J. BIOL. ENVIRON. SCI.,
2016, 10(29), 71-78

Original Research Article

Allelopathy of *Heliotropium europaeum* (Boraginaceae): Influence on Small Grain Cereals

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Received: 25.04.2016; Accepted: 07.10.2016; Published Online: 19.10.2016

ABSTRACT

In this research, probable allelopathic effects of Heliotrope were investigated on early growth of wheat and barley. Aqueous extracts of the plant at 5, 10, 15 and 20% concentrations were prepared from whole dried plants and distilled water was used as control. Results indicated that released allelochemicals from Heliotrope had inhibitory effects on germination and seedling growth of the test seeds when compared with the control. With increasing aqueous concentrations the inhibitory effects were severed and the highest concentration (20%) had maximum inhibitions on both traits. The results also revealed that the sensitivity of the plants to allelochemicals was different. Wheat was more tolerant than barley to the materials. The results also showed that the root growth was more susceptible than shoot growth under chemical stresses. Allelochemicals changed seed reserve remobilization rates. The weight of reserve mobilization and seed reserve depletion percentage decreased while seed reserve utilization efficiency improved. These findings suggest that the catabolism reactions are more susceptible to allelochemicals than anabolic reactions. It was concluded that Heliotrope had a powerful allelopathic effect on the early growth of the crop plants and the interference of Heliotrope was a species-specific phenomenon.

Keywords: Allelopathy, *Heliotropium europaeum*, Seedling Growth, Weeds

INTRODUCTION

Heliotropium europaeum L., belonging to the Boraginaceae family, is a summer annual weed, which successfully grows on road margins, bare and cultivated fields in North West of Iran (Azerbaijan) (Aliloo and Darabinajad 2013). Seeds germinate in the warm, moist conditions after late spring or early summer. In rotation crop systems, the fallow year provides a condition in which the population of this plant increases at a high rate because Heliotrope has a massive seeding potential and the seeds are viable for years in the soil seed bank. The plant contains toxins that exist in its all parts (Saeedi and Semnani 2009). The plant also uses for medicinal purposes to treat warts, stimulate bile secretion, regulate menstruation, lower fever, and soothe insect bites, gouts, and inflammation in joints.

Allelopathy generally refers to any direct or indirect, harmful or beneficial effect of one plant on another through the production of chemical compounds that release into the environment (Rice 1984). Most of allelochemicals in plants classify as secondary metabolites. Allelopathic compounds influence physiological processes such as cellular expansion, cell wall construction, phytohormonal balance, activity of specific enzymes, pollens, spores and seeds germination, mineral uptake, stomatal movement, pigment synthesis, photosynthesis, respiration, protein synthesis, leghemoglobin biosynthesis, N2 fixation, plant water relations, DNA and RNA modification (Rice 1984; Gulzar and Siddiqui 2014). The kind and nature of allelochemicals mostly goes from phenolics to quinones, with a very important diversity in this range. The most identified compounds divide into four groups: phenolic acids, hydroxamic acids, alkaloids, and quinones (sánchez–moreiras *et al*. 2004).

The donor plants may affect germination, growth and development of the recipient plant species (Vyryan 2002). Chemicals with allelopathic potential may exist in all plant tissues (Alam *et al*. 2002). They release through residues, exudates and leaches by leaves, stem, roots, fruit and seeds (Zeng *et al*. 2008). These chemicals products mainly affect plants at seed emergence and seedling growth stages (Naseem *et al.* 2009; Alioo *et al.* 2012). For example, the leaf, stem and roots extracts or leachates from *Eucalyptus globulus* inhibited the germination and seedling growth of rice, sorghum and blackgram (Djanaguiraman *et al*. 2005). Use of the seed germination as a bioassay is valuable because the seed germination is a critical step in the propagation and

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cultivation of most crop and wild species (Ishii-Iwamoto *et al.* 2006). Nowadays, development in the allelopathy science is one of the key points to improve weed managements in agriculture, forestry and rangelands (Pedrol 2002; Naseem *et al.* 2009; Aliloo 2012; Aliloo *et al.* 2012).

Understanding of crop responses to allelopathic plants is very important in weed managements. However, information on allelopathic effects of *Heliotropium europaeum* on wheat and barley is rare. Our earlier study (Aliloo and Darabinajad 2013) illustrated that the seeds of the plant had both physical and physiological dormancy (double dormancy). With respect to same nature of the chemical, that imposes physiological dormancy and allelopathy (in most cases), the author suggests that the whole plant or parts of the plant may be have allelopathic effects on early growth of Poaceae family. Therefore, the objective of this study was to determine the effect of aqueous extracts of *Heliotropium europaeum* plant on germination and seedling growth of wheat and barley.

MATERIALS AND METHODS

In order to determine allelopathic effects of *Heliotropium europaeum* extracts on seed germination and seedling growth of wheat and barley, an experiment was conducted in University of Maragheh, Iran. Factorial complete randomized design (CRD) with four replications was used to arrange the treatments. The plant material was dried then ground to pass a 2-mm sieve. Aqueous extracts (w/v) were prepared by extracting 100 g of dried plant samples with 1000 mL of distilled water in a shaker for 24 h. The mixture was then filtered using a filter paper (Whatman No. 1) to obtain the main extract. The extract was considered as the stock solution and a series of solution with different concentrations (5, 10, 15 and 20%) were prepared by dilution and distilled water was used as a control. The extracts were stored at 4˚C.

A number of 100 seeds of *Triticum aestivum* and *Hordeum vulgare* were placed on filter paper and each solution treatment was poured on papers then rolled filter papers were put in plastic bags to avoid desiccation. Each variant was laid out in four replications. The samples were then placed in a germinator at a temperature of 22±2 ˚C for 12 days. The following characteristics were determined: percentage of germinated seeds (%), root length, shoot length, root to shoot ratio, seedling length, root weights, shoot weights and seedling weights. The inhibition rate on seed germination (GIR) using the formula of Aliloo et al. (2012):

GIR= [(Control-Aqueous extract)/ Control] × 100

The weight of utilized (mobilized) seed (WMSR) reserve was calculated as the dry weight of the original seed minus the dry weight of the seed remnant. Seed reserve utilization efficiency (SRUE) was estimated by dividing seedling dry weight (SLDW) by the utilized seed reserve. The ratio of utilized seed reserve to initial seed dry weight was considered as seed reserve depletion percentage (SRDP) (Soltani et al., 2006).

Statistical Analysis

For statistical analyses, the data of germination percentage was transformed to arcsine$\sqrt{x+0.5}$. Appropriate analysis of variance for experiment was conducted, using SAS software. Means of each trait were compared according to Duncan multiple range test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Seed germination is the most critical stage in plant life, especially under stress conditions. During this stage, modification in biochemical reactions provides the basic framework for subsequent plant growth and development. Potential of allelopathic compounds is often verified by testing their influence on seed germination behavior. The analysis of variance for germination percentage (GP) and germination inhibitory rate (GIR) showed significant differences among extract concentration (Table 1). Aqueous extracts of *H. europaeum* had an inhibitory effect on seed germination of species in compared the control (Figure 1a). Seed germination of barley declined by increasing the extract concentration but this trait was approximately constant in wheat (Figure 1a). Response to the plant extracts in wheat seeds, was initiate at 15% concentration, however, this threshold for barley was commenced earlier at 5% ones. Germination inhibitory rates were significantly increased by increasing the extract concentration, and at 20% concentration, the inhibition on germination percentage reached to ~40% in barley compared with control (Figure 1b). Inhibitory effect of allelochemicals may be connected to the lower water availability for seed germination due to binding water by compounds present in extract (Black, 1989), or belongs to their toxic nature. Iman et al (2006) stated that allelopathy influenced seed germination and seedling development by preventing cell division and inhibiting cell elongation. The inhibition of germination and seedling growth by allelochemicals is also caused by disturbance in hormonal balance, respiration, photosynthesis and interference in cell growth (Li *et al.* 2010; Kumbhar and Dabgar 2012; Amini *et al.* 2014). Aliloo *et al* (2015) and Qasem (1993) also showed that wheat was relatively resistance to allelochemicals than barley at germination stages. The difference in the germination percentage between two species could be related to differences in the selective permeability of the seeds coat and cell membranes to inhibitory substances or metabolizing of allelochemicals by Wheat.

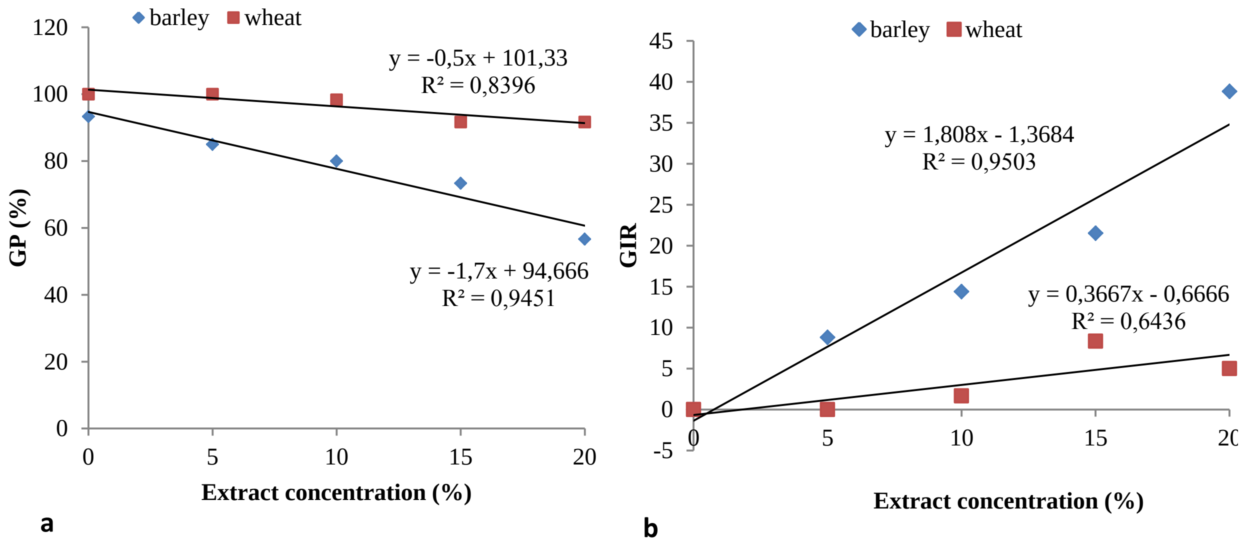

Figure 1. The effects of different *Heliotropium europaeum* extract concentration on germination percentage (a) and inhibitory rate (b) of barley and wheat.

Root (RFW), shoot (SFW) and seedling fresh weight (SeFW) were significantly affected by *H. europaeum* aqueous extracts, but the effects for dry weights were only seen for shoot dry weight (Table 1). Fresh weights of seedlings were slightly increased when exposed to a low concentration of allelochemicals, but when allelochemical contents were severed, the fresh weights were declined sharply. As well as germination percentage, SFW of barley was more sensitive to allelochemicals than wheat (Figure 2a). At mild stress conditions, metabolism of plant commonly increases to modify plant growth, so the low concentration of allelochemicals improved shoot growth compared with control (Table 1). Amendment in the fresh weights usually depends on plant growth rates therefore when allelochemicals reduced the seedling growth, consequently water contents decreased. Jafariehyazdi and Javidfar (2011) showed that osmotic stress induced by allelochemicals play an important role in their mode of action. Seedling dry weights were not significantly changed by allelochemicals nevertheless; reductions were seen at high concentrations (Table 1). These results are in consistence with the results of similar studies (Ziaebrahimi *et al.* 2007; Travlos and Paspatis 2008; Aliloo 2012; Aliloo *et al*. 2012; Pukclai and Kato-Noguchi 2012; Zohaib *et al.* 2014; Aliloo *et al.* 2015).

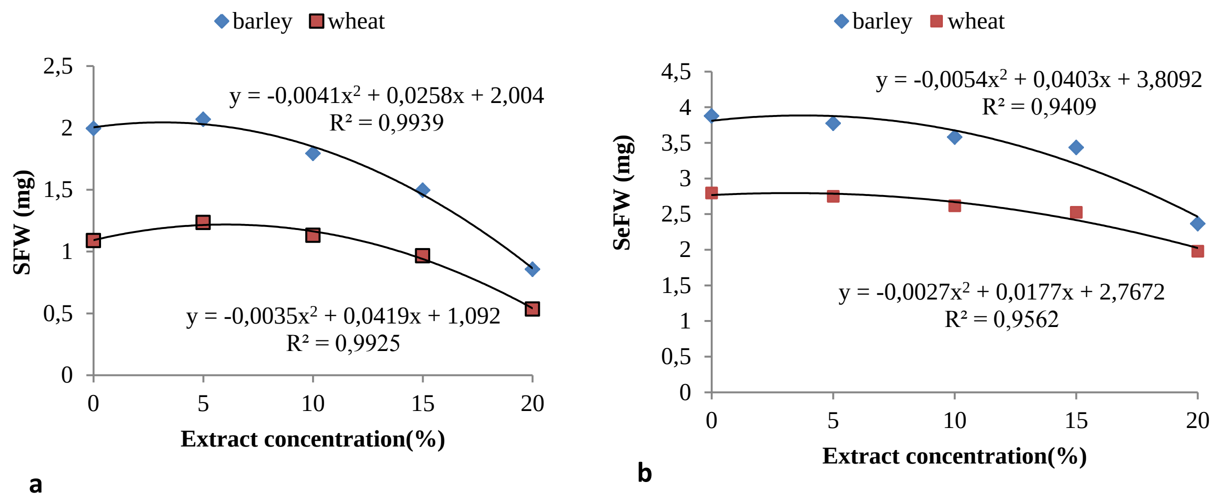

Figure 2. The effects of different *Heliotropium europaeum* extract concentrations on shoot (a) and seedling (b) fresh weights of barley and wheat.

Shoot, root and seedling length were significantly decreased by increasing *H. europaeum* extract concentrations, however, their reduction in wheat was higher than barley (Figure 3a, b and c). Root dry weight of both species was stable under allelopathic conditions, but its elongation was inhibited, so, it seems that roots tip meristemic zones were destroyed by extracts and the thickening was occurred in the roots. In addition, 1-Aminocyclopropane-1-carboxylic acid (ACC) production in response to allelopathic stresses may result ethylene enhancements, phytohormone with growth inhibitory role, may be another reason for root elongation inhibition. Results showed that the ratio of shoot to root length was enhanced by increasing the extract concentration. As we see in Figure 3, concentration at 20% had the greatest positively influence on the SL:RL ratio. Nevertheless, decreasing in shoot and root length under allelopathic condition, SL:RL enhancement showed that roots have been more affected than shoots by extracts. This may be due to the absorption and accumulation of toxic materials along with water by roots. Aliloo *et al*. (2012; 2015) have reported similar results. Turk and Tawaha (2002) reported that the radical length is more susceptible compared with hypocotyl to allelopathic components because the root is first organ of plant which deals with allelopathic material. Iman *et al.* (2006) reported the mechanism of inhibition on the seedling growth caused by allelochemicals could be the result of reductions in cell division rates and/or cell elongation.

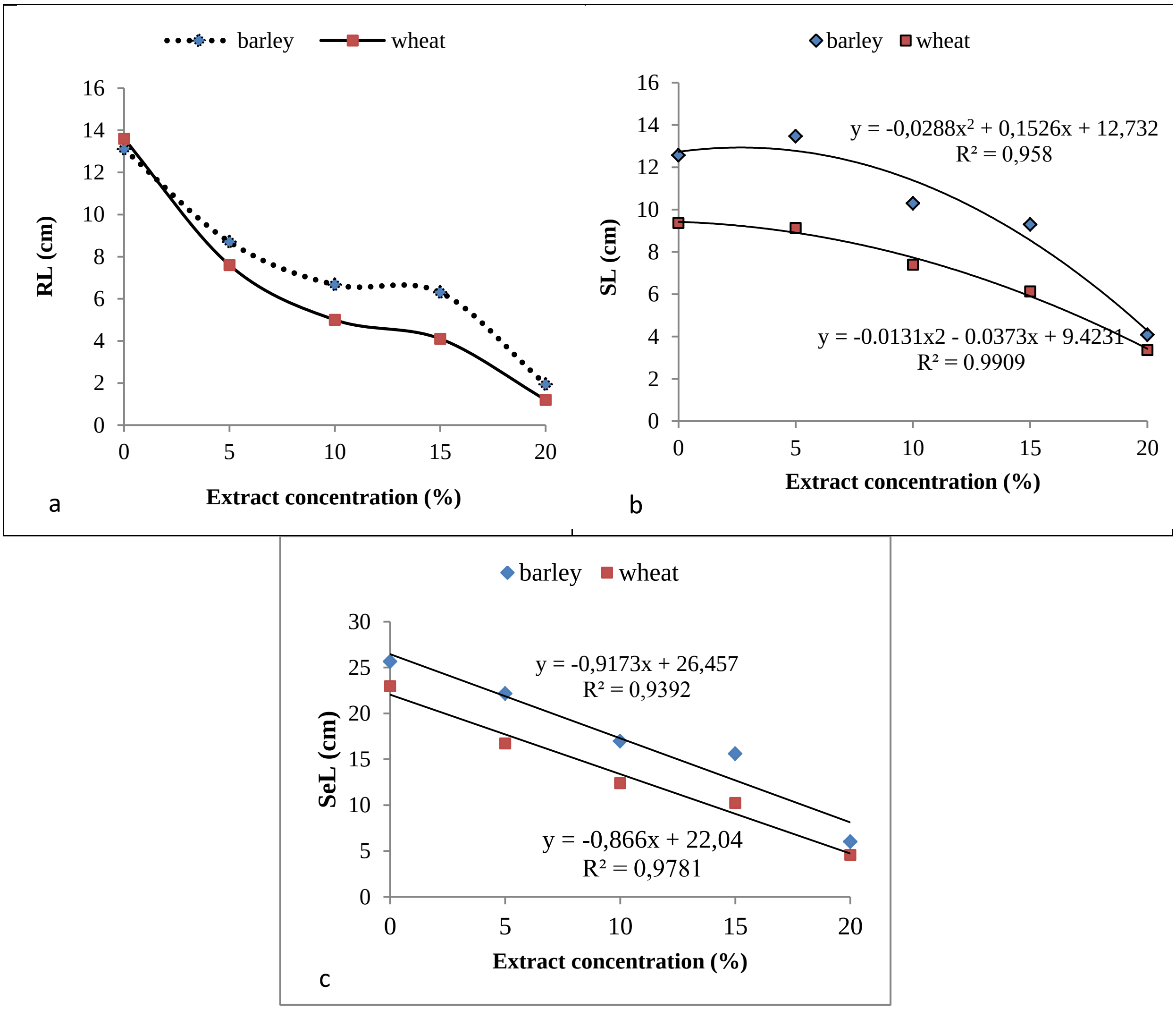

Figure 3. The effects of different *H. europaeum* extract concentrations on root length (a), shoot length (b) and Seedling length (c) of barley and wheat.

Germination consists of several different phases: imbibition, catabolic and finally anabolic phase that are contributing factors in radicle protrusion. During seed germination processes, catabolism of storage reserves and energy production by enzymes and hormones are most important factors. During catabolic phase, stored materials are digested to support respiration and metabolism. Aliloo and Mustafavi (2014) reported that seed germination had very positive correlation with seed reserve remobilization. The weight of utilized (mobilized) seed reserve (WMSR), seed reserve depletion percentage (SRDP) and seed reserve utilization efficiency (SRUE) affected by *H. europaeum* extract concentrations (Table 2). Results showed that allelochemicals reduced seed reserve decomposition (showed in WMSR and SRDP) however, these traits were relatively stable under moderate concentrations. Although alleochemicals reduced seed reserve mobilization, but their conversion to structural material improved. The SRUE increased by the increasing the extract concentrations (Table 2). Our results showed that *H. europaeum* allelochemicals destroyed or delayed catabolism processes, so the mobilization of seed reserves was not take place vigorously. Bogatek (2005) reported that allelochemicals derived from sunflower by influencing catabolism of reserves i.e. blocking or delaying of reserves mobilization, inhibited seedling growth of mustard. An Inhibition in lipid mobilization during germination, in the presence of allelopathic compounds was also detected in canola, sunflower and mustard seeds (Baleroni *et al.* 2000; Bogatek and Stepien 2003; Kupidlowska and Bogatek 2003).

Table 1. The effects of different *H. europaeum* extract concentrations on germination behavior and seedling growth of barley and wheat.

Treatment		GP (%)	GIR	SeL (cm)	SL (cm)	RL (cm)	SeFW (g)	SeDW (g)	RFW (g)	RDW (g)	SFW (g)	SDW (g)	SL:RL	SDW/RDW
Species (A)	**Barley**	77	16.71	17.28	9.94	7.34	3.40	0.70	0.646	0.194	1.64	0.129	1.55	0.97
	Wheat	96	3	13.38	7.08	6.30	2.53	0.63	0.654	0.128	0.99	0.095	1.57	0.92
		**	**	**	**	**	**	ns	ns	ns	**	**	ns	ns
Extract concentration (%) (B)	**Control**	96.6a	0d	24.31a	10.96a	13.35a	3.33a	0.61	0.82a	0.188	1.54a	0.106b	0.82c	0.81b
	5	92.5ab	4.4c	19.45b	11.30a	8.15b	3.2ab	0.55	0.70a	0.133	1.65a	0.126a	1.37b	1.03a
	10	89.1bc	8.02c	14.68c	8.85b	5.83c	3.0ab	0.62	0.64a	0.135	1.46a	0.129a	1.53b	0.99a
	15	82.5c	14.9b	12.91d	7.71c	5.20c	2.97b	0.69	0.68a	0.132	1.23b	0.122a	1.53b	1.06a
	20	74.1d	21.9a	5.29d	3.72d	1.56d	2.17c	0.86	0.38b	0.216	0.69c	0.075c	2.54a	0.82b
		**	**	**	**	**	**	ns	**	ns	**	**	**	**
	A×B	**	**	**	**	*	#	ns	ns	ns	#	#	ns	ns

Different letters indicating significant difference at $p < 0.05$; *, ** significant at 0.05 and 0.01, respectively; percentage of germinated seeds (GP); inhibition rate on seed germination (GIR); seedling length (SeL); shoot length(SL); root length (RL); Seedling fresh weight (SeFW); root fresh weight (RFW); root dry weight (RDW); shoot fresh weight (SFW); shoot dry weight (SDW); seedling dry weight (SeDW) , root to shoot ratio (SL:RL)

Table 2. The effects of different *H. europaeum* extract concentrations on seed reserve mobilization of barley and wheat.

Treatment		WMSR	SRDP	SRUE
Species (A)	**Barley**	0.71	65.34	1.07
	Wheat	0.69	62.75	0.96
		ns	ns	ns
Extract concentration (%) (B)	**Control**	0.78a	71.21a	0.78b
	5	0.80a	72.77a	0.70b
	10	0.74a	67.63a	0.83b
	15	0.66b	60.31b	1.04b
	20	0.53c	48.30c	1.72a
		**	**	**
	A×B	ns	ns	ns

Different letters indicating significant difference at p< 0.05; *, ** significant at 0.05 and 0.01, respectively; Weight of mobilized seed reserve (WMSR); Seed Reserve Depletion Percentage (SRDP); Seed Reserve Utilization Efficiency (SRUE).

CONCLUSION

Our results indicate that Heliotrope contains a wider range of allelochemicals that could be effective on early growth of two important cereal crops, wheat and barley. Both seed germination and seedling growth processes were affected by allelochemicals, herein, the sensitivity of seedling growth was higher than seed germination. We also found a significant difference between the crops in response to extract, wheat was relatively resistance to allelochemicals than barley. Allelochemicals changed seed reserve remobilization rates. The weight of reserve mobilization and seed reserve depletion percentage decreased that suggest susceptibility of catabolism reactions to allelochemicals. The experiment had some limitations, mostly because the experiment conditions were artificial and not conducted in the field or nature. Even though *Heliotropium europaeum* was shown to have allelopathic effects on the test plants.

REFERENCES

Alam SM, Khan S MM, and Shereen A (2002). Influence of aqueous leaf extract of common lambsquarters and Nacl salinity on the germination, growth, and nutrient content of wheat. Acta physiologiae Plantarum, 24: 359-364.

Aliloo AA, and Darabinejad S (2013). Evaluation of Different Techniques for Breaking Seed Dormancy of *Heliotropium europaeum* L. (Boraginaceae). J. Biol. Environ. Sci., 7: 87-91.

Aliloo AA (2012). Allelopathic potentials of *Cupressus arizonica* leaves extracts on seed germination and seedling growth of *Lolium perenne* and *Poa pratensis*. Inter. J. Agri. Crop Sci., 4: 1371-1375.

Aliloo AA, and Mustafavi SH (2014). Does physicochemical pre-treatments can alleviate germination and dormancy of *Sophora alopecuroides* seeds? Azarian J. Agri., 1 : 6-10.

Aliloo AA, Mustafavi SH, and Ezzati F (2015). Allelopathic potential of *Chrozophora tinctoria* on early growth of Barley and Wheat. Azarian J. Agri., 2: 12-18.

Aliloo AA, Shahabivand S, Farjam L, and Heravi L (2012). Allelopathic effects of pine needle extracts on germination and seedling growth of ryegrass and Kentucky Bluegrass. Advances in Envi. Biology, 6: 2513-2518.

Amini S, Azizi M, Joharchi MR, Shafei MN, Moradinezhad F, and Fujii Y (2014). Determination of allelopathic potential in some medicinal and wild plant species of Iran by dish pack method. Theoretical and Experimental Plant Physiology. DOI 10.1007/s40626-014-0017-z.

Baleroni CRS, Ferrarese MLL, Souza NE, and Ferrarese-Filho O (2000). Lipid accumulation during canola seed germination in response to cinnamic acid derivatives. Biologia Plantarum. 43: 313–316.

Black M (1989). Seed research-past, present and future. In: Recent advances in the development and germination of seeds. Taylorson RB (ed.) New York: Plenum. pp. 1–6.

Bogatek R, Gniazdowka A, Stepien J, and Kupidlowska E (2005). Sunflower allelochemicals Mode of action in germinating mustard seeds. In: Proceeding of 3th Alelopathy Congress. Australia, 5-8 June. P:108.

Djanaguiraman M, Vaidyanathan R, Annie sheeba J, Durgadevi D, and Bangarusamy U (2005). Physiological responses of Eucalyptus globulus leaf leachate on seedling physiology of rice, sorghum and blackgram. Inter. J. Agri. Biol., 7:35-38.

Gulzar A, and Siddiqui MB (2014). Allelopathic effect of aqueous extracts of different part of *Eclipta alba* (L.) Hassk. on some crop and weed plants. J. Agri. Exte. Rural Deve., 6: 55-60.

Iman A, Wahab S, Rastan M, and Halim M (2006). Allelopathic effect of sweet corn and vegetable soybean extracts at two growth stages on germination and seedling growth of corn and soybean varieties. J. Agro., 5: 62- 68.

Ishii-Iwamoto EL, Abraham D, Sert MA, Bonato CM, Kelmer AM, and Bracht A (2006). Mitochondria as a site of allelochemical action. In: Allelopathy: A Physiological Process with Ecological Implications .Springer. The Netherlands. P. 267 – 284.

Jafariehyazdi E, and Javidfar F (2011). Comparison of allelopathic effects of some brassica species in two growth stages on germination and growth of sunflower. Plant Soil Envi., 57: 52–56.

Kumbhar BA, and Dabgar YB (2012). Allelopathic effects of aqueous extract of *Chrozophora tinctoria* on seed germination in *Cicer arietinum* L. Bioscience Discovery, 3: 229 -231.

Kupidlowska E, and Bogatek R (2003). Allelopathic potential of sunflower. II. Ultrastructure changes in germinating white mustard (*Sinapis alba* L.) seeds treated with water extract from sunflower (*Helianthus annuus* L.) leaves. Acta Physiologia Plantarum, 25: 89–90.

Li ZH, Wang Q, Ruan X, Pan CD, and Jiang DA (2010). Phenolics and plant allelopathy. Molecules. 15:8933–8952.

Naseem M, Aslam M, Ansar M, and Azhar M (2009). Allelopathic effects of sunflower water extract on weed control and wheat productivity. Pak J Weed Sci Res., 15: 107-116.

Pukclai P, and Kato-Noguchi H (2012). Allelopathic potential of *Tinospora tuberculata* Beumee on twelve test plant species. J. Plant Biol. Res., 1: 19-28.

Qasem JR (1993). Allelopathic effect of nettle-leaved goosefoot (*Chenopodium murale*) on wheat and barley. Dirasat Series B. Pure and Applied Science. 20: 80-94.

Rice EL (1984). Allelopathy. 2th Ed. Academic Press, Orlando, Florida, USA.

Sa´nchez-Moreiras AM, Weiss O, and Reigosa MJ (2004). Allelopathic evidence in Poaceae. Bot. Rev. 69:300-319.

Saeedi M, and Semnani M (2009). Chemical composition and antimicrobial activity of the essential oil of Heliotropium europaeum. Chemistry of Natural Compounds. 45:1, p98.

Soltani A, Gholipoor M, and Zeinali E (2006). Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. Env. Exp. Bot., 55: 195–200.

Vyryan JR (2002). Allelochemicals as leads for new herbicides and agrochemicals. Tetrahedron. 58:1631-1646.

Zeng RS, Mallik, AV, and Luo SM (2008). Allelopathy in sustainable Agriculture and Forestry, Springer-Verlag, Germany.

Zohaib AE. Tabassum T. Abbas T. Rasool T. (2014) Influence of water-soluble phenolics of *Vicia sativa* L. on germination and seedling growth of pulse crops. Scientia Agriculturae. 8: 148-151.