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Allelopathic effects of aqueous extract of liquorice (*Glycyrrhiza glabra* L.) on seed germination and seedling growth of wheat and some weed species

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Abstract

A laboratory experiment using completely randomized design with three replicates was conducted in April 2015, to evaluate the germination and seedling growth of three plant types; Wheat (*Triticum aestivum* L.), Wild oat (*Avena fauta* L.) and Milk thistle (*Silybum marianum* L.) seeds were soaked in distilled water (control), 30 and 50% of liquorice (*Glycyrrhiza glabra* L.) leaf and root aqueous extracts to study the allelopathic potential effects of them. Whereas liquorice leaf and root aqueous extracts concentrations increased from 30 to 50%, the inhibitory effect of allelopathic materials on germination, growth, and seed vigour index, fresh and dry weight of seedling also increased. The results showed that *S. marianum* was the most sensitive to liquorice leaf and root extracts than *A. fauta.,* although both were most sensitive to leaf and root extract. Wheat seeds were the most resistant to liquorice leaf and root extracts than both weed seeds. Leaf and root aqueous extracts showed non-significant different between them for all studied traits. The strong negative relationship was observed among extract concentrations and studied parameters.

Introduction

Conventional methods of weed control are although dependent, but costly and labour intensive. Indiscriminate use of chemicals for controlling weeds may pose environmental problems *[1]*. High degree of weed infestation can cause significant lowering of quantity and quality of production *[2]*. One of the alternatives to overcome these problems is strategies which employ allelopathy, and the use of bio-herbicides for weed management and sustainability of agriculture *[1]*. Liquorice is known to contain water soluble substances that are allelopathic to other plants, and the root of this species is the source of liquorice commercial official in the pharmacopoeias. Iraq, exports liquorice to a certain extent, so far, the exports of drug is limited to Mousl and Kirkuk areas, and both varieties Russian, and Persia liquorice are found in Iraq, but the larger Glycyrrhiza- growing areas in Sulaimani and Erbil districts remain yet unutilized. The use of allelopathy against weeds can be used through biotechnological approaches and exploitation of allelopathy provides unlimited opportunities to contribute to the solution of agricultural problems *[3]*. Reviews of allelopathy are found the residues of several crops have phytotoxic activity on other plants *[4]*. Laboratory studies have often demonstrated allelopathy, but the evidence produced should not be regarded as conclusive of the existence of allelopathy in the environment until it is confirmed by field studies *[5]* and *[6]*. Norsworthy (2003) *[7]* indicated that aqueous extracts of wild radish or incorporated residues suppressed seed germination, radical growth, seedling emergence, and growth of certain crops and weeds. According to

Duke et al. (2002) *[8]* proposed that some of the allelochemical compounds may be useful directly as herbicides or as a template for herbicide development. Production of allelochemicals can occur in any plant organ, but roots, seeds, and leaves are the most common sources for exploitation of allelochemicals, and the amount of their part is important for control purpose *[9]*. Allelochemicals can be produced by weeds and affect crops, but the reverse is also true *[4]*. Wheat is widely grown, almost in all regions in Iraq, conversing un-irrigated area of 1.7 M ha, with the production of 1.94 tonnes/ha *[10]*. Wheat crop usually suffers from stress created by weeds through competition for growth requirement and cause interference by releasing toxic substances into the rhizosphere of the crop plant *[11]*. It was suggested that wheat produces an allelochemical that inhibits the emergence of several broadleaved species *[6]*. So, keeping in view the above facts, the present study was planned to examine the perception of laboratory experiment regarding effect of liquorice root and shoot aqueous extracts on germination and seedling growth of wheat, Oat and milk thistle seeds, as well as give away policy recommendation for effective allelochemical finding of appropriate herbicides on the basis of the research findings.

Materials and Methods

To study the allelopathic effect, different parts of liquorice extracts on seed germination and seedling growth of wheat (*Triticum aestivum* L.), wild oat (*Avena sativa* L.) and milk thistle (*Silybum marianum* L.) are tested. An experiment was conducted based on a completely randomised design with three replicates, in the laboratory of the College of Agricultural Sciences, University of Sulaimani – Iraq in 2015. The test was performed as the following:

A. Preparing of water extracts solution

The whole plant of liquorice was collected from Bakrajo's farms at maturity stage. Leaves and roots of plants were separated. The fresh materials were ground in a grinder and passed through a 40mesh screen. To prepare the extract, of each plant parts 100 ml of distilled water with 10gm obtained from grinded fresh substances of organs were separately mixed and put for 24 hours via Shaker device that operate at 300 rpm performance and subsequently after passing materials through a Whatman filter paper No.1 the obtained substances were diluted in order to achieve the desired treatments. Treatments concentrations were prepared using control 0%, 30% and 50% of the original dosage for fresh material. Wheat Aras cultivar and tested weed seeds were sterilised with sodium hypochlorite (1%) and washed thrice with distilled water. Ten seeds of wheat, wild oat and milk thistle were sown in 9 cm Petri dishes lined with filter paper, and 10 ml of each treatment solution was added, while autoclaved distilled water was used as a control. Seeds were incubated to germinate at 25 ± 1°C for 10 days. Germination was calculated when the roots were2 mm long *[12]*.

B. Collection of data

Germination percentage was registered every 24 hours for 10 days. Root and shoot length of seedling and seedling fresh and dry biomass were calculated at the end of the experiment. Seed vigour index was calculated according to Abdul-Baki and Anderson (1973) *[13]* by multiplying mean of germination percentage with mean seedling length.

C. Statistical analysis

The experimental design was factorial, contains three factors (2×3×3), arranged in a completely randomized design with three replicates .Data were analysed statistically using analysis of variance with XLSTAT version 11. Comparisons of the treatment mean were performed using a Duncan's multiple choice. The differences were accepted as significant if $P < 0.05$.

Results and Discussion

Germination Test (%)

The effect of leaf and root aqueous extract concentrations, on wheat and weed seeds germination percentage is shown in (tables 1 and 2). Plant part extracts (leaf and root) resulted in non-significant ($P<0.05$) between them on germination percentage was 42.96 and 40.74 %, respectively. A similar finding was observed by Mubeen et al. (2011) *[14]* , who indicated that soaking of rice seed in water extracts of root and leaf of *Dactyloctenium aegyptium* L. had same effects. It can be inferred from the data presented in (table 2) that germination percentage reduced significantly from 62.22 to 30.00% as aqueous extract concentration increased from Zero to 50%. This might be because liquorice, release allelochemical compounds like tannins, wax, flavonoides, glycyrrhizic acid and phenolic acids into the environment imparting significant interference on the growth and development of neighbouring weeds and plants. These results are agreed with the finding of Putnam and Duke, (2002) *[15]*; Bora et al., (2007) *[16]* and Fag and Stewart, (1994) *[17]*, who reported that the inhibitory effect of leaf and root extracts on germination of some crops was proportional to the concentration of the extract. The effect of plant types is shown in (table-2). Wheat had significantly higher seed germination percentage than wild oats and wild milk thistle seeds; it was 83.88, 15.55 and 26.11%, respectively. This result could be attributed to wheat seed ability to create chemical materials which increase allelopathic potential against liquorice leaf and root extracts. These results were supported by Inderjit et al., (2001) *[6]*, Zimdahl, (2007) *[4]* and Jalali et al., (2012) *[9]* , who suggested that wheat, produced an allelochemical that inhibits the emergence of several weed species. The interaction between all categories was observed for germination percentage. The original data points, however, root x wheat x controls 96.66% interaction gave significantly higher germination percentage.

Shoot Length (cm)

Table (1) showed a significant effect of liquorice extracts on test seeds shoot length. Shoot length for wheat increased significantly by 5.961 and 8.271% compared with wild oat and wild milk thistle, respectively (Table3). These results could be due to that, the allelopathic effect by various parts of liquorice extract on weed seedling growth through decreasing photosynthesis and respiration, and finally reducing the permeability of cell membrane hence inhibiting the enzymes activity. This agree with the results that reported by (Katoch et al., 2012) *[18]* , who observed a significant stimulation effect on the plumule length of wheat seedling when wheat seeds were exposed to Eupatorium adenphorum aqueous extract (50 %) as compared to the respective control. The results also showed a significant reduction in shoot length with an increase in aqueous extract concentrations (Table 3). Increasing concentrations from 0 to 50 reduced shoot lengths from 7.097 to 1.940 cm, respectively. The reason may be related to the responsibility of liquorice aqueous extract in inhibiting the growth of seedling shoot length by encouraging the production of cell division inhibitor hormones. This is confirmed by the significant negative value of the correlation coefficient between concentrations and the shoot length reaching to (R^2=0.992) (Fig. 1A). A similar finding was observed by (Ankita and Mittal, 2012) [19]. The results also showed significant increasing of shoot length in term of the interaction. The values of interaction were ranged from 11.711 cm (Leaf x Control x Wheat) to 0.000 cm (Root x C_{50} x Milk thistle). Non-significant differences effect between leaf and root of liquorice extract for shoot length were observed.

Table - 1: Analysis Of Variance Of Different Studied Traits.

		Mean Squares					
Source	***df***	***Germination%***	***Shoot length***	***Root length***	***Vigour Index***	***Fresh plant weight***	***Dry plant weight***
Plant parts	*1*	*66.667ns*	*0.0951ns*	*4.497ns*	*101.635ns*	*0.088ns*	*0.012ns*
Concentrations	*2*	*5651.851***	*122.168***	*262.484***	*29230.272***	*6.556***	*0.030ns*
Plant type	*2*	*24357.407***	*327.858***	*39.494***	*69476.061***	*17.853***	*0.290***
Plant parts*Concentrations	*2*	*88.889ns*	*7.600ns*	*32.575***	*3067.448**	*0.038ns*	*0.024ns*
Plant parts*Plant type	*2*	*72.222ns*	*19.028***	*19.871***	*4322.153***	*0.139ns*	*0.0092ns*
Concentrations*Plant type	*4*	*474.074**	*28.938***	*19.871***	*2408.380***	*0.354ns*	*0.0143ns*
Plant parts*Concentrations*Plant type	*4*	*344.444**	*3.153ns*	*11.908***	*605.948ns*	*0.301ns*	*0.0069ns*
Error	*36*	*103.704*	*2.689*	*2.800*	*607.139*	*0.288*	*0.012*

*: Significance at 0.05 level
**: Significance at 0.01 level
Ns: Non-significance

Table- 2: Effect Of Plant Parts, Aqueous Extract Concentrations Of Liquorice, Plant Types And Their Interactions On Germination Percentage Of Wheat And Two Weed Species Seeds

Plant Parts	Extract concentrations (%)	Wheat	Wild Oat	Wild milk thistle	Plant part effect	Extract concentration effect
	0	93.33ab	23.33f	60.00de		62.22A
Leaf	30	83.33abc	10.00fg	10.00fg	42.96A	
	50	76.67bcd	3.33g	6.66fg		33.33B
	0	96.66a	50.00e	50.00e		
Root	30	83.33abc	6.67fg	6.67fg	40.74A	30.00B
	50	70.00cd	0.00g	23.33f		
Plant type effect		83.889A	15.556C	26.111B		

Data followed by the same letter (s) (Capital letters for the main effects; Plant parts, concentration, and Plant types, and small letters for the interaction effects) are not differ significantly at P≤ 0.05.

Root Length (cm)

Data in (Table 4), indicated that wheat seeds recorded the highest value of 5.746 cm root length compared with wild oats and wild milk thistle which was 3.228 and 3.135 cm, respectively. The tested crop and weed species could be responded differently by the extracts. These results are supported by (Fag and Stewart, 2005) *[17]*, who reported that the different response between test seeds related to allelochemicals. The influence of extract concentrations on root length is shown in (Table 4), which clarified that the highest concentration of 50% significantly reduced length root value 1.527 cm compared to distilled water treatment which recorded the highest root length 8.431 cm. This could be assigned to negative influences of the liquorice concentrations as it increased from zero to 50%. The decreasing response of root length observed with increasing concentration percentage is in agreement with (Putnam and Duke, 2002 *[15]*; Mubeen et al., 2011*[14]* and Navaey et al., 2013 *[20]*). This confirms by the significant negative relationship coefficient between root length and concentration percentage which was (R^2=0.897) (Fig. 1B). The data obtained clearly showed non-significant effect between leaf and root aqueous extracts of liquorice on root length trait were 3.748 and 4.325 cm, respectively (Table 4). This could be resulted from reflected effects of those parts on germination and shoot length traits. In respect of interaction effect, data obtained revealed a significant effect of the interaction (Table 4). Using root x control x wild milk thistle gave the highest values for root length (12.188 cm), while the interaction root x concentration $_{50\%}$ x wild milk thistle revealed minimum value of root length (0.000 cm)

Table- 3: Effect Of Plant Parts, Aqueous Extract Concentrations Of Liquorice, Plant Types And Their Interactions On Shoot Length (Cm) Of Wheat And Two Weed Species Seeds.

Plant Part	Extract concentration (%)	Wheat	Wild Oat	Wild milk thistle	Plant part effect	Extract concentration effect
Leaf	0	11.711a	5.583d	1.877e	4.345A (Leaf)	7.097A(C0)
	30	11.844a	0.444e	0.711e		
	50	6.422cd	0.00e	0.511e		3.871B(C50)
Root	0	11.033ab	10.822ab	1.555e	4.261A (Root)	
	30	8.561bc	1.666e	0.00e		1.940C(C50)
	50	4.711d	0.00e	0.00e		
Plant type effect		9.047A (wheat)	3.086B (Wild type)	0.776C (Wild milk thistle)		

Data followed by the same letter (s) (Capital letters for the main effects; Plant parts, concentration, and Plant types, and small letters for the interaction effects) are not differ significantly at P≤ 0.05.

Vigour Index

The results in (Tables 1 and 5) showed that the plant parts and extract concentration significantly affected the vigour index. Wheat seeds registered the highest vigour index value 128. 443 compared with 24.220 and 17.744 for wild oat and milk thistle, respectively. The reduction in seed vigour index of tested weeds indicates the accumulation of toxic allelochemical substances of the donor plant, which is harmful to the germination and growth of seedling for weed plants. This is in agreement with Katoch et al., (2012) *[18]* and Ghafarbi et al., (2012) *[21]*. The results showed a significant decrease in vigour index with an increase in the aqueous extract concentrations (Table 5). Increasing concentration from 0 (control) to 50% decreased the vigour index from 101.633 to 23.590. Vigour Index largely affected by high concentration, the reason could be resulted to suppressive effects with an increase in extract concentrations. Researchers indicated that the effect of plant extracts depended very much on their concentrations. Similar observation was found by Turk and Tawaha (2002) *[22]*. The effect of liquorice parts (root and shoot) had a non-significant effect on vigour index, were 55.431 for leaf while it was 58.175 for root extract. This result was in accordance with Mubeen et al. (2011) *[14]* ,while Ghafarbi et al. (2012) *[21]* recorded reversible results, who stated that the radicle growth was more sensitive to allelochemicals than the coleoptile growth. The interaction between all categories was observed for vigour index. The original data points, however, revealed that the following interaction gave significantly higher vigour index; leaf x wheat x control (194.177).

Table - 4 : Effect Of Plant Parts, Aqueous Extract Concentrations Of Liquorice, Plant Types And Their Interactions On Root Length (Cm) Of Wheat And Two Weed Species Seeds.

Plant Part	Extract concentration (%)	Wheat	Wild Oat	Wild milk thistle	Plant part effect	Extract concentration effect
Leaf	0	8.977bc	6.777cd	4.044de	3.748A	8.431A
	30	6.455cd	0.322f	0.655f		
	50	4.577de	0.00f	1.922ef		2.150B
Root	0	6.944cd	11.655ab	12.188a	4.325A	
	30	4.855de	0.616f	0.00f		1.527B
	50	2.666ef	0.00F	0.00f		
Plant type effect		5.746A	3.228B	3.135B		

Data followed by the same letter (s) (Capital letters for the main effects; Plant parts, concentration, and Plant types, and small letters for the interaction effects) are not differ significantly at $P \leq 0.05$.

Table - 5 : Effect Of Plant Parts, Aqueous Extract Concentrations Of Liquorice, Plant Types And Their Interactions On Vigour Index Of Wheat And Two Weed Species Seeds.

Plant Part	Extract concentration (%)	Wheat	Wild Oat	Wild milk thistle	Plant part effect	Extract concentration effect
Leaf	0	194.177a	27.022fg	34.366fg	55.431A	101.633A
	30	150.688bc	1.488g	1.366g		
	50	87.333de	0.000g	2.433g		45.184B
Root	0	173.688ab	112.244cd	68.300ef	58.175A	
	30	112.944cd	4.566g	0.000g		23.590C
	50	51.777ef	0.000g	0.000g		
Plant type effect		128.443A	24.220B	17.744B		

Data followed by the same letter (s) (Capital letters for the main effects; Plant parts, concentration, and Plant types, and small letters for the interaction effects) are not differ significantly at $P \leq 0.05$.

Seedling fresh weight (g)

Seedling fresh weight of tested seeds was significantly affected by plant parts of liquorice aqueous extracts (Tables 1 and 6). Wheat seeds experienced significantly higher seedling fresh weight (2.186 g) compared to wild oat (0.491 g) and (0.431 g) of milk thistle seeds, respectively. This was because of increased its roots and shoots length (Table 2 and 3). This is in accordance with the results reported by Mubeen et al. (2011) *[14]*, who reported that the reduction in seedling fresh weight was due to a reduction in root length and thickness. Seedling fresh weight decreased by 68.79% when the extract was used in a concentration of 50% comparing with no extract. Increasing the concentration led to decrease all the categories under this study. The effect of concentrations on seedling fresh weight is similar to the results obtained by Putnam and Duke, (2002) *[15]* and Navaey et al , (2013)*[20]*). This result is supported by the significant negative relationship between the seedling fresh weight and concentrations which amounted to (R^2=0.985) (Fig. 2A). It was found from comparison pair-wise that the effect of liquorice plant parts, between roots and leaves extracts on SFW was not significant which they recorded 0.995 and 1.077g , respectively. The results also indicated that there was significantly higher interactions effect for leaf x wheat x control (3.21g). .

Seedling dry weight (g)

Table (7) showed that plant types and extract concentrations had a significant effect on seedling dry weight. The higher value was 0.273 (g) for wheat seeds while it was 0.099 and 0.026 (g) for wild oats and wild milk thistle, respectively. Zimdahl, (2007) *[4]* and Jalali et al., (2012) *[9]* reported an increase of seedling dry weight in wheat seedling compared with those of weeds. The highest seedling dry weight for wheat could be attributed to an increasing in wheat shoot length 9.047 cm and 5.746 cm for root length (Table 3 and 4). Aqueous extract concentrations of liquorice had significantly affected on seedling dry weight (Table 7). The seedling dry weight reduced from 0.168 to 0.087 (g), (48.21%) when the concentration raised from 0 to 50%. The reducing response of seedling dry weight observed with increased concentrations is in agreement with Ghafarbi et al. (2012) *[21]* and Mlakar et al. (2012) *[23]*. Increasing the concentration led to a reducing shoot and root length and this leads to a reduce in light interception and thereby reduces the efficiency of photosynthesis which reduces the dry weight of seedling. This trend supports the significant negative relationship between the fresh and dry weight of seedling which was R^2=0.902 (Fig. 2B). Among regression analyses of extract concentration with all parameters studied, the highest value of regression coefficient (b=7.868) was observed with root length, whereas the lowest value of regression coefficient (b=0.174) was seen with seedling dry weight (Fig. 1 and 2). The results also indicated that there was significant higher interaction for leaf x $C_{(30)}$ x wheat (0.436 g).

Table- 6 : Effect Of Plant Parts, Aqueous Extract Concentrations Of Liquorice, Plant Types And Their Interactions On Seedling Fresh Weight (Gm) Of Wheat And Two Weed Species Seeds

Plant Parts	Extract concentrations (%)	Wheat	Wild Oat	Wild milk thistle	Plant parts effect	Extract concentrations effect
Leaf	0	3.210a	0.643ef	1.223de	1.077A	1.705A
	30	2.156bcd	0.603ef	0.050f		0.871B
	50	1.583cde	0.093f	0.126f		0.532B
Root	0	2.640ab	1.326cde	1.186de	0.995A	
	30	2.246bc	0.170f	0.000f		
	50	1.276de	0.113f	0.000f		
Plant type effect		2.186A	0.491B	0.431B		

Data followed by the same letter (s) (Capital letters for the main effects; Plant parts, concentration, and Plant types, and small letters for the interaction effects) within are not differ significantly at P≤ 0.05.

Table- 7: Effect Of Plant Parts, Aqueous Extract Concentrations Of Liquorice, Plant Types And Their Interactions On Seedling Dry Weight (Gm) Of Wheat And Two Weed Species Seeds

Plant Part	Extract concentration (%)	Wheat	Wild Oat	Wild milk thistle	Plant part effect	Extract concentration effect
	0	0.290ab	0.120b-f	0.036def		0.168A
Leaf	30	0.436a	0.143b-f	0.010ef	0.148A	
	50	0.216b-e	0.033def	0.043def		0.142AB
	0	0.260abc	0.236bcd	0.066c-f		
Root	30	0.230bcd	0.033def	0.000f	0.118A	0.087B
	50	0.206b-f	0.026def	0.000f		
Plant type effect		0.273a	0.099b	0.026c		

Data followed by the same letter (s) (Capital letters for the main effects; Plant parts, concentration, and Plant types, and small letters for the interaction effects) are not differ significantly at P≤ 0.05.

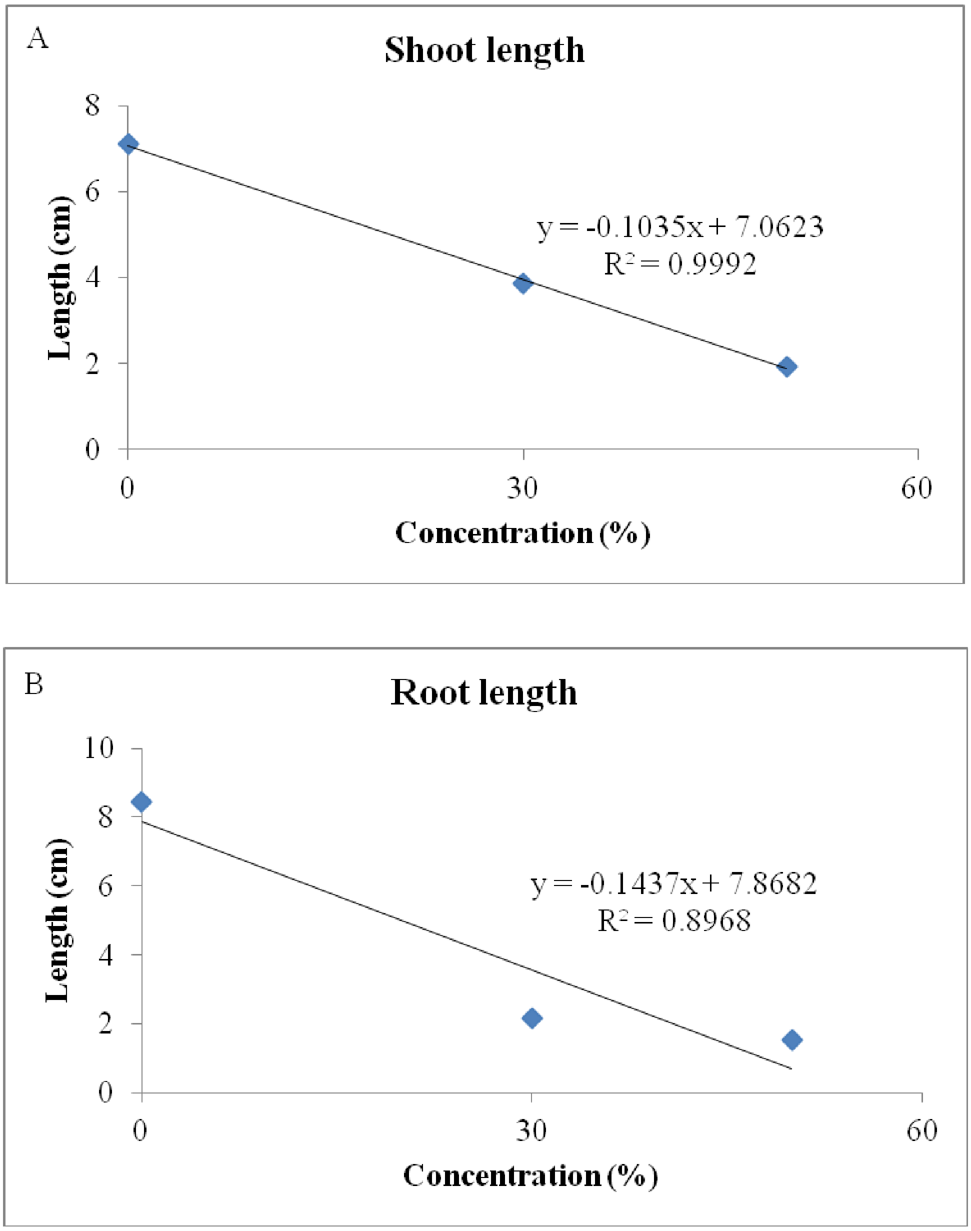

Figure – 1: Regression analysis of shoot (A) and root (B) length of plant species as affected by extract concentration.

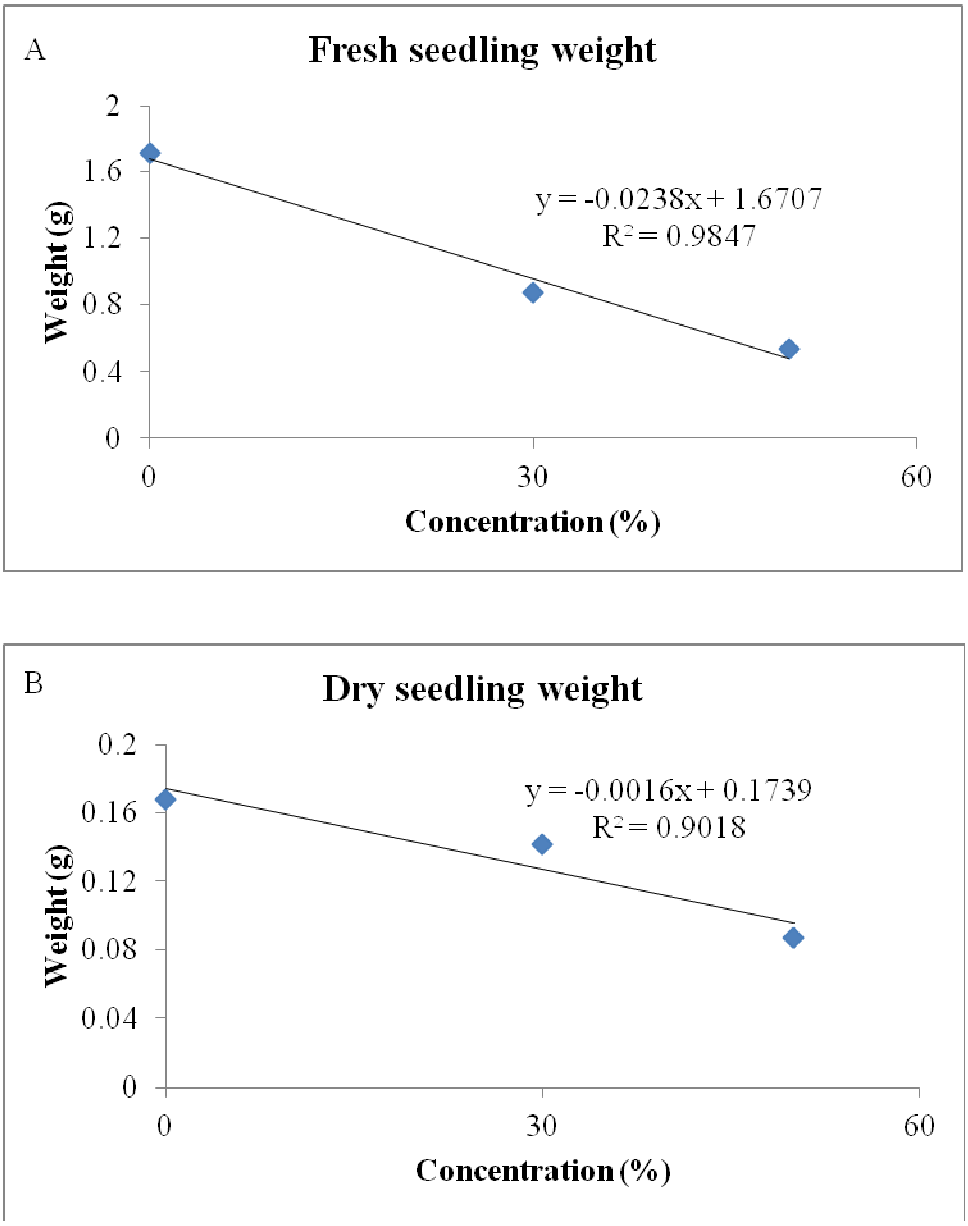

Figure – 2:

Regression analysis of fresh (A) and dry (B) seedlings weight of plant species as affected by extract concentration.

Conclusion

Present results showed that leaf and root aqueous extracts, significantly influences tested seeds germination and seedling growth, but wheat was most resistant than weed species seeds. The degree of this effect responds considerably to extract concentration. Hence, it can be concluded that exist of inhibitory substances in aqueous extract leaves and roots of liquorice would be responsible for the observed effects. So, further investigations are needed to identify the active compound(s) in the liquorice extract responsible for their activity against a wider range of receptor weeds. The effect of Liquorice parts on the germination and seedling growth of wheat crop and associated weeds in the natural environment where additive or synergistic effects become between significant even at low concentrations should also be investigated.

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