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Allelopathic potential of *Chrozophora tinctoria* on early growth of Barley and Wheat

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ABSTRACT

A laboratory bioassay was conducted to investigate the allelopathic effects of *Chrozophora tinctoria* on germination and seedling growth of barley and wheat. Aqueous leave extracts of *C. tinctoria* at 5, 10, 15 and 20 % concentrations were prepared and distilled water was used as a control. Results showed that germination percentage of two species decreased with increasing the extract concentrations; however, wheat germination was relatively resistant to allelochemicals than barley. In contrast to germination behavior, seedling traits showed different responses. The extracts improved seedling dry weights, particularly barley, whereas seedling lengths were inhibited. Roots of both species were more affected than shoots by extracts. The extracts reduced seed reserve mobilization significantly ($p \leq 0.05$). It was concluded that the used extract had inhibitory effects on seed germination of the crops; however, at seedling stages the effects were severely reduced.

INTRODUCTION

Weeds are a serious everlasting pest in agricultural ecosystems that damage crop yields. Among the pests, they cause more loses in quantity and quality of agriculture products. Their minimum reductions in gross production is about 10% annually (Dhole et al. 2013) So, understanding the nature of weeds and mode of action of them in environment is necessary in order to learn how to reduce their effects on agricultural crops. Weeds compete with crop for resources (i.e. water, nutrients, space …) by many means including physically and chemically or both. There are several reports that show some weed species have allelopathic effects on seed germination and seedlings growth of economically important crop plants (Delabays et al. 2004; Mulatu et al. 2009). Allelopathy is an interference mechanism in which live or dead plant materials release chemical substances, which inhibit or stimulate the associated plant growth (Pellissier 2013). Chemicals with allelopathic potential are present in a variety of plant tissues, including leaves, flowers, fruits, stems, roots, and seeds (Farooq et al. 2011).

Although allelopathy is often considered as a problem for agriculture but there is now considerable evidence to alleviate weed problems by plant bioactive substances (Cheema et al. 2013). There are positive trends which have focused on the possibility of using allelochemicals as natural pesticides in sustainable agriculture (Kropff and Walter 2000). The use of allelopathic compounds has several benefits compared to synthetic herbicides. Due to their natural origin, researchers have suggested that most allelopathic compounds will be biodegradable and also less polluting than traditional pesticides (Khanh et al. 2013). Since more than half of the total volume of agricultural pesticides are used as herbicides (Dayan et al. 2009) therefore any success in new allelochemical recognitions might help to environments safety. This approach can also reduce problematic weeds which occurs due to synthetic herbicides (Pimentel et al. 2001)

Chrozophora tinctoria (Euphorbiaceae) is an annual summer plant and it is the only species of the Chrozophora genus found in East-Azerbaijan, Iran. It is used in dye industry. The growth and development of the root, stem and leaf of C. tinctoria was observed under various soil, light and watering conditions.. Chrozophora contains a wider range of phenolic components, including tannins, saponins (Usman et al. 2007) coumarins,

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chromones , phenylpropanoid glycosides (Mohamed 2001) and certain flavonoids. Usually, the defense compounds have bioactive influences on life cycle of other plants. Einhelling (2004) reported that phenolic compounds are the most active compounds which involved in allelopathy. Their initial actions are on cell membranes, resulting in nonspecific permeability changes that alter ion fluxes and also interact with several phytohormones and enzymes, causing deviations from typical patterns for biosynthesis and flow of carbon into metabolites (Einhelling 2004). Several investigations showed allelopathic effect of these compounds on plants. For instance, Berhow and Vaughn (1999) reported that flavonoids drived from *Tithonia diversifolia* inhibited germination of radish, cucumber (*Cucumis sativus*), and onion (*Allium cepa*) seeds. Flavones from *Celaenodendron mexicanum* (Euphorbiaceae) were shown to inhibit the growth of seeds and shoots of *Amaranthus* and *Echinocloa* species. Kumbhar and Dabgar (2012) showed that plant extracts from *Chrozophora tinctoria* showed inhibitory effects on chickpea seed germination. It's also reported that leaf extract of *Chrozophora tinctoria* had inhibitory effect on growth of lettuce seedling (Amini et al, 2014). One of the most important factors that must be determined prior to selecting the special plants as natural selective herbicide is that, they had not inhibitory effect on goal crops growth. The previous documents did not strongly illustrate inhibitory effect of *Chrozophora tinctoria* extracts on crops especially monocotyledons; so, in this research we examined the plant extracts on two important monocotyledons crops, wheat and barley.

MATERIAL AND METHODS

In order to determine the allelopathic effect of *Chrozophora tinctoria* extracts on seed germination and seedling growth of wheat and barley, a laboratory 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, ground plant samples with 1000 mL of distilled water in a shaker for 24 h. The mixture was then filtered using filter paper (Whatman no. 1) to obtain 10% extracts. The extract was considered as stock solution and a series of solution with different concentrations (5, 10, 15 and 20%) were prepared for each species by dilution and distilled water was used as a control. The extracts were stored at 4 ˚C.

A number of 100 seeds of *T. aestivum* and *H. 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. The seed surface was sterilized before use. Each variant was laid out in four replications. The samples were then placed in a germinator at a temperature of 22 ˚C±2 ˚C for 12 days. The following characteristics were determined: percentage of germinated seeds (%), root length, shoot length, root/shoot ratio, seedling length, root dry weight, shoot dry weight and seedling dry weight. The inhibition rate on seed germination (GIR) using the formula of Aliloo et al. (2012):

$$GIR= [(Control-Aqueous\ extract)/\ Control] \times 100$$

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).

The measured parameters (germination percentage and seedling dry weight) were analyzed individually. All experimental data were statistically processed using the Statistical Analysis System (SAS) program.

RESULT AND DISCUSSION

The effects of leaf dry powder aqueous extracts of *Chrozophora tinctoria* on germination behavior and seedling growth of two target plant species (*T. aestivum* and *H. vulgare*) are shown in Table 1. Germination percentage (GP) and germination inhibition rate (GIR) were significantly affected by extracts application. Water extracts from *C. tinctoria* leaves had an inhibitory effect on both species seed germination (Figure 1a). The degree of inhibition increased with the extract concentration enhancement (Table 1). In two cereals species, the trend was similar, however the rate of germination reduction for barley was higher than wheat seeds (fig 1a). Response to the plant extracts in wheat seeds, initiate from 15% concentration, however this threshold for barley commenced earlier at 5%. It seems that between the crops, seed germination of barley is more sensitive than wheat to allelochemicals derived from *C. tinctoria*. Germination inhibitory rate was 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 due to the lower water availability for in presence of extract (Black, 1989), or belongs to their toxic nature. The inhibitory effects of *C. tinctoria* are in consistence with reports of Amini et al. (2014) on lettuce and Kumbhar and Dabgar (2012) on chickpea.

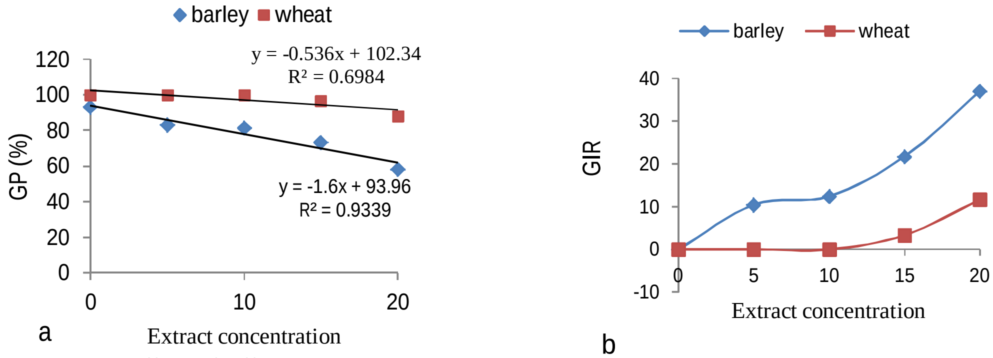


Figure 1. The effects of different Chrozophora tinctoria extract concentrations on germination percentage (a) and inhibitory rate (b) of barley and wheat.

Allelochemicals could deteriorate cell membranes and resulting high electrolyte leakage, also the accumulation of malondialdehyde in seeds exposed to allelochemicals has been reported (Bogatek et al 2006). 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).

Aqueous extracts of C. tinctoria significantly affect seedling dry weight (SDW). Seedling growth (showed by SDW) improved with increasing the extract concentration (Table 1), however, the response of crop growth were different, with respect to Figure 2a. Changing in SDW under extract application may be attributed to the seedling density effect on seed bed and therefore competition for resources was severed for wheat seedling (see results of germination percentage). In other words, under allelopathic treatments, presence of fewer seedlings has enhanced the ratio of individual seedling for water uptake. Bogatek et al. (2006) reported that osmotic stress induced by allelochemicals play a minimal role as the extract mode of action. Whereas, Jafariehyazdi and Javidfar (2011) showed that greatest reduction in seedling growth depending to water uptake reduction by increasing the concentration of aqueous extract. Different response of species to allelochemical is also reasonable but it seems, the number of seedlings is more effective, because each seedling has used media solution for a long time, so did not exposed to osmotic stress. Qasem (1993) reported that *Chenopodium Murale* reduced the germination rates and seedling growth of wheat and barley, and the results showed that barley was more sensitive to allelochemicals than wheat. Another reason may be related to plant stage sensitivity, the sensitivity of plant parts may vary to allelochemicals among species (Aliloo 2012; Aliloo et al. 2012:Pukclai and Kato-Noguchi, 2012; Zohaib et el. 2014). The results illustrated that barley at germination stage was more sensitive than wheat to allelochemical, but at the following stages it is gained resistance to it, subsequently, response of species to environmental factors such as allelochemical may be differ by stage developing of the plants. Many of the researches reported that plant resistance to stress is relative and altered by their growth and development (Patakas 2012; Farooq et al. 2012).

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

		GP (%)	GIR	SL (cm)	PL (cm)	RL (cm)	SFW (g)	SDW (g)	RFW (g)	RDW (g)	PFW (g)	PDW (g)	PL/RL	PDW/ RDW
Species (A)	barley	78	16.32	21.68	11.66	10.02	3.55	0.58	0.822	0.155	1.80	0.150	1.193	0.97
	Wheat	97	3	18.88	9.52	9.36	2.74	0.54	0.797	0.120	1.16	0.108	1.044	0.92
		**	**	**	**	*	**	*	ns	**	**	**	**	ns
Extract concentration (%) (B)	Control	96.6a	0c	23.00a	10.61	12.38a	3.23	0.49b	0.86	0.141a	1.51ab	0.11b	0.86c	0.81b
	5	91.6ab	5.23c	19.36bc	10.00	9.36b	2.93	0.54b	0.77	0.118b	1.31b	0.12ab	1.05b	1.03a
	10	90.8ab	6.21c	20.50b	11.05	9.45b	2.95	0.55b	0.76	0.142a	1.42ab	0.14a	1.17ab	0.99a
	15	85.0b	12.53b	18.38c	10.35	8.03c	3.29	0.61a	0.82	0.132b	1.51ab	0.14a	1.19ab	1.06a
	20	73.3c	24.33a	20.16bc	10.96	9.20b	3.33	0.63a	0.81	0.152a	1.65a	0.12ab	1.29a	0.82b
		**	**	**	ns	**	ns	**	ns	*	#	#	**	**
	A×B	*	**	ns	ns	*	ns	*	*	**	*	*	ns	ns

Different letters indicating significant difference at $p < 0.05$. Germination percentage (GP), Germination Inhibitory Rate (GIR), Seedling Length (SL), Shoot Length (PL), Root Length (RL), Seedling Fresh Weight (SFW), Seedling Dry Weight (SDW), Root Fresh Weight (RFW), Root Dry Weight (RDW), Shoot Fresh Weight (PFW) and Shoot Dry Weight (PDW).

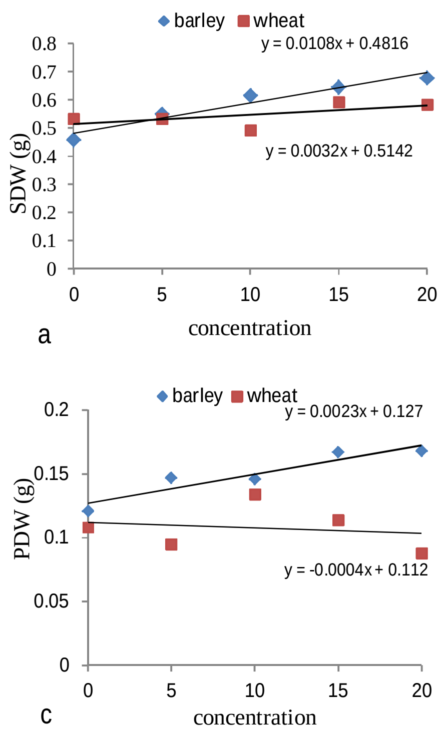
Table 2. The effects of different *Chrozophora tinctoria* extract concentrations on seed reserve mobilization of barley and wheat.

		WMSR	SRDP	SRUE
Species (A)	barley	0.81	74.12	0.73
	Wheat	0.78	71.00	0.71
		*	*	ns
Extract concentration (%) (B)	Control	0.86a	78.33a	0.58b
	5	0.79bc	72.48bc	0.67b
	10	0.82ab	75.31ab	0.68b
	15	0.75c	68.53c	0.82a
	20	0.74c	68.16c	0.84a
		**	**	**
	A×B	*	*	*

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

Among the root and shoot fresh and dry weights, only the root dry weight (RDW) was significantly affected by extracts, but all of these traits significantly affected by species × extract concentration interaction. Root and shoot dry weight of barley increased by extract thickening; however growth of roots was more slightly than shoot (Figure 2b c). In comparison with barley, wheat root and shoot slightly decreased by allelochemicals (Figure 2b c). It seems that allelochemicals altered assimilate partitioning and materials loading to roots especially in barley. Root is the first organ which contact with stresses in the rhizosphere (such as allelopathic materials), thus supplying assimilate for this organ may be a best strategy for their resistance (Xie et al. 2008).

Aqueous extracts of Chrozophora tinctoria applied to both cereal species negatively influenced seedling length and root length compared with control; however they had not significant effect on shoot length (Table 1). Also, there was not statistically significant difference among the extract concentrations for these traits (Table 1). Root length (RL) also was significantly affected by species × extract interaction. Wheat root elongation was more inhibited by allelochemical than barley (Figure 3b). It is also noticeable that chrozophora tinctoria restrained root elongation more than shoot. Root dry weight of barley stimulated by allelochemicals, but its elongation was inhibited, so, it seems that roots tip meristemic zones of this



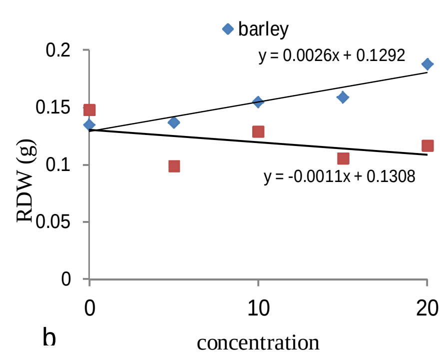


Figure 2. The effects of different *Chrozophora tinctoria* extract concentrations on seedling dry weight (SDW), root dry weight (RDW) and shoot dry weight (PDW) of barley and wheat, a, b and c, respectively.

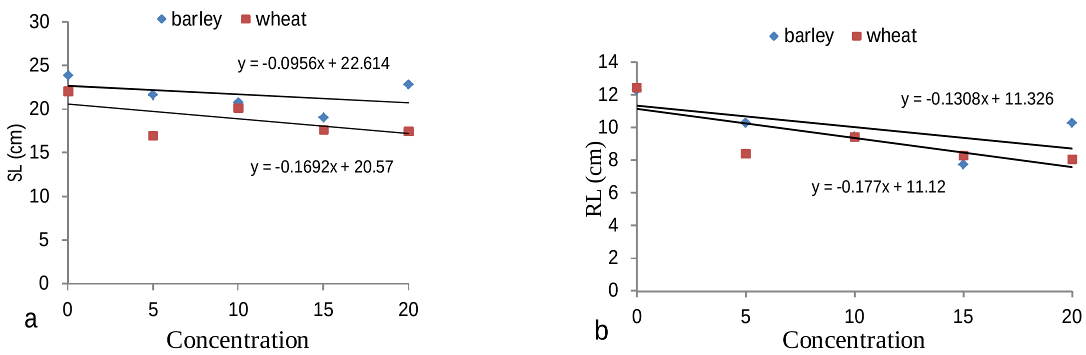


Figure 3. The effects of different *C. tinctoria* extract concentrations on seedling length (a) and root length (b) of barley and wheat.

species destroyed by extracts and the thickening was occurred in the roots. Shoot length to root length ratio enhanced when exposed to allelochemicals. It might be due to the direct contact of root tips with the extract (Quasem 1995). Nishida et al. (2005) found that root growth requires both proliferation and elongation of cells; allelochemicals with monoterpenoids combinations did not inhibit cell expansions, whereas they did inhibit cell proliferation and DNA synthesis in the root apical meristem, so, decreased root length. The decrease in the xylem transport which, affected by allelochemicals, might be responsible for shoot stability under this conditions.

The results indicated a significant effect of species × extract concentrations interaction on weight of mobilized seed reserve (WMSR), seed reserve depletion percentage (SRDP) and seed reserve utilization efficiency (SRUE). Under control conditions (without allelochemicals), barley seed metabolic activity (showed by SRDP) was higher than wheat, and it was depleted reserves earlier than wheat, but when exposed to allelopathy conditions, their responses were different (Figure 4a). Therefore, barley seeds were more sensitive to allelochemicals than wheat, and its respiration has reduced earlier, however by increasing the extract concentration, barley seed reserve mobilization tended to be stable but wheat ones commenced to reduce. For both species, seed reserve utilization efficiency was enhanced by media application of extracts; however barley had better values than wheat from this view point (Figure 4b).

Germination consists of several different phases: imbibition, catabolic and finally anabolic phase resulting in radicle protrusion. Catabolism of storage reserves and energy production due to participation of several enzymes and hormones are most important factors of seed germination. During catabolic phase storage materials are digested supporting substrates for biosynthetic processes and respiration. Aliloo and Mustafavi (2014) reported that seed germination had very positive correlation with seed reserve remobilization. Our results showed that *C. tinctoria* allelochemicals destroyed or delayed catabolism processes, so mobilization of seed reserve was not take place vigorously, however factors controlling the catabolism (i.g. enzymes, hormones) in wheat were stable than barley. Bogatek (2005) reported that allelochemicals by influencing the catabolism of storage reserves blocked or delayed reserve mobilization and in consequence decreases seed germination and seedling growth. Inhibition in lipid

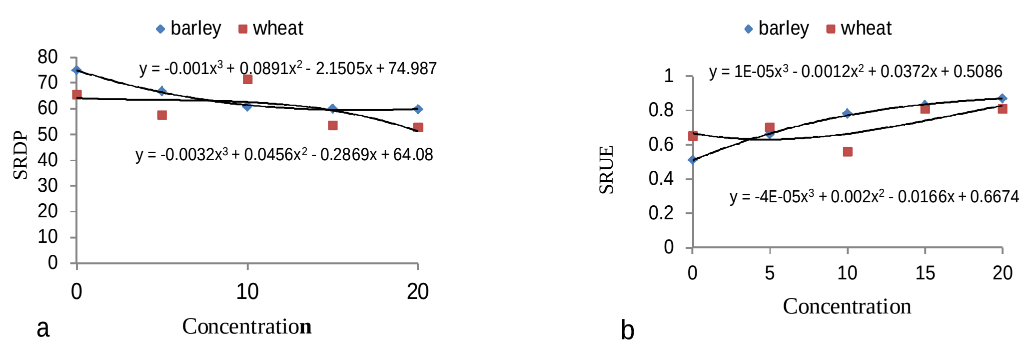


Figure 4. The effects of different *C. tinctoria* extract concentrations on seed reserve depletion percentage (SRDP) and seed reserve utilization efficiency (SRUE) of barley and wheat, a and b respectively.

mobilization during germination of fat-storing seeds, in the presence of allelopathic compounds was detected in canola, sunflower and mustard (*Sinapis alba*) seeds (Baleroni et al. 2000, Bogatek and Stepien 2003, Kupidlowska and Bogatek 2003).

CONCLUSION

Chrozophora contains a wider range of phenolic components that mainly had allelopathic effect on neighborhood plants. Our results showed that *Chrozophora tinctoria* extract had inhibitory effects on germination percentage of both of species, especially barley, however, seedling traits showed different responses; extracts improved seedling dry weights, particularly barley, whereas seedling lengths were inhibited. Therefore, this research supply two important practicable results, one of them is that, when we decide to cultivate in late summer or early fall, the presence large amount of C. *tinctoria* residues might influence seed germination of crops and their emergence. The other, post-germination uses of *C. tinctoria* extract as natural pesticides for controlling of weeds is recommended.

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