

INHIBITORY EFFECTS OF LAVENDER, ABSINTHIUM AND WALNUT ON GERMINATION AND SEEDLING GROWTH OF *Convolvulus arvensis*, *Portulaca oleracea* AND *Triticum aestivum*

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ABSTRACT

One means of reducing utilization of herbicides is allelopathic effect that is found in some plant species. Therefore, allelopathic effects of different doses (0, 5, 7.5 and 9 g dried leaves/plot) of Walnut, Lavender and Absinthium on germination and seedling growth of Convolvulus arvensis, Portulaca oleracea and Triticum aestivum were evaluated in greenhouse conditions. This study was conducted in a factorial experiments based on a randomized complete design with three replications in 2012. The experimental treatments included amounts of allelopathic plants that were mixed with 2 kg soil in each pot. Shoot dry weight, shoot fresh weight, root dry weight, root fresh weight, seedling height, leaf number and germination percent were measured. Results showed that main effects of allelopathy on shoot fresh and dry weight, root dry weight and seedling height were significant ($p < 0.05$). Lavender had more reducing effects than Walnut and Absinthium on all traits. Dose had significant effect on all parameters and higher doses (7.5 and 9 g/plot) were more effective. In general, results showed that all allelopathic plants reduced shoot fresh weight of portulacaoleracea significantly and 9 g/plot of Walnut and Absinthium had significant reducing effect on convolvulus arvensis. Allelopathic plants had significant effect on shoot fresh and dry weight, root fresh and dry weight and seedling height of Triticumaestivum and not significant effect on germination percentage. It can be concluded that the allelopathic effect of Lavender disrupted the germination and seedlings of Portulacaoleracea by producing allelopathic chemicals.

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INTRODUCTION

Earth's population has been increasing permanently. It is predicted that the Iran's population in the year 2021 based on the population rate of 2 percent reach more than 100 millions. In views of agricultural production experts, increasing food production is the only way to solve the problem of hunger; especially in developing countries, it is necessary to invest more in food production (FAO2010). Wheat is the universal cereal of old world agriculture (Gustafson *et al.* 2009) and the world's foremost crop plant (Gustafson *et al.*, 2009), followed by rice and maize. According to FAO (2010), world and Iran production of wheat was 647 and 14.5 million tons, respectively. Crop yield losses such as wheat due to weeds interference is about 15% in fields (Iqbal and Wright, 1999). Allelopathy is negative interference in crops life that its harmless effect is obtained of chemical materials from allelopathic plants. "Allelopathy", for the first time in 1937 was created by Hans Mulish and was defined as biochemical- stimulating interactions or inhibitory among plants and microorganisms (Putnam 1985). Makkizadeh *et al.* (2009) reported the allelopathic potential of medicinal plants on seed germination of three species of weeds. There are various bioassays about allelopathy that most of them are about change in the rate or percentage of seed germination. Then change of seedling growth caused by allelopathic plants has been reported (Bere and Kazinczi, 2000). Different plant organs, including leaves, stems, bark, fruit bark, roots and various parts of them able to have allelopathic effects (Malik, 2005). Allelopathy involving secondary metabolites is produced by plants, micro-organisms, viruses, and fungi that influence growth and development of biological systems (Oussama, 2003). Azizi and Fuji (2006) studied on effect of essential oil germination inhibition and the extract some of medicinal plants on *Amaranthus retroflexus* and *Portulaca oleracea*.

Inhibitory effect of Walnut leaves to near plants is one of the oldest examples about allelopathic effects. When number of plants grew near or under walnut shade, they wilted and died finally. This is due to produce chemical substance is named "Hydrojuglone" that is nontoxic and pale composition. Hydrojuglone is found in leaves, stems, fruit, bark and roots and when it is encountered with air or soil,

isoxidized to Juglone (5-hydroxy-1, 4-Naphtha quinine), the allelopathic substance, which is highly toxic (Bertin *et al.*, 2003). Falling leaves in autumn or washing Juglonewith rain caused that plants are damaged under walnut tree canopy. Although Juglone has low solubility in water, but in small amounts can damage the susceptible plants. Some experiments showed that Juglone had inhibitory for respiration and could reduce necessary energy for metabolic activities in susceptible plants. Kocacaliskan and Teriz (2001) reported that Juglone and extract of walnut leaves reduced germination and seedling growth of tomato, cucumber and alfalfa, extremely, but germination of wheat, barley, corn, melons and beans was not affected. Their investigation revealed that walnut extract significantly increased seedling growth in melon. Results of Ercisli *et al.* (2005) showed that vegetative and reproductive plant growth was inhibited strongly by the treatment of both juglone and undiluted walnut leaf extracts. Allelopathic effect of extract of *Artemisia annua* on germination and growth of *Plantago ovate* was reported (Moussavi-Niket *al.* 2011). Also, allelopathic effect of *Artemisia biennison* germination of *Solanum melanocerasum* has been reported (Kegodeet *al.* 2012). In bioassay carried out by Haig *et al.* (2009) it was determined that the stem and leaf extract of *Lavandula intermedia* cv. *Grosso* ranked highest and had the potential to reduce significantly the root growth of several plant species. Several studies have been carried out about allelopathic effects of medicinal plants on the growth of weeds and crops separately, but few studies have been done on allelopathic effects of medicinal plants on weeds and crops, simultaneously. This study was carried out based on allelopathic effects of medicinal plants such as lavender (*Lavandula angustifolia*), Absinthium (*Artemisia absinthium*) and walnut (*Juglones regia*) on germination and growth of Ivy weed (*Convolvulus arvensis*), Purslane (*Portulaca oleracea*) and Wheat (*Triticum aestvium*).

MATERIALS AND METHODS

This study was conducted based on factorial experiments in a completely randomized design with three replications in greenhouse of Natural Resources and Agriculture Research Center of Hamedan in 2012. The first factor was allelopathic plants at three levels (including Lavender, Absinthium and Walnut), the second factor was dosage of various plants at four levels (including: 0, 5, 7.5, 9 g dry leaves of allelopathic plants pot^{-1}) and the third factor was weeds or crop at three levels (including *C. arvensis*, *P. oleracea*, and *T. aestivum* cv. *alvand*). Leaves of allelopathic plants were collected and dried in shade conditions. Weed and wheat seeds were obtained from Natural Resources and Agriculture Research Center of Hamedan and tested for

germination vigor. However, before testing the *C. arvensis* seed germination, they were soaked in 95% sulfuric acid for 20 minutes to break seed dormancy.

Soil gathered from the field and analyzed for sowing the weed seeds and wheat (Table-1). Two kilograms of soil added to each pot and mixed with dried leaves of allelopathic plants based on treatments and remained for 2 months in order to release gradients of allelochemical substances in soil. Three days before sowing, pots were irrigated until the soil was wet to plant. For each pot, 30 seeds were sown and sowing depth was two or three times size of the seeds. The first irrigation was done on the first day of sowing. Statistical sampling was performed 4 days after sowing. Seedling length, number of leaf and number of germinated seeds in every day were recorded. After 17 days, shoot and root separated and weighted. Then, they were dried in oven for 24 h in 70 °C and dry weight of seedling and root were measured. Data were analyzed by SAS 9.01 software and the Duncan test at 5% level (SAS Institute Inc, 2003).

RESULTS AND DISCUSSION

Analysis of variance (Table-2) showed that the triple interaction effects (allelopathic plant × dosage × weed/crop) had significant effect on fresh and dry weight of shoot and root and seedling length in green house conditions (at the 1% level).

Shoot dry weight

In this study, shoot dry weight of *Triticum aestivum* decreased by increasing in amount of allelopathic plant dosage (Lavender, Absinthium and walnut) in the soil. Comparison of means showed that Absinthium and Walnut had similar effect on shoot dryweight of weed/crop (0.998 and 0.943 g) and placed at the same group, but Lavender had different effect (0.779 g) and placed at the other group (Table-3). Interaction effect between allelopathic plant × weed/crop showed different results, so that all allelopathic plants reduced shoot dry weights of *P. oleracea* and *C. arvensis* significantly, but increased *T. aestivum* (Fig. 1c). Plant residues in soil had allelopathic reverse effect on shoot dryweight of *T. aestivum* (Fig. 1b). Treatments 7.5 and 9g/pot of Lavender had the most negative effect in comparison to the other two plants (level d)(Fig. 1a). Bajalan et al. (2013) reported that the seedling's dry weight of *Hordeum vulgare* remarkably reduced under the influence of different extracts of walnut's organs. It also has been reported that the aqueous extract (1g fw / 10ml HO₂) which was obtained from leaves of *Sorghum halepense* clearly prevented seedling growth of 8 plants and the root extract and remnant of this plant in amount of 1.2 g of leaves dry weight was dried in 454 g soil or 1.85 g leaf and stem, and it prevented growing of several species of plant in

equal amount of soil (Gonzalez *et al.*, 2002). Destroying hormonal balances is one of the most important reasons for shoots and roots growing in plants. Some mechanisms for action of allelopathic substances are like plant hormones. For example, phenolic acids and polyphenols reduced growth induced by auxin based on stop oxidative decarboxylation of it (Chon *et al.*, 2005). Their studied plants had no significant effect on the shoots dry weight of *Convolvulus arvensis*, *Juglones regia*. At the lower concentrations, positive effect on germination was observed, because allelopathy correlate to concentration of allelopathic chemical substances and different inhibitory and stimulating effects may obtain with changing in concentration that this issue confirms results of Chon and Kim (2002). Ismail and Chong (2002) reported that allelopathic substances in low concentrations may have positive or negative effects on target plants, but in high concentrations always have inhibitory effects. Mousavi-Nik *et al.* (2011) also declared that seedlings dry weight had been decreased and these results are in agreement with our results. Also, Akbarzadeh *et al.* (2013) observed similar results about reduction of dry weight of Purslane and velvet flower the influence of various concentrations of *Lavandula officinalis*.

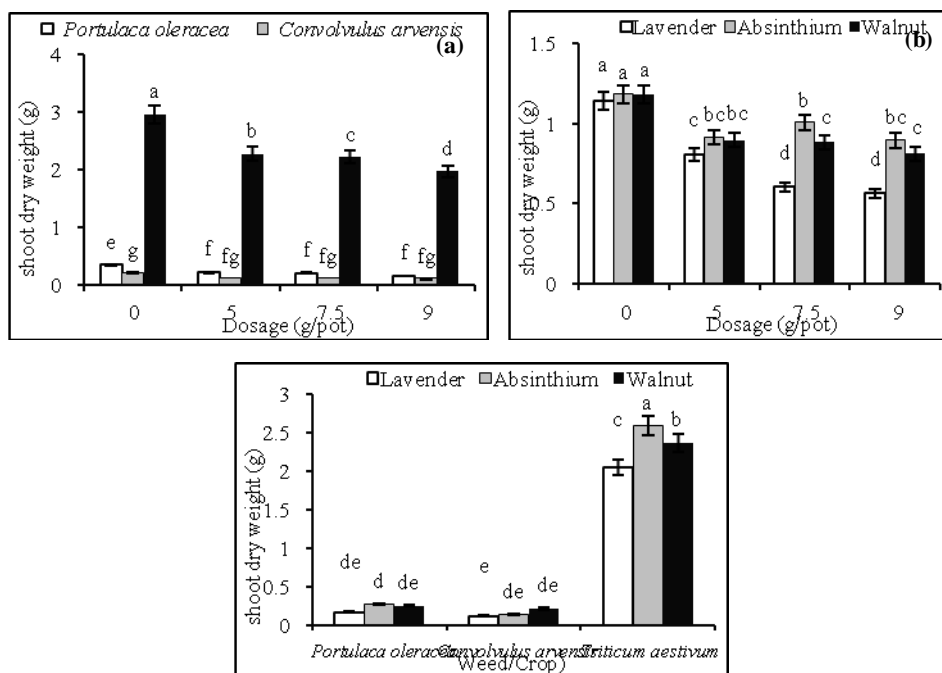


Figure 1. Interaction effects of Dosage x Allelopathic plant (a), Dosage x Weed/Crop (b) and Weed/Crop x Allelopathic plant (c) on shoot dry weight.

Root dry weight

Concentration of 7.5 and 9g/pot of dry Lavender leaves was the most effective on root dry weight of weed/crop, and statistically Absinthium and Walnut placed in a group (Fig. 2a). Treatment of 9 g/pot of Lavender in the soil reduced root dry weight of *Triticum aestivum* from 2.58 mg (control) to 1.33 g (Data not shown). Results were consistent with examines of Sheng and Bao (2006) that observed the maximum inhibitory effect on shoot and root length of *Triticum aestivum*. Dosage of allelopathic plants had no significant effect on root dry weights of *Portulaca oleracea* and *Convolvulus arvensis* but it was obtained significant for *Triticum aestivum* (Fig. 2b). Allelopathic plants reduced root dry weights of *Portulaca oleracea* and *Convolvulus arvensis* significantly, but increased *Triticum aestivum* (Fig. 2c). Zuk and Fry (2006) reported that Zoysia grass seedling emergence and root mass were reduced by 60% and 66%, respectively, when irrigated with solutions in which perennial ryegrass shoots were soaked. Terzi (2008) reported that both the root and the stem dry weight of muskmelon seedlings in all juglone and other decomposed walnut leaf dilutions decreased slightly.

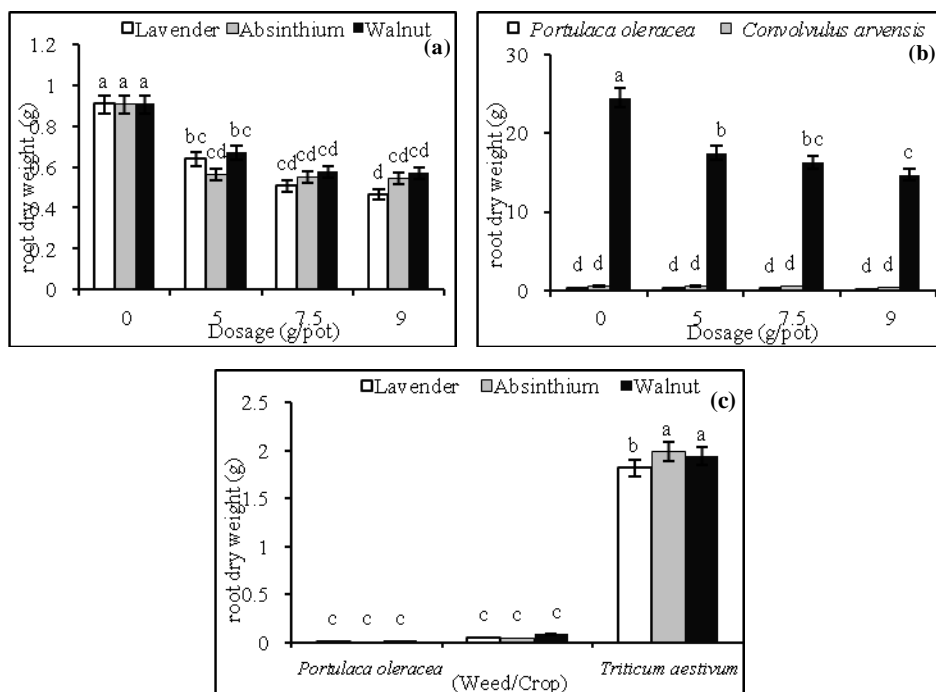
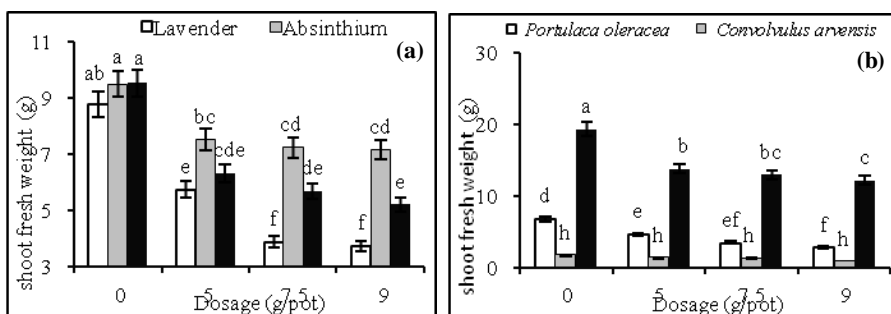


Figure 2. Interaction effects of Dosage × Allelopathic plant (a), Dosage × Weed/Crop (b) and Weed/Crop × Allelopathic plant (c) on root dry weight.

Shoot fresh weight

All allelopathic plants reduced shoot fresh weight of *Triticum aestivum* with increasing amount of dosages that 7.5 and 9 g/pot of Lavender had more reducing effect on this parameter. Treatment 9 g/pot of Walnut had more reducing effect in compared with Absinthium (Fig. 3a). There was significant difference between Allelopathic plants ($P < 0.05$) (Table-3). Percent reduction of shoot fresh weight of *Triticum aestivum* (9 g/pot of Lavender, Absinthium and Walnut) in compared to control was 9.08, 15.14 and 12.16 grams, respectively. Treatments 7.5 and 9 g/pot of Lavender and Walnut reduced shoot fresh weight of *Portulaca oleracea* (Data not shown). Shoot fresh weight of *Triticum aestivum* was more than other tow weeds in all dosages (Fig. 3b). All allelopathic plants reduced this parameter of *Portulaca oleracea* and *Convolvulus arvensis* (Fig. 3c). This is consistent with Ciernia and Kegode (2003) that in preliminary studies, greenhouse soil amended with aboveground *A. biennis* biomass reduced fresh weights of selected crops (*Zea mays*, *Glycine max*, *Helianthus annuus*, and *Triticum aestivum*) by 10% and selected weed species (*Setaria viridis*, *Solanum melanoceasum*, *Amaranthus retroflexus*, and *Avena fatua*) by 18%. Sheng and Bao (2010) also had similar results for allelopathic effect of *Artemisia frigid* on *Leymus chinensis*, *Stipa krylovii*, and *Cleistogenes squarrosa*. Moussavi-Nik *et al.* (2011) found that by increasing extract concentration of *Artemisia annua*, wet weight of Isabgol decreased. Kocacaliskan *et al.* (2009) also reported that the growth parameters as elongation, fresh and dry weights of the seedlings were decreased significantly by juglone in both intact and coatless seeds.

In previous studies, it was found out that juglone and walnut leaf extracts increased the dry weights of muskmelon seedlings (Terzi, 2008). These results are consistent with results of Kegode and Ciernia (2005) who found that greenhouse soil containing below ground *A. biennis* biomass reduced crop and weed fresh weight on average by 39% and 61%, respectively.



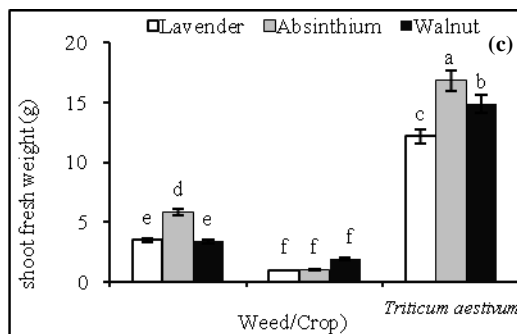


Figure 3. Interaction effects of Dosage \times Allelopathic plant (a), Dosage \times Weed/Crop (b) and Weed/Crop \times Allelopathic plant (c) on shoot fresh weight.

Root fresh weight

Root fresh weight of *Triticum aestivum* decreased with the increasing in amount of dosages of allelopathic substances in pots (Fig. 4b). So as hairy roots are the first part of seedling that encounter with allelopathic chemicals, it is possible that allelopathic effect of extract on it has been studied more than other traits. It has reported that reduction of hairy roots growth by allelopathic chemicals is due to their effect on decreasing in cell division, reduction of inducing auxin for root growth and interfering with respiration and oxidative phosphorylation (Indergit and Foy, 1999). Results showed that root fresh weight was decreased with increasing in dosage of allelopathic plants per pot (Fig. 4a). Allelopathic chemicals can reduce water absorption in plants by influencing on root growth through reduction of hairy roots formation and growth of main roots. Allelopathic plants had no significant inhibitory effect on root fresh weight of the *Convolvulus arvensis* and *Portulaca oleracea* (Table-5). Ercisli et al. (2005) obtained similar results on strawberry. It seemed that with increasing in allelopathic plant residues in soil, excreted allelopathic chemicals from their residues increased and inhibited root and shoot growth of *Triticum aestivum* and reduced it in comparison with control. All three allelopathic plants had the same effect on the inhibition of *Triticum aestivum* root fresh weight, statistically (Fig. 4c). Similar results were obtained in results of Kocacaliskan et al. (2009).

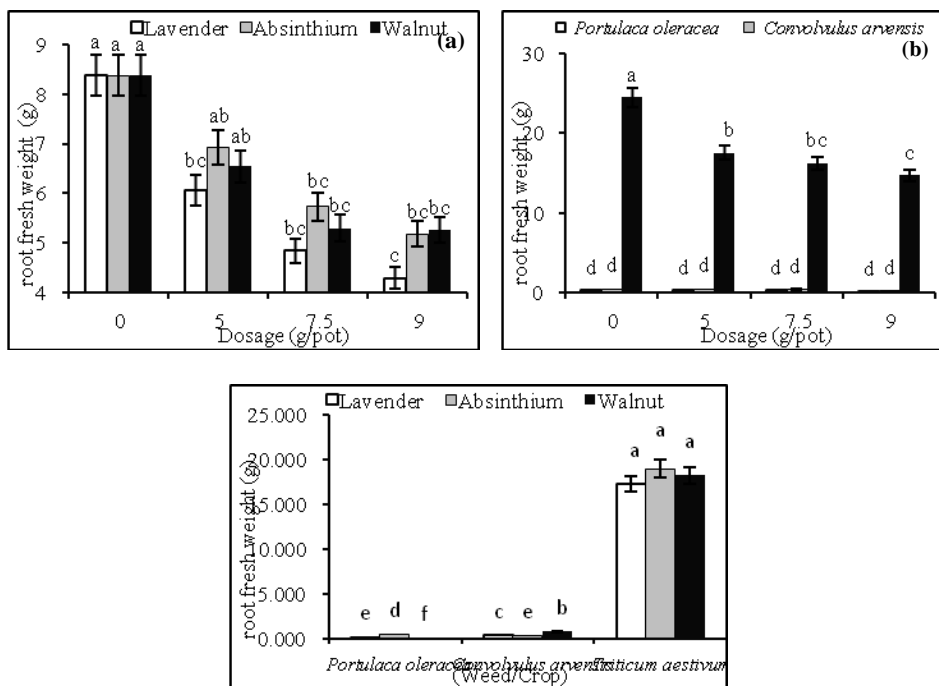


Figure 4. Interaction effects of Dosage × Allelopathic plant (a), Dosage × Weed/Crop (b) and Weed/Crop × Allelopathic plant (c) on root fresh weight.

Seedling length

Lavender had just inhibitory effect on seedling length of *Portulaca oleracea* that adding 5 g/pot Lavender in soil had no significant effect on seedling length of *Portulaca oleracea* but with increasing the amount of plant residues in soil reduced *Portulaca oleracea* seedling length which the value of 6.70 cm (control) reached to 3.66 cm (9 g/pot treatment) (data not shown). Results showed that increasing in dosage had effect on reduction of seedling length of Lavender more than *Convolvulus arvensis* and *Triticum aestivum* (Fig. 5a). Lavender and Walnut leaves had a negative influence on *Triticum aestivum* seedling length that the amount of 7.5 and 9 g/pot of Lavender had greater inhibitory effect than Walnut. Allelopathic effect of Lavender on *Triticum aestivum* was more than Absinthium and Walnut (Fig. 4c). Dosage had no significant effect on seedling length of *Convolvulus arvensis* (Fig. 5b). Moosavi *et al.* (2011) reported that reduction pattern in seedling growth was highly similar to root growth. The most effective inhibitor for seedling growth was sorghum stem extracts. At concentration of 35 g l⁻¹ stem extract, seedling length was decrease around 80% compared to control. Turk *et al.* (2003) made

similar observations on other plant species. Dongre and Yadav (2005) found inhibition in the length of plumule and radicle, a reduction in their dry weights and total seedling weights in wheat, pea and lentil with water extracts of various weeds. Kegode *et al.* (2012) declared that increasing the amount of *A. biennis* root and stem biomass to soil from 5 to 20 g caused 75% or greater reduction in average *S. melanocerasum* plant height compared to the untreated control. Several related species within the *Artemisia* genus are allelopathic. For example, extracts from *A. annua* (annual wormwood) leaves have been shown to inhibit growth of *A. retroflexus* and *Chenopodium album*. These results are in agreement with our results.

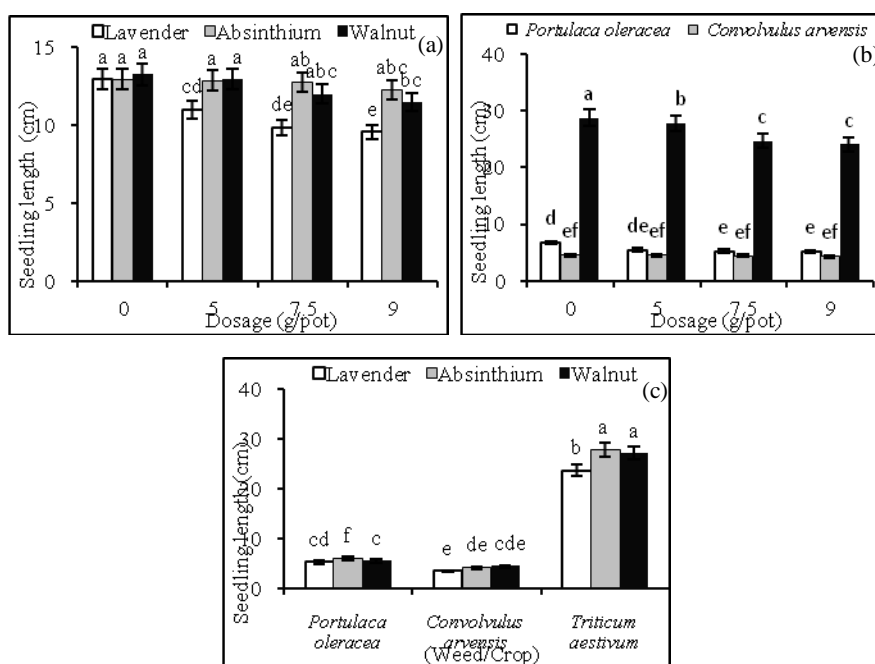
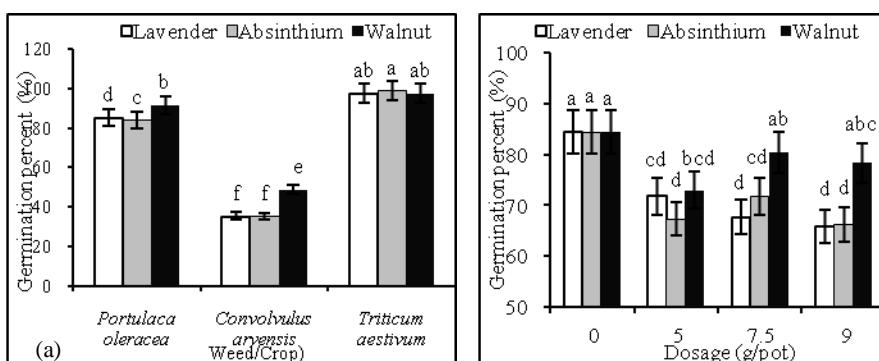


Figure 5. Interaction effects of Dosage \times Allelopathic plant (a), Dosage \times Weed/Crop (b) and Weed/Crop \times Allelopathic plant (c) on seedling length.

Germination Percent

Lavender and Absinthium had inhibitory effect on germination percent of *Convolvulus arvensis* (Fig. 6a). Also Absinthium and Walnut had no effect on germination of *Triticum aestivum* and all treatments were placed in same group (Fig. 6b). Germination percent reduced with increasing in dosage of allelopathic plants, especially about *Convolvulus arvensis* (Fig. 6c). Treatments 7.5 and 9 g/pot of

Absinthium had similar effect on germination percent (Fig. 6b). Zukalova and Vasak (2002) stated that species of Brassicaceae possessed ability to inhibit the germination and growth of plants. Samedani and Baghestani (2005) showed that the inhibitory effect of *A. auchari* on germination of wild oat was greater than other Lavender species that is consistent with the results of this study. Maighany *et al.* (2005) reported that the maximum inhibitory effect of aqueous and organic of Persian clover, Bersim and wild mustard was observed on germination of *Sinapis arvensis* and germination of *Convolvulus arvensis* was less impressed. Kocacaliskan and Terzi (2001) stated that extract of Walnut had severe inhibitory effect on germination of alfalfa, but had little inhibitory effect on germination of wheat, barley, maize and bean. Arouiee *et al.* (2010) found that germination percent, germination rate influenced by plant extracts (*Thymus vulgaris*, *Lavandula sp.*, *Rosmarinus officinalis* and *Eucalyptus citriodora*) in comparison of control, so that the all germination parameters decreased. Kocacaliskan and Terzi (2001) indicated that juglone and leaf extracts of Walnut inhibited seed germination and seedling growth of tomato, cucumber, garden cress and alfalfa. Also, Yarnya *et al.* (2009) found that, bindweed had negative allelopathic effects on seed germination, growth and yield of wheat. Siddiqui *et al.* (2009) demonstrated that Inhibitory effect of *Prosopis juliflora* on seed germination and radicle length of wheat may be related to the presence of allelochemicals including tannins, wax, flavonoides and phenolic acids. It has reported that allelopathic materials of the walnut leaf reduced Barley germination (Roohi *et al.*, 2009; Bajalan *et al.*, 2013). Also, Akbarzadeh *et al.* (2013) reported that *Lavandula officinalis* reduced germination percent of velvet flower.



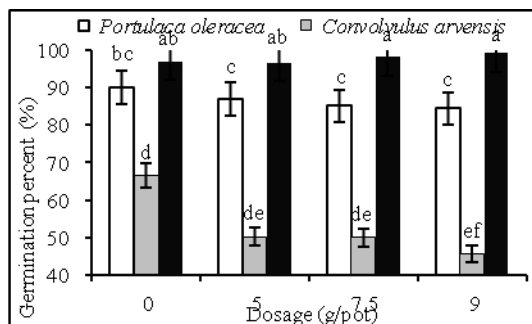


Figure 6. Interaction effects of Dosage × Allelopathic plant (a), Dosage × Weed/Crop (b) and Weed/Crop × Allelopathic plant on germination percent (%).

CONCLUSION

Results showed that allelopathic plants had significant effect on more evaluated traits. Also dosages of allelopathic plants had significant effect on all traits (Table 4). In general, all plants reduced shoot fresh weight of *Portulaca oleracea*, so that with increasing in dosages of allelopathic plants, this parameter reduced more. It is considered that allelopathic plants reduced shoot and root fresh and dry weights of *Triticum aestivum* cv. *Alvand*. Also Absinthium and Walnut reduced seedling length of *Triticum aestivum*, but had no effect on germination percent. Results showed that in order to reducing germination, number of leaves and shoot fresh weight of *Portulaca oleracea*, it is recommended that Lavender cultivated in infected field by rotation. Also it is recommended that Absinthium cultivated by rotation for reducing germination of *Portulaca oleracea*.

Table 1. Condition of physical and chemical soil test plan (0-30 cm depth)

Soil texture	Soil particles (%)			K2O (ppm)	P2O5 (ppm)	N (%)
	Sand	Silt	Clay			
Sandy-loam	52.5	25.4	22.1	462.0	11.1	0.05

Table-2. Analysis variance of allelopathic effects of some medicinal plants extracts on seedgermination and growth of evaluated plants.

S.O.V	df	shoot DW	shoot FW	root DW	root DW	Seedling length	Number of leaf	Germination percent
Allelopathic plant	2	0.467**	49.324**	0.036 ^{ns}	4.133 ^{ns}	36.497**	0.343**	518.621**
Dosage	3	0.874**	79.291**	0.732**	56.316**	16.88**	1.96**	1243.621**
weed/crop	2	54.775**	1741.888**	42.094**	3820.957**	5475.374**	59.676**	34302.881**
Dosage × Allelopathic plant	6	0.071**	5.214**	0.051**	5.215 ^{ns}	5.498**	1.775**	104.630 ^{ns}
weed/crop × Allelopathic plant	4	0.241**	18.891**	0.033 ^{ns}	3.354 ^{ns}	14.214**	1.107**	212.603**
Dosage × weed/crop	6	0.443**	21.893**	0.605**	57.262**	16.618**	4.96**	1034.156**
Dosage × weed/crop × Allelopathic plant	12	0.073**	6.026**	0.050**	5.878**	3.686**	1.205**	37.912 ^{ns}
Error	72	0.017	1.684	0.015	3.989	1.715	0.102	56.79

Table-3. Effect of Allelopathic plants on germination parameters of evaluated plants

Allelopathic plant	shoot DW(g)	shoot FW(g)	root DW(g)	root FW(g)	Seedling length(cm)	Number of leaf(N)	Germination percent (%)
Lavender	0.779 ^b	5.537 ^c	0.631 ^a	5.892 ^a	10.832 ^b	5.028 ^a	72.500 ^b
Absinthium	0.998 ^a	7.868 ^a	0.688 ^a	6.548 ^a	12.706 ^a	4.944 ^{ab}	72.500 ^b
Walnut	0.943 ^a	6.892 ^b	0.683 ^a	6.366 ^a	12.408 ^a	4.833 ^b	79.074 ^a

Table-4. Effect of Allelopathic plants on germination parameters of evaluated plants

Weed/Crop	shoot DW (g)	shoot FW (g)	root DW (g)	root DW (g)	Seedling length (cm)	Number of leaf (N)	Germination percent (%)
<i>Convolvulus arvensis</i>	0.233 ^b	4.419 ^b	0.020 ^b	0.193 ^b	5.650 ^b	6.361 ^a	86.667 ^b
<i>Portulacaoleracea</i>	0.157 ^c	1.287 ^c	0.067 ^b	0.450 ^b	4.102 ^c	4.444 ^b	39.630 ^c
<i>Triticum aestivum</i>	2.330 ^a	14.591 ^a	1.915 ^a	18.164 ^a	26.194 ^a	4.000 ^c	97.778 ^a

Table-5. Effects of different levels of some medicinal plants extract on seed germination parameters of evaluated plants

Dosage (g/pot)	shoot DW (g)	shoot FW (g)	root DW (g)	root DW (g)	Seedling length (cm)	Number of leaf (N)	Germination percent (%)
control	1.167 ^a	9.283 ^a	0.908 ^a	8.371 ^a	12.929 ^a	5.333 ^a	84.444 ^a
5	0.686 ^b	6.414 ^b	0.620 ^b	5.924 ^b	12.321 ^a	4.852 ^b	68.765 ^c
7.5	0.835 ^b	5.715 ^{bc}	0.606 ^b	5.684 ^b	11.476 ^b	4.815 ^b	73.333 ^b
9	0.756 ^c	5.650 ^c	0.535 ^c	5.096 ^b	11.203 ^b	4.741 ^b	72.222 ^{bc}

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