Allelopathic Potential of Aerial and Root Extracts of Alfalfa Against Germination and Seedling Related Traits of Four Weed Species.

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Abstract:

In this study, we investigated the effect of aerial and root water extract of Alfalfa (*Medicago sativa*) plant on seed germination and seedling growth of four weed species. This study was carried out in the phytochemistry laboratory/Faculty of Agricultural Sciences/Sulaimani University. Factorial experiment conducted in Completely Randomized Design (CRD) with 3 replications, including five concentrations 2,4,6,8 and 10 g of aerial and root dry tissues of alfalfa (*Medicago sativa* L.) plants per 100ml of MeOH 70% v/v, against germination and seedling growth of four problematic weeds, which widely distributed in wheat fields, namely cephalaria (*Cephalaria syriaca* L.), rye grass (*Lolium perenne* L.), bitter vetch (*Lathyrus linifolius* L.) and black mustard (*Brassica nigra* L.). Results of experiment showed that extracts from dry tissue of aerial and root parts of alfalfa greatly inhibited seed germination and seedling growth of weeds at all concentrations compared to water control. Root extracts had the higher allelopathic potential toward imbibitions and germination of weeds compared to aerial extracts. Root extract reduced imbibitions by 61, 54, 61, 54 % and caused germination inhibition I%, by 51, 37, 34, and 47 % in cephalaria, rye grass, bitter vetch and black mustard respectively. Aerial extracts, significantly reduced hypocotyls, radicle length and dry weight compared to root extracts. The reduction of seedling traits of cephalaria, rye grass, bitter vetch and black mustard respectively due to aerial extracts compared to water control were 52, 52, 32, 35% hypocotyls length reduction, 41, 44, 33, 48% radicle length reduced, 58, 61, 64, 76% hypocotyls dry weight losses and 60, 57, 58, 77% were losses in radicle dry weight. Allelopathicity increased for aerial and root tissues with increase in concentrations and the greatest inhibition was 80% equally for imbibitions and I%, which obtained by 10g dry root tissue per 100 ml MeOH extract occurred in cephalaria, while 10 g dry aerial tissues per 100 ml MeOH extracts shows the highest inhibition percentages of hypocotyls length by 78% in rye grass, radicle length by 79% in cephalaria, hypocotyls dry weight and radicle dry weight by 90% both in black mustard.

Keywords: Allelopathy, Medicinal plants, Saponins, Phenolic compounds, Bio-herbicides, problematic weeds.

1. Introduction

Allelopathy is any process, which involve secondary products of plants, algae, fungi or micro-organisms that effect the growth of surrounding biological system. Allelochemicals are secondary products released by a series of processes such as leaching from plant parts, root exudates and decomposition of plant residues in the soil [1]. Because of that the screening of allelochemicals from medicinal plants is easier than other plants [2], many researchers around the world reported that medicinal plants have growth inhibitory effects towards noxious weed species and successfully acts as bioherbicides [3,4 ,5, 6, 7 and 8]. The land plants, which is formed by weed is only one percentage, but still it can cause severe damage [9, 10], thus the practices of weed control are necessary to avoid yield and quality reduction. The allelopathy is one of them, which is recently ecologically acceptable, and environment friendly method for weed control [11]. Alfalfa (*Medicago sativa* L.) has a long history as a feeding crop for animal [12], while the intake of alfalfa by human is generally very low, but it was reported by [13], that medicinally using alfalfa in the form of tablets or juice or as green salad in the diets, control hypo cholesterol and cardiovascular disease. Alfalfa contains biologically active substances like phenolic compounds mainly chlorogenic and salicylic acids, saponins and toxic amino acids [14, 15]. Regarding to the phenolic compounds [16] recorded that chlorogenic acid occurs in relatively large amount (0.39 mg/g) in fresh alfalfa leaf aqueous extracts as compared to salicylic acid (0.03mg/g), and bioassay suggest that chlorogenic acid is involved in alfalfa autotoxicity. Also it was observed that highest amount of allelochemicals were detected in leaf extract, when [17] determined the autotoxicity of aqueous extract from leaves, stems, roots and seeds of alfalfa. The highest inhibition percentage found from the leaves, extract at 40g/L from alfalfa leaves were 15.4, 17.5 and 28.7 times more toxic to alfalfa root growth than the same concentration of those from roots, stems and seeds, respectively, due to the highest concentration of phenolic compounds observed in aerial parts (leaf and stem) was 5.63 mg g-1 as compared to 1.77 mg g-1 in roots and 0.11 mg g-1 in seeds. The triterpenoid saponins in alfalfa are called monodesmosidic glycosides, which shows biological activity compared to the other type bidesmoside glycoside, which have no biological activity [14]. Saponins leach into the soil from the epidermal cells of root and cause a major reduction in the yield of the crops, are known to inhibit the germination, exerting their action by interfering with oxygen uptake through the seed coat and seed membrane. These effects can be attributed to the surface activity of saponins [18 and 19]. It has been found that different parts of alfalfa plants contain different amount of saponins, the highest content occurred in the root was 30 mg g^{-1} [20]. The concentration and the type of the extracts are important to the suitable performance of the allelopathic potential of a plant [21 and 22] reported that the most reduction on germination, rye grass by extract from alfalfa leaves obtained, compared to other involved plants, and alfalfa extract from vegetative stage had more effect that

reproduction stage. There are many winter weed species have been reported that commonly distributed in wheat farms, but the more problematic weed in our region are; 1- Syrian cephalaria (*Cephalaria syriaca*), which is due to similarity of their fruit to a wheat grain in size and shape, it is difficult to separate from the wheat grain when it is harvested. It is not toxic and adding Cephalaria flour in small amounts improved the rheological properties of wheat flour dough [23], but the contamination of wheat seeds by Cephalaria seeds with 2% decrease the grade and quality due to the bitter test of the wheat flour dough, 2- Rye-grass (*Lolium perenne* L.), it is a fibrous rooted perennial grass that grow in rich soil and considered to be stressful for soil and interfere strongly in competition with surrounding plants, 3- Bitter vetch (*Lathyrus linifolius* L.), which is a noxious weed, seeds and all parts of plant are poisonous if ingested, and 4- Black mustard (*Brassica nigra* L.), which is beside the competition with the wheat crop, it contains glycosinolates, sulfur compounds that can irritate the digestive tract and cause thyroid dysfunction when consumed in large quantities over time. It has been reported that wild mustard transpires about four times more water than a crop plant [24]. The aim of this laboratory bioassay was to evaluate the sensitivity of germination and seedling related traits of those weeds towards different alfalfa plant parts extract and to distinguish the proper concentration to be act as an alternative natural herbicide.

2. Material and Methods

2.1. Sampling and preparation of extracts:

To study the allelopathic effects of *Medicago sativa* L. extract on seed germination and seedling growth, an experiment was carried out in the phytochemistry laboratory/Faculty of Agricultural Sciences/Sulaimani University. Alfalfa (*Medicago sativa* L.) was selected as a test plant, which contains allelochemical compounds, while cephalaria (*Cephalaria syriaca* L.), rye grass (*Lolium perenne* L.), bitter vetch (*Lathyrus linifolius* L.) and black mustard (*Brassica nigra* L.), were chosen for bioassay because they are common noxious weeds. Alfalfa plants were pulled out at a vegetative stage of growth, exactly before flowering from the fields in June 2012. Fresh alfalfa plants were washed by tap water and separated into aerial (leaves and stems) part and roots. These two parts separately were oven-dried at 65 ^{o}C for 72 h. The dried samples were ground to pass a 1 mm sieve then stored in a refrigerator at 2 ^{o}C to avoiding chemical reactions until used. To prepare the concentration of 0.02 extracts, 2 g of each of above dried powdered plant parts was separately soaked in 100 ml of 70% (v/v) aqueous methanol (MeOH) for 48 h, and filtered through whatman no.1 filter paper. The filtrate of each sample evaporated using rotary evaporator at 40 ^{o}C for 2 h or when third of the extract was remained. Repeating this extract using 4, 6, 8 and 10 g of powdered samples represents concentrations of 0.04, 0.06, 0.08 and 0.10 respectively [25].

2.2. Imbibition: One- gram samples of the four weed species were soaked for 24 h in alfalfa aerial and root extracts at the above concentrations. Distilled water was used as the control. After soaking, seeds were taken from

the extracts, blotted for 2 h and re-weighed. The imbibition measured as (final seed weight minus original weight) and expressed in milliliters of water uptake by one-gram of seeds of a weed species.

2.3. Germination and inhibition percentage: The germination test was carried out at room temperature in petridishes of 9 cm diameter placing two layers of whatman no.1 filter paper on petridishes. Twenty seeds of each weed species were placed in a petridish. 10 milliliters of methanolic extract from each treatment combination was added to petridishes and distilled water was used as a control, then distilled water was added to each petridish, whenever it was needed to keep the seed moist enough to get favorable condition for germination, which was measured at the end of 7 days from the starting. Inhibition percentage (I %), calculated by the following formula:

$I\ \% = (1 - G_2 / G_1) \times 100$

G_2: No. of germinated seeds in any extract.

G_1: No. of germinated seeds in the control.

2.4. Seedling trait measurements: The germinated seeds in each petridish, which belong to any treatment combination, were kept and serviced by adding distilled water to maintain the seedling growth up to the end of 14 days from the starting. The hypocotyls and radicles of the seedling were separated to measure their length (cm), then they were oven-dried at 65 ^{o}C for 72 h, weighed, their average dry weight were recorded in (mg).

2.5. Statistical analysis: The whole experiment was factorial conducted in Completely Randomized Design with three replications, ANOVA as a general test for the effect of alfalfa plant parts, concentration of MeOH extracts, and their interactions, while Least Significant Difference ($p \leq 0.01$) used for significant differences between means [26].

3. Result and discussion:

3.1. Imbibition rate: Data in (Table 1) showed significant effect of different extract concentration from dry tissues of aerial and roots of alfalfa on the imbibitions of one-gram of the four species of weeds and it was shown that the highest reduction in imbibitions occurred significantly in root extracts compared to the aerial extracts. The amount of reductions in imbibitions due to socking for 24 h in all root extracts arranged in order were 1.11, 0.99, 0.89 and 0.78 ml in bitter vetch, cephalaria, black mustard and rye grass respectively, in contrast and for the same weed species respectively the reductions in imbibitions were 0.85, 0.77, 0.68 and 0.57 ml due to aerial extracts. Results of the (Table 1), also showed that increasing in extract concentration as averages of both aerial and root dry tissues of alfalfa caused higher and significant reduction in the numbers of milliliters of water imbibited by one-gram of seeds of the different weed species without exceptions. The interactions of different plant extracts and concentrations also were highly significant affected on the imbibitions of weed seeds. The highest significant reduction occurred by 10% MeOH root extract it was exceeded 75% reductions in all weed species

compared to their water control treatment, while the lowest reductions of imbibitions observed in 10% MeOH aerial extracts compared to their water control, which were 17% in cephalaria, bitter vetch and black mustard, while it was only 10% in rye grass. These results may be due to the higher content of saponons and allele-chemicals like flavonoids, phenolic and coumaric acids compounds in the root of alfalfa compared to the aerial part, which was reached up to 30mg g^{-1} according to [19] and saponins due to their hydrophobic property reduced the adsorption and absorption of water by the weed seeds. In addition, primarily saponins were synthesis in leave in palisade cell and then transported to root for storage via phloem. So leaves acted primarily as synthesis, and not storage location. On the other hand, the increase in extract concentrations increase the osmotic pressure of the extract and reduce the rates of imbibitions, these results are in accordance with [15].

Table 1: Effect of alfalfa-parts, concentration of alfalfa-part extracts and their interactions on water imbibitions (ml) by 1g seeds of some weed species.

Weed Species	Alfalfa Part Extracted	Control	Conc. g / 100ml MeOH 70 %					P.P. Mean	$LSD_{0.01}$
			2	4	6	8	10		
Cephalaria	Aerial	1.60	1.33	1.10	0.80	0.50	0.40	0.83	0.11 P.P.
	Root	1.62	0.93	0.83	0.63	0.50	0.33	0.63	0.17 Conc.
Conc. Mean			1.13	0.97	0.72	0.50	0.37		0.24 P.P.*Conc.
Rye grass	Aerial	1.45	1.30	1.20	0.90	0.53	0.47	0.88	0.11 P.P.
	Root	1.43	0.93	0.83	0.63	0.50	0.33	0.65	0.17 Conc.
Conc. Mean			1.12	1.02	0.77	0.52	0.40		0.24 P.P.*Conc.
Bitter Vetch	Aerial	1.80	1.50	1.10	0.83	0.70	0.60	0.95	0.01 P.P.
	Root	1.83	1.40	0.90	0.53	0.40	0.37	0.72	0.16 Conc.
Conc. Mean			1.45	1.00	0.68	0.55	0.48		0.22 P.P.*Conc.
Black Mustard	Aerial	1.53	1.27	1.03	0.90	0.63	0.43	0.85	0.13 P.P.
	Root	1.59	1.17	0.90	0.73	0.50	0.37	0.73	0.21 Conc.
Conc. Mean			1.22	0.97	0.82	0.57	0.40		0.30 P.P.*Conc.

P.P.: Plant Parts, Conc.: Concentrations, P.P.*Conc.: Interaction between Plant Parts and Concentrations, N.S: Not Significant ($\alpha = 0.01$).

3.2. Germination Inhibition percentage I%: Different concentrations of extracts from aerial and roots of alfalfa and their interactions had a significant effect on I% (Table 2). Results showed that I% of the four weed species greatly occurred by the root dry tissue compared to the aerial parts. The reduction in germination of cephalaria was 50.57% occurred by root extract of alfalfa, while the lowest reduction in germination of bitter vetch was 23.21% obtained by aerial extract of alfalfa. The I% increased in the four weed species without exception for all tissues extract with increase in extract concentration from 2 up to 10g per 100 ml of MeOH 70%. I% ranged between 21 and 73% in cephalaria, 16

and 64% in rye grass, 18 and 40% in bitter vetch and 26 and 71% in black mustard, all the high degrees of I% were due to higher concentrations. Finally, data of (Table 2) refers to the significant effects of the interactions of alfalfa part extracts and concentrations on I% for all weed species except bitter vetch, which was not significantly affected. Among the interactions the 10% of dry root tissue extract of alfalfa shows superiority in I%, it was 80% in cephalaria followed by 76% in black mustard and 69% in rye grass, while the lowest effect of this interaction on I% was 45% observed in bitter vetch. These results may be also referred to the higher contents of saponins and some allele-chemicals like flavonoids, scopoletin and coumaric acids compounds in root tissue of alfalfa plants and as it was discussed, the higher concentration of saponins increase the osmotic pressure in an extract and reduce imbibitions, therefore reduce the germination. In addition the biological action of saponins against germination, referred to the interfering with oxygen uptake through the seed coat and seed membrane. These results agreed with [18, 19].

Table 2: Effect of alfalfa-parts, concentration of alfalfa-part extracts and their interactions on germination inhibition percentage (I %) of some weed species.

Weed Species	Alfalfa Part Extracted	Conc. g / 100ml MeOH 70 %					P.P. Mean	$LSD_{0.01}$
		2	4	6	8	10		
Cephalaria	Aerial	17.03	23.67	31.83	40.17	65.67	35.67	1.80 P.P. 2.85 Conc. 4.03 P.P.*Conc.
	Root	24.57	38.10	46.69	63.20	80.27	50.57	
Conc. Mean		20.80	30.88	39.26	51.68	72.97		
Rye grass	Aerial	13.47	18.17	23.37	44.67	59.30	31.79	1.11 P.P. 1.76 Conc. 2.49 P.P.*Conc.
	Root	18.97	22.30	27.77	48.67	69.40	37.42	
Conc. Mean		16.22	20.23	25.57	46.67	64.35		
Bitter Vetch	Aerial	12.63	16.80	20.87	30.07	35.70	23.21	2.48 P.P. 3.92 Conc. (N.S) P.P.*Conc.
	Root	22.60	29.03	33.47	41.20	44.73	34.21	
Conc. Mean		17.62	22.92	27.17	35.63	40.22		
Black Mustard	Aerial	21.77	27.07	34.27	56.17	65.53	40.96	1.36 P.P. 2.15 Conc. 3.04 P.P*Conc.
	Root	31.10	38.27	42.07	48.07	75.73	47.05	
Conc. Mean		26.43	32.67	38.17	52.12	70.63		

P.P.: Plant Parts, Conc.: Concentrations, P.P.*Conc.: Interaction between Plant Parts and Concentrations, N.S: Not Significant ($\alpha = 0.01$).

3.3. Hypocotyl length (cm): All extracts from dry aerial and root tissues of alfalfa reduced hypocotyls length at all concentrations (Table 3). The aerial extracts significantly increase inhibition of hypocotyls length of 14- day-old seedling compared to the root extracts in cephalaria and rye grass, while there difference were not significant toward inhibition of hypocotyls length of bitter vetch and black mustard. Reduction in hypocotyls length due to aerial extracts compared to water control were 52% in cephalaria and rye grass, 32% in bitter

vetch and 34% in black mustard. For all alfalfa organ extracts, hypocotyls length of the four weed species reduces significantly with increase in concentration and at all concentrations the reduction was greatest with dry aerial tissue extracts compared to dry root tissue extracts of alfalfa. Results of (Table 4) showed the highest effect on hypocotyls length , which reached to 78% obtained by 10% aerial extract compared to water control in rye grass and the lowest inhibition was only 6% observed in hypocotyls length of black mustard treated with 2% dry root extract of alfalfa compared to water control. The higher allelopathic potential of aerial (leaf and stem) compared to the roots of alfalfa confirmed by many researchers [16, 17, 19, 21 and 22]. They reported that alfalfa contains relatively large amount of phenolic compounds in the leaf and stem compared to root, especially chlorogenic acid, which is biologically more active than salicylic acid. The degree of allelopathic potential of the aerial and root extract of alfalfa increase with increase in concentration due to more concentration of allelochemical compounds

Table 3: Effect of alfalfa-parts, concentration of alfalfa-part extracts and their interactions on hypocotyls length (cm) of 14- day- old seedling of some weed species.

Weed Species	Alfalfa Part Extracted	Control	Conc. g / 100ml MeOH 70 %					P.P. Mean	$LSD_{0.01}$
			2	4	6	8	10		
Cephalaria	Aerial	5.48	3.87	2.90	2.53	2.23	1.67	2.64	0.18 P.P.
	Root	5.37	4.43	3.90	3.10	2.53	2.23	3.24	0.28 Conc.
Conc. Mean			4.15	3.40	2.82	2.38	1.95		0.40 P.P.*Conc.
Rye grass	Aerial	5.72	4.60	3.63	2.40	1.90	1.27	2.76	0.16 P.P.
	Root	5.68	4.53	3.27	2.70	2.53	2.10	3.03	0.26 conc.
Conc. Mean			4.57	3.45	2.55	2.22	1.68		0.36 P.P.*Conc.
Bitter Vetch	Aerial	4.73	3.80	3.97	3.10	2.70	2.53	3.22	(N.S) P.P.
	Root	4.65	3.80	3.73	3.53	2.77	2.50	3.27	0.32 Conc.
Conc. Mean			3.80	3.85	3.32	2.73	2.52		(N.S) P.P.*Conc.
Black Mustard	Aerial	6.27	5.80	5.03	4.07	3.10	2.53	4.11	(N.S) P.P.
	Root	6.20	5.80	5.03	4.07	3.37	2.60	4.17	0.34 Conc.
Conc. Mean			5.80	5.03	4.07	3.23	2.57		(N.S)P.P.*Conc.

P.P.: Plant Parts, Conc.: Concentrations, P.P.*Conc.: Interaction between Plant Parts and Concentrations, N.S: Not Significant ($\alpha = 0.01$).

3.4. Radicle length (cm): Extracts from dry tissue of aerial and root parts of alfalfa caused a marked reduction in radicle length of 14-day-old seedling of the four weed species (Table 4). The aerial extracts similarly to their effect on hypocotyls length reduction, caused more reductions in radicle length of cephalaria and rye grass compared to root extract i.e. they were more sensitive, while the difference in radicle length reduction of bitter vetch and black mustard were not significant i.e. they were less sensitive toward alfalfa extract. Again, inhibitory effects of different plant part extract of alfalfa increase with increase in

extract concentrations. Data in (Table 4) confirmed that gradually radicle length of seedling of all weed species reduced as extract concentration increased, significant difference in radicle length between minimum concentration and maximum concentration of extracts were 2.61, 3.26, 2.78 and 3.62 cm in cephalaria, rye grass, bitter vetch and black mustard respectively. The effect of interactions between alfalfa organ extract and concentration on radicle length showed highly significant in cephalaria and rye grass, while their effects were not significant in bitter vetch and black mustard. However, the highest inhibition percentages in radicle length compared to water control obtained by extract of 10% dry aerial tissue, were 71%, 65%, 62% and 53% in rye grass, cephalaria, black mustard and bitter vetch respectively, while radicle length inhibitions were lowest with the same concentration of root extracts. Also these results as they were discussed due to higher content of allele-chemical compounds such as benzoxazolinones, L-tryptophan, and coumaric acid in aerial parts of alfalfa compared to the roots and they were agreed with the results that recorded by [16, 17, 19, 21 and 22].

Table 4 Effect of alfalfa-parts, concentration of alfalfa-part extracts and their interactions on radicle length (cm) of 14- day- old seedling of some weed species.

Weed Species	Alfalfa Part Extracted	Control	Conc. g / 100ml MeOH 70 %					P.P. Mean	$LSD_{0.01}$
			2	4	6	8	10		
Cephalaria	Aerial	7.37	5.43	4.97	4.63	4.10	2.60	4.35	0.26 P.P. 0.41 Conc. 0.59 P.P.*Conc.
	Root	7.37	6.53	5.67	5.23	4.13	4.13	5.14	
Conc. Mean			5.98	5.32	4.93	4.12	3.37		
Rye grass	Aerial	7.63	6.10	5.23	4.23	3.40	2.23	4.24	0.20 P.P. 0.32 Conc. 0.45 P.P.*Conc.
	Root	7.50	6.17	4.97	4.17	3.73	3.50	4.51	
Conc. Mean			6.13	5.10	4.20	3.57	2.87		
Bitter Vetch	Aerial	6.53	5.80	5.40	4.13	3.53	3.03	4.38	(N.S) P.P. 0.41 Conc. (N.S) P.P.*conc.
	Root	6.56	5.77	5.33	4.37	3.60	2.97	4.41	
Conc. Mean			5.78	5.37	4.25	3.57	3.00		
Black Mustard	Aerial	8.80	6.97	6.77	5.17	4.10	3.33	5.27	(N.S) P.P. 0.44 Conc. (N.S) P.P.*Conc.
	Root	8.70	7.33	6.53	5.43	4.17	3.73	5.44	
Conc. Mean			7.15	6.65	5.30	4.13	3.53		

P.P.: Plant Parts, Conc.: Concentrations, P.P.*Conc.: Interaction between Plant Parts and Concentrations, N.S: Not Significant ($\alpha = 0.01$).

3.5. Hypocotyls dry weight (mg): According to the results in (Table 5), different alfalfa part extracts was greatly affected the hypocotyls dry weight of the 14-day-old seedlings of the four weed species. Although the influences difference between aerial and root extract were not significant in rye grass and bitter vetch, but still the higher reductions in hypocotyls dry weight were occurred with aerial extracts at all concentrations compared to root extracts. The reduction percentages in dry weight of weeds obtained by aerial extracts were 64%, 62%,

52% and 59% in cephalaria, rye grass, bitter vetch and black mustard respectively. For all alfalfa tissue extract, hypocotyls dry weight decrease as the extract concentration increase in all weed species without exception, the quantity of the reductions caused by highest concentrations compared with their lowest concentrations were 1.22, 1.28, 0.98 and 0.76 mg occurred in cephalaria, rye grass, bitter vetch and black mustard respectively. Although the interaction effect was significant only in cephalaria, but the obtained results confirmed that the highest concentration 10% of aerial dry tissue extract recorded greatest reduction over all weed species, ranging between 87 and 89%. Root extract with 10% concentrations also recorded reductions in hypocotyls dry weight 88% equally occurred in cephalaria and rye grass and 83% in bitter vetch, but it showed only 26% inhibition in dry hypocotyls dry weight compared to water control in black mustard. Generally, the results confirmed that aerial part extracts of alfalfa, which contained higher levels of allelochemicals such as L-tryptophan that had affected on cell division, elongation, cell wall structure and permeability of the membrane, lead to reduce seedling growth, more than that occurred by root extracts and these results in accordance with [19, 21 and 22].

Table 5: Effect of alfalfa-parts, concentration of alfalfa-part extracts and their interactions on hypocotyls dry weight (mg) of 14- day- old seedling of some weed species.

Weed Species	Alfalfa Part Extracted	Control	Conc. g / 100ml MeOH 70 %					P.P. Mean	$LSD_{0.01}$
			2	4	6	8	10		
Cephalaria	Aerial	1.75	1.53	0.93	0.57	0.40	0.23	0.73	0.11 P.P. 0.23 Conc. 0.33 P.P.* Conc.
	Root	1.67	1.37	1.30	1.13	0.43	0.23	0.89	
Conc. Mean			1.45	1.12	0.85	0.42	0.23		
Rye grass	Aerial	1.98	1.50	1.10	0.63	0.40	0.27	0.78	(N.S) P.P. 0.24 Conc. (N.S) P.P.* Conc.
	Root	2.03	1.57	1.20	0.87	0.63	0.23	0.90	
Conc. Mean			1.53	1.15	0.75	0.52	0.25		
Bitter Vetch	Aerial	1.63	1.10	0.93	0.50	0.27	0.13	0.59	(N.S) P.P. 0.27 Conc. (N.S) P.P.* Conc.
	Root	1.60	1.37	0.80	0.57	0.37	0.20	0.66	
Conc. Mean			1.23	0.87	0.53	0.32	0.17		
Black Mustard	Aerial	1.90	0.87	0.67	0.33	0.23	0.13	0.45	0.10 P.P. 0.16 Conc. (N.S) P.P.* Conc.
	Root	1.82	1.40	1.17	0.90	0.57	0.40	0.89	
Conc. Mean			1.13	0.92	0.62	0.40	0.27		

P.P.: Plant Parts, Conc.: Concentrations, P.P.*Conc.: Interaction between Plant Parts and Concentrations, N.S: Not Significant ($\alpha = 0.01$).

3.6. Radicle dry weight (mg): The obtained data from (Table 6) showed that the effects of the aerial part extract of alfalfa on the reduction radicle dry weight of the 14-day-old seedling at all concentrations were greater than root extracts. The reduction percentage respectively for aerial and root extracts compared to water control, were ranging between 69 and 60% in cephalaria, which was not significant, 64 and 49% significant in rye

grass, 58 and 46% not significant in bitter vetch and 60 and 40% significant in black mustard. Again, the reduction in radicle dry weight of the four weed species increase significantly as concentration of the alfalfa extracts increase. The losses due to increase from lowest to highest concentration in aerial extract was 0.47 mg occurred in cephalaria, 0.30 mg in rye grass, 0.37 mg in bitter vetch and 0.33 mg in black mustard. The interactions effect of plant parts with concentrations on radicle dry weight of the weed species were significant except that occurred in cephalaria, but still the greatest loss in radicle dry weights of the four weed species occurred with 10% of aerial extracts of alfalfa, which recorded 90, 84, 86 and 83% reductions in radicle dry weight of cephalaria, rye grass, bitter vetch and black mustard respectively. Radicle dry weight appeared more affected by the aerial extract compared to root extracts of alfalfa at highest concentrations, rather than the hypocotyls dry weight, may be due that the phenolic compounds such as hydroquinone, trans-cinnamic acid, gentisic acid, vanillic acid and syringic acid have greater effects on radicle, rather than hypocotyls. The fact that radicle is the first to come in contact with the allelochemicals may be another attribute factor [17, 19, 21 and 22].

Table 6 Effect of alfalfa-parts, concentration of alfalfa-part extracts and their interactions on radicle dry weight (mg) of 14- day- old seedling of some weed species.

Weed Species	Alfalfa Part Extracted	Control	Conc. g / 100ml MeOH 70 %					P.P. Mean	$LSD_{0.01}$
			2	4	6	8	10		
Cephalaria	Aerial	0.93	0.59	0.51	0.42	0.24	0.12	0.37	(N.S) P.P.
	Root	0.83	0.73	0.63	0.33	0.17	0.10	0.39	0.09 Conc.
Conc. Mean			0.66	0.57	0.38	0.20	0.11		0.12 P.P.* Conc.
Rye grass	Aerial	0.69	0.43	0.47	0.27	0.17	0.13	0.29	0.07 P.P.
	Root	0.73	0.53	0.43	0.40	0.33	0.20	0.38	0.11 Conc.
Conc. Mean			0.48	0.45	0.33	0.25	0.17		(N.S) P.P.* Conc.
Bitter Vetch	Aerial	0.72	0.47	0.45	0.32	0.17	0.10	0.30	(N.S) P.P.
	Root	0.61	0.57	0.47	0.33	0.17	0.10	0.33	0.11 Conc.
Conc. Mean			0.52	0.46	0.33	0.17	0.10		(N.S) P.P.* Conc.
Black Mustard	Aerial	0.98	0.43	0.30	0.20	0.13	0.10	0.23	0.09 P.P.
	Root	0.85	0.53	0.43	0.33	0.30	0.23	0.37	0.14 Conc.
Conc. Mean			0.48	0.37	0.27	0.22	0.17		(N.S) P.P.* Conc.

P.P.: Plant Parts, Conc.: Concentrations, P.P.*Conc.: Interaction between Plant Parts and Concentrations, N.S: Not Significant ($\alpha = 0.01$).

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