and gram, respectively (Anonymous, 2005). Some weed species cause damage to crops by giving asylum to pest and disease agents (Dangwal et al., 2010). Due to lack of financial resources, the small farmers neither have enough money to remove weeds from their fields nor can they afford expensive synthetic herbicides. This badly influences yield and quality of the crop and increases production costs, resulting in high economic losses (Jabeen and Ahmed, 2009). Weed control by herbicides is expensive practice with detrimental effect on the environment. Allelopathy allows sustainable weed management while reducing the impact of agriculture on the environment (Vincenzo et al., 2008). Beside these negative environmental effects, health and ecological problems related with synthesized pesticides increase the importance of organic agriculture (Dayan et al., 1999). The ability of plants to inhibit germination of other plants is generally defined as allelopathy, but it is least used for weed control in crops. It can be used in both conventional and organic agriculture (Machado, 2007).

In conventional agriculture, weeds can build up resistance to pesticides, making pesticides less effective (Prado and Franco, 2004). Allelopathy is a natural and an environment-friendly approach for weed control, increases crop yields, decreases our dependence on synthetic pesticides, and improves the ecological environment (Hegab et al., 2008). The agro-ecological applications of allelopathy provide alternatives to synthetic herbicides for weed management which increase attention towards allelopathy (Putnam, 1985). The search for natural weed control methods is emphasized world over. Allelopathy has been recognized now-a-days as a natural weed control approach. Different plants possess allelochemicals, which could be utilized for suppressing weeds (Randhawa et al., 2002). About 50, 89 and 112 weed species that may possess allelopathic properties were listed by Putnam (1985), Patterson (1986) and Inderjit and Keating (1999), respectively. *Salvia moorcroftiana* Wall. ex Benth.(Labiatae), a perennial herb commonly known as "sage" is distributed in the temperate Himalayas from Kashmir to Kumaun. It is present in Chitral, Lowari, Jehlum, Quetta and Suliman Range in Pakistan. Several flavonoids are found in this plant. These flavonoids comprise chalcones, uvaritine, isouvaritine and anthocyanines which have anti-microbial, anti- fungal, anti-viral, cytotoxic properties and also inhibited larval growth in insects (Asim, 2002). Crude acetone extracts of aerial parts of *S. moorcroftiana* was found to have strong herbicidal activity against *Leman aequinoctials* at 500 µg/ml which completely repressed its growth (Khan et al., 2002). *Verbascum thapsus* L. commonly known as "mullein" belongs to the family Scrophulariaceae. The plant is herbaceous densely covered with soft grayish-yellow tomentum (Chopra and Siddiqee, 1969). *V. thapsus* is a weed of pastures, abandoned fields, and roadsides. Reproduces only by seed and produce 100,000 to 180,000 seeds per plant. Common mullein is also a host for numerous diseases and insect pests. Hybridization is known within the genus. *V. thapsus* is a declared noxious weed in Colorado and Hawaii. It is also a class C noxious weed in Washington (National Plants Database, 2003). Some *Verbascum* species, such as *V. thapsus* and *V. sinuatum*, have strong allelopathic activity owing to various chemical substances; these substances comprise verbascoside, luteolin, ajugol, sinuatol, aucubin, iridoid glycosides (Sunar et al., 2009). *Chenopodium album* L. commonly known *is* "lamb׳s quarters" belongs to family Chenopodiaceae, growing in fields and waste places. From medicinal point of view the plant is laxative, anthelmintic, digestive, carminative and extract of boiled leaves taken twice a day for 2-3 days is useful against diarrhoea and jaundice (Jhade et al., 2009). *C. album* produces and releases a phenolic acid which adversely affects seed germination, mitotic cell division and coleoptiles elongation of several weeds. They concluded that high leaf extract concentration (30% v/v) decreased *Cuscuta* seed germination while 50% leaf extract reduced *Echinochola* and *Amaranthus* seedling growth (Jafari et al., 2007). The current experiment is to study both stimulatory and inhibitory effect of the aqueous extract and powder of *S. moorcroftiana*, *V. thapsus* and *C. album* on the germination and growth of different plants. The main objective of the study is "identification of water soluble plant-driven natural herbicides exhibiting biological activity at low concentrations".

MATERIALS AND METHODS

Laboratory based experiments were conducted at the Weed Management Programme (WMP) Institute of Plant and Environmental Protection (IPEP) National Agriculture Research Center (NARC) Islamabad Pakistan.

Collection of plants materials

Plants were collected from Dargai District Malakand, identified and preserved in paper bags in the laboratory for further use.

Drying of plant leaves

The leaves were detached from collected plants and dried under shade conditions for 2-3 days and then in an oven at 60°C for 24 h. These dried leaves were ground and then sieved by sifter of mesh size consecutively 20 and 50 mm. The acquired powder was kept in reagent bottles separately till further use.

Methodology

Aqueous extract laboratory bioassay method was used to evaluate the allelopathic activity of leaves of these plants in aqueous form.

Aqueous extract method

In this method, powder of 20 mm mesh size was used to prepare aqueous extract of each plant. Ten gram of each plant material was weighted by using electronic digital balance and was soaked for 48 h in 200 ml of distilled water in a clean beaker (Samreen et al., 2009). After that, each solution was filtered through Whatman's No 1 filter paper in a conical flask. These filtrates were considered as the stock solutions (Barkatullah et al., 2010).

Filter paper

In the first experiment, three concentrations 30, 60 and 100% of each aqueous extract of *S. moorcroftiana*, *V. thapsus* and *C. album* were used on the seed of *A. fatua, E. helioscopia,* wheat (*T. aestivum* Wafaq 2001) and sunflower

(*Helianthus annus* L. local var. K.S.E 7777). Ten seeds of each plant species were placed in each sterilized glass Petri-dish. Then 5 ml of each plant extracts was applied to petri-dishes individually soon after placing the seeds in each petri-dish while distilled water was used as control. The glass petri-dishes were incubated in the growth chamber for 15 days. Each experiment was replicated five times.

Soil

In this experiment, soil weighing 25 g was used as medium in each Petri- dish. Only 100% aqueous extract of each plant was used on direct and pre- germinated seeds of tested weeds and crops.

Direct seeding

In first experiment, ten seeds of each plant species were placed in each Petri-dish (lining with 25 g soil) while only 15 ml of 100% of each plant extracts was applied to Petri-dishes individually soon after placing the seeds in each Petri-dish. Control (distilled water) was also included for comparison.

Pre-germinated seeds

In the second experiment, the plant seeds first were germinated in the growth chamber in control conditions. After three days when the plumule and radical just emerged, the ten seedlings were transferred to each Petri-dish lining with 25 g soil. After that, 15 ml of 100% of each plant extract was applied to Petri-dishes individually soon after placing the seeds in each Petri-dish. Distilled water (15 ml) was used as control in each Petri-dish for comparison. The glass Petri dishes were incubated in the growth chamber for 15 days. Each experiment was replicated five times.

Photography

Plants were selected and labeled for photography before the data were recorded.

Parameters to be calculated

The following parameters were studied during the experiment.

i. Germination Percentage: Seedlings were counted daily after three days and percentage germination was recorded by using the following formula:

$$\text{Percentage germination} = \frac{\text{No. of germinated seeds}}{\text{Total no. of seeds}} \times 100$$

ii. Radical length (cm): Radical length of all the seedlings was measured in centimeter. At the end average per dish was calculated.

iii. Plumule length (cm): Plumule length of all the seedlings was recorded in centimeter. At the end average per dish was worked out.

Statistical analysis

The average data recorded for each replication was subjected to the ANOVA technique using Statistix-9 computer software and the significance was tested by using Least Significant Differences (LSD) at P = 0.05 (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

Aqueous extract method was used to study both stimulatory and inhibitory effect of the aqueous extract of *S. moorcroftiana* (Plate no.1), *V. thapsus* (Plate no.2) and *C. album*(Plate no.3) on germination and growth of *T. aestivum* (wheat), *H. annuus* (sunflower), *A. fatua* (oat) and *E. helioscopia* (Sun spurge).

Effect of different concentrations of *S. moorcroftiana* extract on filter paper

Aqueous extract of 30, 60 and 100% concentrations of S. *moorcroftiana* were used on seed germination, plumule and radical length. Data on percentage seed germination in wheat and sunflower under the different extract concentration of *S. moorcroftiana* on filter paper showed no significant difference as compared to control while A. *fatua* and *E. helioscopia* was significantly reduced in all concentrations. Maximum inhibition was evident in *E. helioscopia* among species. The degree of percent germination inhibition in different species was in the order of *E. helioscopia*> *A. fatua*> Sunflower> Wheat (Figure 1). Present findings are also in agreement with the study of Azizi and Fujii (2006) that undiluted extract of Sage *(S. officinalis)* had a significant inhibitory effect on the seed percentage germination and germination rate for *A. retroflexus.* Plumule length of wheat also did not show any significant difference in the extract but at high concentration its growth was decreased (Table 1). The results are similar to the work of Qasem and Abu-Irmaileh (1985), that shoot and rhizome extract of *Salvia syriaca* L.

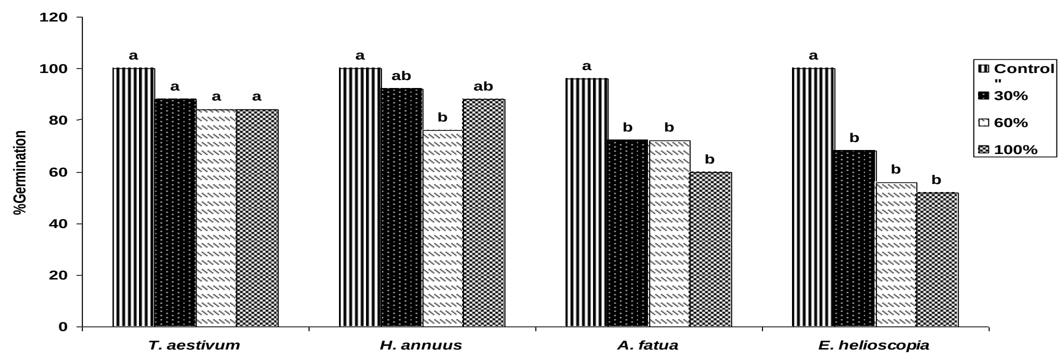

Figure 1. Effect of *S. moorcroftiana* leaf extract on germination of seeds.

Table 1. Effect of *S. moorcroftiana* leaf extract on plumule length of seeds.

	Tested species			
Treatment	***T. aestivum***	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	9.08^{a}	9.91^{a}	3.66^{a}	6.74^{a}
30%	8.18^{ab}	8.88^{a}	3.84^{a}	4.91^{ab}
60%	6.67^{bc}	6.64^{b}	3.52^{a}	4.70^{ab}
100%	5.89^{c}	5.46^{b}	1.06^{b}	3.36^{b}
LSD	1.80	0.39	1.01	2.13
F-value	5.76	49.21	15.40*	3.82*

Means sharing a common letter in the respective column are not significantly different by LSD test at P=0.05.

Table 2. Effect of *S. moorcroftiana* leaf extract on radical length of seeds.

	Tested species			
Treatment	***T. aestivum***	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	11.53^{a}	6.97^{a}	7.78^{a}	1.86^{a}
30%	9.40^{a}	5.98^{b}	6.84^{a}	1.09^{b}
60%	5.99^{b}	3.22^{c}	7.05^{a}	0.86^{bc}
100%	3.71^{b}	2.54^{c}	2.40^{b}	0.42^{c}
LSD	2.98	0.94	2.19	0.60
F-value	12.33*	46.63*	11.15	9.06*

Means sharing a common letter in the respective column are not significantly different by LSD test at P=0.05.

decreased development of wheat seedling and also delayed its germination in laboratory. In case of sunflower, plumule length was not affected in 30% extract but significantly in 60 and 100% concentrations, respectively. There was reduction in plumule length with concentration increase but for *A. fatua* only the highest concentration was effective. The plumule length of *E. helioscopia* was not affected significantly in this extract (Table 2). Broadleaf plants were more sensitive to *S. moorcroftiana* extract than grasses. Even 30% concentration of the extract was effective in reducing radical. In wheat, reduction was evident at 60 and 100% concentrations, while in *A. fatua* at 100% only. Sunflower was badly affected in all extract concentrations (Table 3). The present finding was similar to the work Khan et al. (2002), who reported that *S. moorcroftiana* completely inhibited the growth of *L. aequinoctials* at 500 µg/ml.

Allelopathic effect of different concentrations of *V. thapsus* extract on filter paper

The negative effect of *V. thapsus* extract was found on the

Table 3. Effect of *V. thapsus* leaf extract on plumule length of seeds.

	Tested species			
Treatment	***T. aestivum***	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	9.08^{a}	9.91^{a}	3.66^{ab}	6.74^{a}
30%	7.04^{b}	7.62^{b}	4.02^{a}	5.19^{ab}
60%	5.12^{c}	7.36^{b}	2.20^{c}	3.49^{bc}
100%	4.12^{c}	5.14^{c}	2.38^{bc}	2.46^{c}
LSD	1.40	1.76	1.43	2.24
F-value	22.10*	11.08*	3.67*	6.37*

Any two means not sharing a common letter in column differ significantly at P=0.05 probability level.

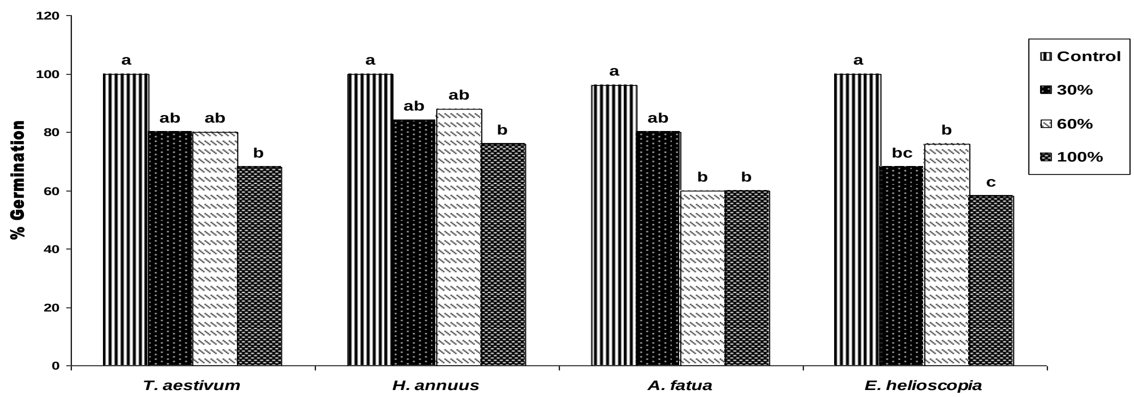

Figure 2. Effect of *V. thapsus* leaf extract on germination of seeds.

Table 4. Effect of *V. thapsus* leaf extract on radical length of seeds.

	Tested species			
Treatment	***T. aestivum***	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	11.53^{a}	6.97^{a}	7.78^{a}	1.86^{a}
30%	7.52^{b}	5.92^{a}	7.75^{a}	0.57^{b}
60%	4.10^{c}	2.97^{b}	4.17^{ab}	0.57^{b}
100%	3.51^{c}	2.15^{b}	3.65^{b}	0.25^{b}
LSD	2.35	1.66	3.83	0.46
F-value	22.30*	17.47*	3.05	21.54**

Any two means not sharing a common letter in column differ significantly at P=0.05 probability level.

germination of wheat, sunflower and *A. fatua* at high concentrations respectively while *E. helioscopia* significantly reduced at all concentrations (Figure 2). Plumule length of crop species in all concentrations was reduced significantly. Effect on *A. fatua* was not clearly significant while *E. helioscopia* plumule length was reduced at higher concentrations (Table 3). Radical length of wheat and *E. helioscopia* was significantly affected in all concentration but sunflower at 60 and 100% extract concentrations. So, 30% of *Verbascum* extract was effective for the control of *E. helioscopia* in sunflower (Table 4).

Allelopathic effect of different concentrations of *C. album* extract on filter paper

In *C. album* extract, germination of wheat was affected only at 100% extract while other species were significantly inhibited by all concentrations (Figure 3). Significance inhibition of plumule was observed in all tested species but maximum inhibition was recorded in *A. fatua.* Plumule was completely inhibited by 60 and 100% extract (Table 5). These results are in line with the findings of Jafari et al. (2007) who reported that high concentration of leaf extract

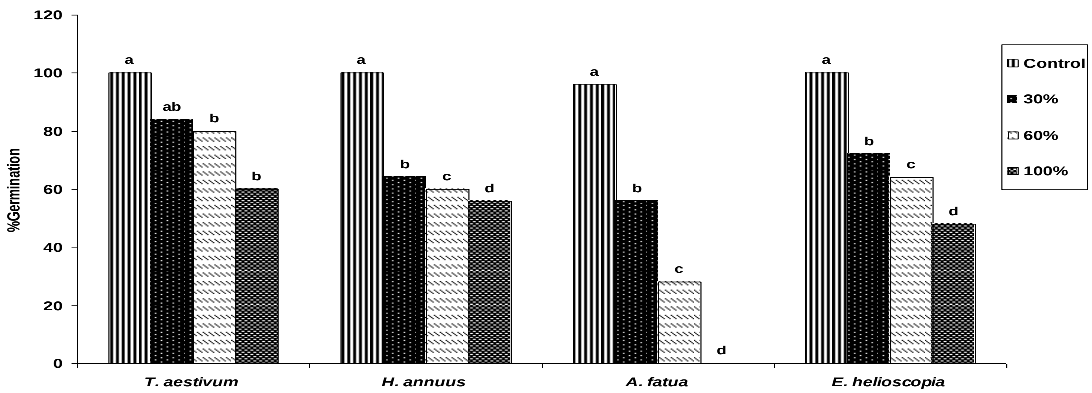

Figure 3. Effect of *C. album* leaf extract on germination of seeds.

Table 5. Effect of *C. album* leaf extract on plumule length of seeds.

Treatment	Tested species			
	T. aestivum	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	9.08^{a}	9.91^{a}	3.66^{a}	6.74^{a}
30%	5.78^{b}	3.46^{b}	0.86^{b}	2.68^{b}
60%	3.06^{c}	3.48^{b}	0.17^{c}	0.00^{c}
100%	1.14^{d}	2.90^{b}	0.00^{c}	0.00^{c}
LSD	1.37	1.64	0.34	0.86
F-value	56.80**	37.17**	222.87**	122.58*

Any two means not sharing a common letter in column differ significantly at P=0.05 probability level.

of *C. album* decreased *Cuscuta, Echinochola* and *Amaranthus* seedling growth. Radical length of all test species was also reduced by all extract concentrations but *A. fatua* was completely inhibited by 100% only (Table 6). Extracts of *C. album* on filter paper reduced the germination, plumule and radical length of weeds and crops in this study. Similar results were reported by Alam et al. (2002) that the leaf extract of *C. album* alone or in combination with NaCl affected shoot and root seedling length of wheat.

Allelopathic effect of different plant extract in soil on direct seeding

In this experiment, only 100% aqueous extract of each plant was studied on direct and pre-germinated seeds of weeds and crops. All treatments showed most promising results in soil. Even 100% extract of all treatments showed no significant effect on tested crop species while significantly reduced weed species. Among the tested species, *A. fatua* showed more inhibition followed by *E. helioscopia*. The degree of percentage germination inhibition in different species was in order of *A. fatua*> *E. helioscopia*> Sunflower> Wheat, while *C. album* showed most promising results among the treatments. The degree of percentage germination inhibition among treatments was in the order of *C. album*> *V. thapsus*> *S. moorcroftiana*> Control (Figure 4). *S. moorcroftiana* extract enhanced plumule length of wheat while no significant difference was observed in *V. thapsus* extract. *C. album* extract showed a decrease in plumule length. In case of sunflower and *E. helioscopia*, plumule length was not reduced in any treatment but in *C. album* extract the growth of *E. helioscopia* declined. In case of *A. fatua* the plumule length was significantly decreased in all extract concentrations in order of *C. album*>*S. moorcroftiana*>*V. thapsus*> Control (Table 7). Radical length of tested crop species showed no significant difference among all treatments, while in the weed species it was significantly reduced. *C. album* indicated detrimental effect on *A. fatua* and *E. helioscopia* among treatments. The degree of radical length inhibition in *A. fatua* among the treatments was in the order of *C. album*> *S. moorcroftiana*> *V. thapsus*> Control. Among test species, the highest inhibition was in the order of *A. fatua* > *E. helioscopia*> Sunflower > Wheat (Table 8).

Allelopathic effect of different plant extract in soil on seedling stage

Survival of wheat seedling was affected by *V. thapsus* extract while sunflower was affected by *C. album*. Survival

Table 6. Effect of *C. album* leaf extract on radical length of different species.

Treatment	Tested species			
	T. aestivum	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	11.53[a]	6.97[a]	7.78[a]	1.86[a]
30%	4.28[b]	1.58[b]	2.33[b]	0.58[b]
60%	1.79[c]	1.96[b]	0.38[c]	0.09[c]
100%	0.34[c]	0.87[b]	0.00[c]	0.08[c]
LSD	1.58	1.33	1.81	0.37
F-value	89.05**	39.42**	35.17**	45.24**

Any two means not sharing a common letter in column differ significantly at P=0.05 probability level.

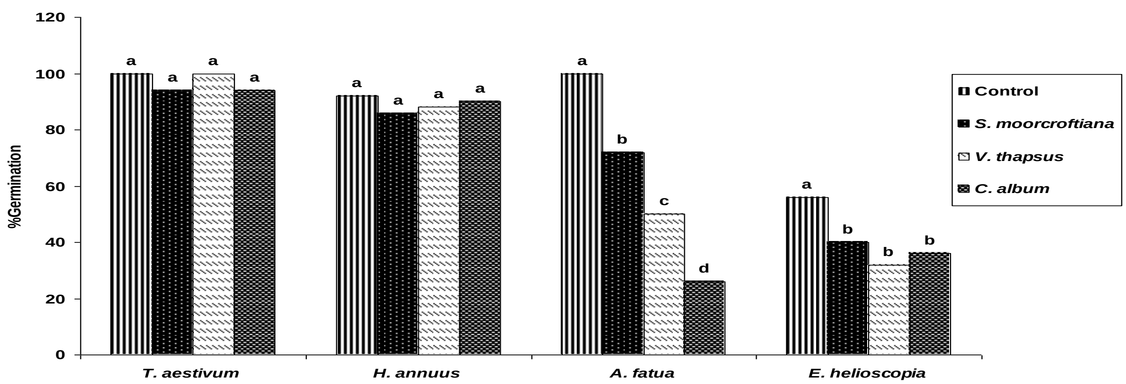

Figure 4. Effect of different plant extracts on germination of seeds in soil.

Table 7. Effect of different plant extracts on plumule length of seeds in soil.

Treatment	Tested species			
	T. aestivum	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	15.12[ab]	13.56[ns]	7.86[a]	6.35[a]
S. moorcroftiana	16.86[a]	12.58	3.90[b]	4.13[b]
V. thapsus	13.28[ab]	12.45	4.54[b]	4.46[ab]
C. album	11.90[b]	10.93	0.14[c]	2.60[b]
LSD	3.86	2.69	0.95	2.09
F-value	2.82	1.46	99.16**	4.85**

Any two means not sharing a common letter in column differ significantly at P=0.05 probality level. ns: non significant.

Table 8. Effect of different plant extracts on radical length of seeds in soil.

Treatment	Tested species			
	T. aestivum	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	8.49[ns]	10.90[a]	9.64[a]	2.50[a]
S. moorcroftiana	7.89	8.04[b]	1.68[c]	0.23 [b]
V. thapsus	8.34	8.89[ab]	3.80[b]	0.30[b]
C. album	7.89	9.52[ab]	0.07[d]	0.08[b]
LSD	0.96	2.46	0.75	0.55
F-value	0.93	2.16	282.29**	39.58**

Any two means not sharing a common letter in column differ significantly at P=0.05 probability level ns: non significant.

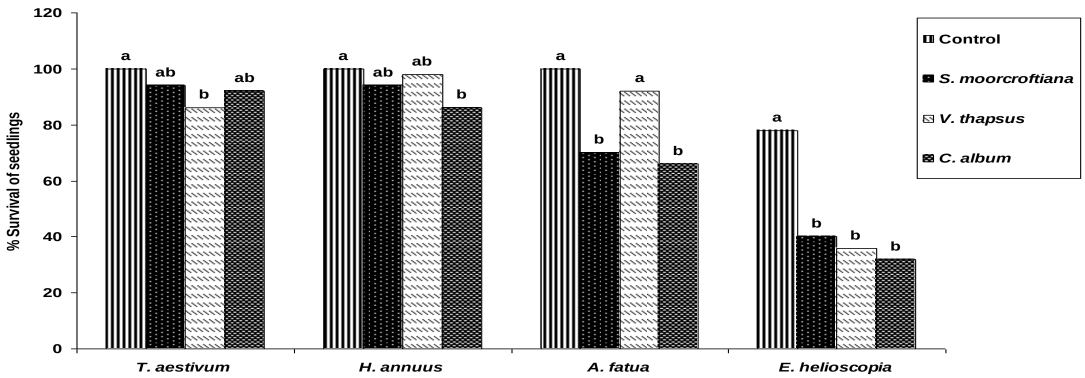

Figure 5. Effect of different plant extracts on survival of seedling in soil.

Table 9. Effect of different plant extracts on plumule length of seedling in soil.

Treatment	**Tested species**			
	T. aestivum	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	9.08 ns	3.06 a	13.53 a	6.02 a
S. moorcroftiana	7.74	2.16 b	9.65 b	3.09 b
V. thapsus	7.70	2.40 ab	11.89 ab	3.51 b
C. album	8.76	2.49 ab	10.07 b	1.02 c
LSD	1.46	0.83	3.41	0.98
F-value	2.10	1.90	2.45	39.65**

Any two means not sharing a common letter in column differ significantly at P=0.05 probability level. ns: non significant.

Table 10. Effect of different plant extracts on radical length of seedling in soil.

Treatment	**Tested species**			
	T. aestivum	***H. annuus***	***A. fatua***	***E. helioscopia***
Control	8.15 ns	3.17 ns	5.79 a	1.63 a
S. moorcroftiana	7.33	2.85	2.75 bc	0.46 b
V. thapsus	6.99	2.88	3.77 b	0.67 b
C. album	7.24	2.70	2.16 c	0.02 c
LSD	1.25	0.99	1.14	0.32
F-value	1.46	0.36	17.52*	39.75**

Any two means not sharing a common letter in column differ significantly at P=0.05 probability level. ns: non significant.

of *A. fatua* seedling was affected in *S. moorcroftiana* and *C. album* extracts. *E. helioscopia* seedling survival was reduced in all treatments otherwise; survival of *E. helioscopia* seedling was very difficult because its seedlings were sensitive to disturbance (Figure 5). Similarly no significant effect was observed on wheat while *A. fatua,* sunflower and *E. helioscopia* plumule length was reduced in *S. moorcroftiana* which showed that plumule length of broadleaf plant seedling was more sensitive (Table 9). Present findings are similar with the work of Qasem (2001) who reported that foliage leachates or root exudates of *S. syriaca* L. reduced seedling growth of broadleaf tomato and cabbage when mixed into soil. *V. thapsus* only reduced *E. helioscopia* while *C. album* extract was negatively affected by both weeds while no significant effect on crop species was observed. Radical length of tested crop species of seedling showed no significant difference among all treatments, while weed species were significantly reduced. *C. album* showed depressing effect on *A. fatua* and *E. helioscopia* among treatments. The degree of radical length inhibition among treatments was in order of *C. album*> *S. moorcroftiana*> *V. thapsus*> Control. Among the test species, most inhibition was in order of *E. helioscopia*>*A. fatua*>Sunflower>Wheat (Table 10). Aqueous extract and leaf powder of all treatments have the ability to suppress germination, root and shoot length of *A. fatua* and *E. helioscopa,* but have no detrimental effect on *T. aestivum* and *H. annuus.* However, aqueous extracts and leaf powder of *C. album* and *S. moocroftiana* have strong allelopathic properties which can successfully inhibit germination and growth of *A. fatua* and *E. helioscopa* in *T. aestivum* and *H. annuus.*

Conclusion

Further study should be conducted under field conditions on *C. album* and *S. moocroftiana* leaf extracts and powder before they can be utilized as natural environment- friendly weedicide for the control of noxious weeds in field crops.

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