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Allelopathic inhibitory potential of some crop species (wheat, barley, canola, and safflower) and wild mustard (*Sinapis arvensis*)

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Abstract

Allelopathic effects of four crops including wheat, barley, canola, and safflower were studied on seed germinations and embryonic growth of wild mustard using different aqueous extract concentrations of the crops. The effects of wild mustard aqueous extracts were also studied on germinations and embryonic growth of the crops. Wild mustard germinations and embryonic growth were significantly affected by different crop aqueous extract concentration treatments. The germinations of wild mustard were terminated by 10w/v of all crop extracts**.** The allelopathic effect of barely on wild mustard germinations were more influential than wheat. Among examined crops, stronger inhibitory allelopathic effects were observed on wild mustard germination when aqueous extracts of safflower were applied. The germination of wild mustard were entirely failed to occur at 5 w/v concentration of safflower aqueous extracts. Crop species responded differently to allelopathic effects of wild mustard extracts as safflower was able to be more resistant. The more intensive inhibitory effects on wild mustard germinations due to the application of safflower aqueous extracts can demonstrate that they can be suggested as biological control agents in field management. It can be further suggested to use safflower in crop rotations because of the better performance of this species against alleclochemicals in areas that wild mustard is dominant. The future researches can be considered to find the exact components of allelochemicals in safflower and the target cells on which these substrates may influence in other plants.

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Introduction

The harmful effect of weeds on crops and horticultural plants which is called interference consists of parasitism, competition and allelopathy. The negative effects of weeds reduce the yield of commercial plants annually (Dawson *et al.*, 1994). The reduction is estimated between 10-100% depending on weeds, crops, and field management (Montazeri, 2005). Some weedy and crop species are able to exude chemicals such as phenol, alkaloids, fatty acides and flavenoids into their rhizosphere that can motivate, decrease, or even terminate the germination and growth of vegetations growing in their vicinities (Rice, 1995; Nilsen *et al.*, 1999; An *et al.*, 2003; Verma and Rao, 2006; Aleksieva and Serafimov, 2008). The ability is known as allelopathic effect that can be used as a biological weed control method (Hussain *et al.* 2009; Iqbal *et al.* 2009). Indeed these biochemicals can function as natural pesticide agents to surpass weeds (Wu *et al.*, 1999). Crop and weed scientists traditionally have viewed allelopathic interactions as detrimental impacts in agricultural sciences (Putnam and Weston, 1986). Many of the world's weeds have been reported to have allelopathic properties which reduce crop growth and yield. In fact, 13 of the world's 18 "worst weeds" have been reported to produce allelochemicals (Patterson, 1986). Allelopathic potential has been recently suggested for about 90 species of weeds (Putnam, 1988). The effect can negatively influence some growth indicators including radicle and coleoptiles growth of other species (Kurse *et al.*, 2000).

Wild mustard (*Sinapis arvensis*) is one the most unfavorable weeds belonging to *Brassicaceae* family and growing in temperate to tropical regions. It is one the major broadleaf weeds in canola (*Brassica napus; Brassicaceae*), safflower (*Carthamus tinctorius*; *Asteraceae*), wheat (*Triticum* spp*; Poaceae*) and barely (*Hordeum vulgare; Poaceae*) cultivations in Iran particularly in agricultural areas of south west of the country (Zand *et al.,* 2008). Studies showed the negative allelopathic effect of black mustard on different species of wheat (Al- Sherif *et al.*, 2013), and wild mustard on physiological factors including chlorophyll content and other characteristics including radical length of canola (Hadadchi and Masoodi Khorasani, 2006).

In recent years, more attention has been given to possible ways of exploiting allelopathic effects in weed management (Farhoudi and Lee, 2012; Oveysi *et al.*, 2008). For instance, family *Brassicaceace* including canola contains allelochemical compound called glucosinolate (containing sulphur and nitrogen) which can be released by the plant and reduce the germination and growth of other plants growing in its vicinity (Abasi *et al.*, 2007). The aqueous extract of canola decreased the emergence rate in some weedy species such as *Amaranthus* spp, *Chenopodium album*, and *Avena fatua* (Abassi *et al.*, 2003). Moreover, the allelopathic effect of safflower (Miri, 2009), barley (Kermer and Ben-Hammoud, 2009) and wheat (Putnam and Weston, 1986) on wild mustard has been reported. In addition, cereals including wheat and barley are considered as allelopathic species. Different varieties of barley could reduce the germination rate, radical and coleoptile lengths of wild mustard by producing allelochemicals (Oveysi, 2007). Wheat also contains phenolic substrates (Wu *et al.*, 1998), and hydroxamic acid (Perez and Ormenonunez, 1991), which can cause allelopathic effects on other weed plants.

Study of weed and crop allelopathic interactions is substantial to improve our knowledge in weed management and crop production. Thus, the purpose of the present study is to investigate and compare the allelopathic effects of four economically important crops including wheat, barley, canola, and safflower on wild mustard to probe which one of them has stronger allelopathic effects on the wild mustard. It may eventually lead us to understand better the interaction between plants and the environment and assist researchers in finding biological control methods to deal with this weed. Furthermore, the allelopathic effect of wild mustard will be discussed on these four crops to find proper plants in plant rotations.

Materials and methods

Culture conditions and experimental design

Allelopathic effects of four crops including wheat (*T. aestivum* L., cv. Atyila), barley (*H. vulgare,* cv. Jonoob), canola (*B. napus,* cv. Hayola), and safflower (*C. tinctorius,* line IL111) were studied on seed germination and embryonic characteristics of wild mustard designed as separate completely randomized design (CRD) with three replicates. The allelopathic effect of wild mustard was also studied on germination rate and embryonic growth of the crops designed as four separate experiments based on CRD with three replicates. Examined aqueous extract concentrations of the crops and the weed were 0 (control), 2.5, 5, and 10 w/v. To prepare the aqueous extracts, the crops and the weed were planted in the experimental field in blocks of 3x3. Plants were entirely harvested (root and shoot) 90 days after emergence (Farhoudi and Lee, 2012). Sampled plants were washed and dried at room temperature of 25 °C. Dried samples were powdered and sieved. The amount of 25, 50, and 100 g of powdered sample were mixed with 1000 ml of distilled water for 24 hours on a shaker. The mixture were filtered and centrifuged at 3000×g for 45 min.

The crop and weed seeds were separately mixed with a fungicide (Mancozob) to avoid any contaminations. The seeds were sunk into distilled water for two hours. 20 seeds of each species were collected and placed on filter papers Whatman No.1 in sterile Petri dishes (9 cm diameter). 5ml of prepared solutions was added into each Petri dish containing the seeds. The Petri dishes were placed in a growth chamber with 25°C, 60% humidity and continuously dark. Germination was determined by counting the number of germinated seeds at 24-h intervals over a 5 (Mutu and Atici, 2009) and 12 (Moradi *et al.*, 2011) day period for crops and wild mustard, respectively. After mentioned days, root and shoot length, dry matter weight of the seedlings, germination rate and percentage was measured and calculated. Germination percentage was calculated on the based of the equation 1 (Farhoui and Lee, 2012):

$$PG=100(n/N) \quad (1)$$

Where, PG is the germination percentage, n is the number of germinated seeds, and N is total number of the seeds. The average time of germination was measured using equation 2 (Scott *et al.*, 1984):

$$MGT=\Sigma fixi/N \quad (2)$$

Where, f_i is the number of days, x_i is the number of germinated seeds at day f, and N is the total number of germinated seeds. To calculate the germination rate, the equation 3 was applied (Miri, 2009):

$$RS=(x+a)^n=\sum_{i=1}^{n} Si/di \quad (3)$$

Where, RS is germination velocity (the number of germinated seeds per day), S_i is the number of germinated seeds at per measurement, and d_i is the number of days until 1/n calculation. Five seeds were collected from each petri dish to measure the radicle and coleoptile lengths.

Statistical analysis

All collected data were analyzed by SAS Ver.9.1 and mean comparisons were performed using Doncan's multiple range test at $\alpha = 0.05$.

Results and discussions

Allelopathic effects of wheat and barley on wild mustard

Characteristics of wild mustard including germination rate, radicle and coleoptile lengths were significantly ($\alpha = 0.01$) affected by different concentration of aqueous extracts of wheat and barley (Table 1). Mean comparisons showed that the wild mustard germination were reduced when 2.5 and 5 w/v of wheat and barley were applied by 65 and 91 percent for wheat aqueous extracts; 80 and 96.6 percent for barely aqueous extracts. The effective surpass was reported in annual broadleaf weeds because of the allelopathic effects of wheat, barley and oats (Baghestani *et al.*, 1999). The germination of wild mustard was terminated by 10w/v of both crops (Fig 1). It can be noticed that the allelopathic effects of barley on wild mustard germination is more influential than wheat due to barley germplasm which contains higher allelopathic substances, such as phenolic acids, than wheat germplasm (Baghestani *et al.*, 1999). Phenolic compounds have been reported in

barley with stronger allelopathic influences than those in wheat species (Ma *et al.*, 1999).

Embryonic characteristics including radicle and coleoptile lengths of wild mustard were remarkably reduced by increasing in the concentration of aqueous extracts. In such a case also the allelopathic effect of barley was noticeably stronger than wheat (Table 1). The lengths of coleoptiles were more affected than the length of radicle. Several chemicals including Alkaloides (Yoshida *et al.*, 1993), Phenolic Acids (Yu *et al.*, 2001), Flavonoides (Liu *et al.*, 1995) and Polyamines (Walter and Wylie, 1986) have been identified as potential allelochemicals that contribute to barley allelopathic activities. Physiological effects of Alkaloids on susceptible plants include cell wall damage, increased cell vacuolation, damage to mitochondrial structure and disruption in cellular metabolism (Liu and Lovett 1993) can reduce the radicle and coleoptile length. Moreover, Worshan (1984) extracted chemical compounds from wheat with inhibitory effects on weeds. Several categories of allelochemicals for wheat allelopathy have been identified including phenolic acids, hydroxamic acids, and short-chain fatty acids which are toxic to seed germinations and root growth of some weed species (Wu *et al.*, 1999).

Table 1. Effect of wheat, barley, canola and safflower extracts on germination and seedling growth of wild mustard.

Crops Extracts (%)	GP	GR (Seed.day^{-1})	ATG (day)	RL (mm)	CL (mm)	SDM (mg)
Wheat						
0	100a	8.0a	2.8a	40.0a	41.5a	10.0a
2.5	37b	1.7b	4.4a	3.6b	3.8b	2.0b
5	8c	0.3c	3.2a	2.3c	1.3c	0.7c
10	-	-	-	-	-	-
Barley						
0	100a	8.1a	2.8a	39.0a	43.3a	10.0a
2.5	20b	0.9b	3.8a	2.5b	2.3b	2.0b
5	3c	0.1c	4.0a	2.0b	0.5c	0.8c
10	-	-	-	-	-	-
Canola						
0	100a	7.2a	3.3b	39.3a	36.7a	8.0a
2.5	18b	0.8b	5.0a	3.0b	2.5b	2.0b
5	7c	0.2c	6.5a	2.0c	0.8c	1.0c
10	-	-	-	-	-	-
Safflower						
0	100a	7.7a	3.0b	36.2a	40.6a	10.0a
2.5	11b	0.5b	5.7a	2.2b	1.6b	1.0b
5	-	-	-	-	-	-
10	-	-	-	-	-	-

In each column, means which have similar letters do not have significant difference based on multiple-range test at 5% probability level. GP, GR, ATG, RL, CL and SDM: germination percentage, germination rate, average time of germination, coleoptiles length and seedling dry matter, respectively.

Allelopathic effect of canola on wild mustard

The data showed that allelopathic effects of different aqueous extracts concentration of canola had significant effects on germination and embryo growth of wild mustard. Increasing in the extract concentrations of canola caused drastic reductions in wild mustard germinations from 100% germination in control treatment to 7% and 18% in 5 and 2.5 w/v of canola, respectively (Fig 1). The reduction can be because of the inhibitory effects of *Brassica* spp. on germination of some weed species has been reported due to isothiocyonamatic compounds which can block

the germination mechanism (Peterson *et al.*, 2001). The germination of wild mustard was terminated by 10w/v of canola extract (Fig 1). Embryo characteristics including radicle and coleoptiles lengths were also negatively affected in germinated seeds of wild mustard from 39.3 and 36.7 mm in control treatment to 2.0 and 0.8 mm in 5% treatment, respectively.

Allelopathic effect of safflower on wild mustard

A stronger inhibitory effect was observed on germination rate and embryo growth of wild mustard due to the application of safflower aqueous extracts. While reductions were reported in germination and embryo growth of wild mustard affected by allelopathic effects of safflower (Miri, 2009; Farhoodi and Lee, 2012) our findings indicated that germination of wild mustard seeds were entirely failed at 5% w/v concentration of safflower aqueous extract (Table 1 and Fig 1). Dramatic reduction occurred in wild mustard germination rate and embryo growth indicators including radicle and coleoptiles length even in lower extract concentration (2.5 w/v) compared to canola while germination timing were increased by 47% (Table 1). As Bonamigo *et al.* (2013) demonstrated that allelopathic effect of safflower aqueous extracts detrimentally affected the seedling emergence and early growth of canola, the same phenomenon occurred in wild mustard on basis of our findings. Farhoodi and Lee (2012) showed that the Safflower extracts inhibited the induction of α-amylase in wild mustard seeds and the inhibition increased in higher extract concentrations.

Table 2- Effect of wild mustard extracts concentrations on germination and seedling growth of wheat, barley, canola and safflower.

Extract concentrations (%)	GP	GR (Seed.day^{-1})	ATG (day)	RL (mm)	CL (mm)	SDM (mg)
Wheat						
0	100a	8.7a	2.5c	124.3a	130.4a	0.32a
2.5	43b	3.0b	3.6b	18.7b	55.1b	0.21b
5	15c	0.9c	3.9a	2.0c	16.3c	0.14c
10	-	-	-	-	-	-
MS	5835.4**	46.1**	8.99**	10545.4**	10095.9**	0.05**
Barley						
0	100a	8.4a	2.6c	128.8a	122.3a	0.46a
2.5	63b	3.9b	3.6b	22.93b	94.7b	0.25b
5	17c	0.8c	4.4a	4.6c	29.6c	0.16c
10	-	-	-	-	-	-
MS	6188.9**	43.21**	11.06**	11026.5**	9592.4**	0.11**
Canola						
0	100a	8.2a	2.6c	63.8a	46.3a	0.11a
2.5	33b	1.1b	6.1b	4.5b	5.6b	0.07b
5	18c	0.5c	7.2a	1.9b	3.1b	0.06b
10	-	-	-	-	-	-
MS	5696.5**	44.6**	32.8**	2860.4**	1431.3**	0.006**
Safflower						
0	100a	9.2a	2.3d	29a	59.0a	0.47a
2.5	82b	6.4b	2.9c	10b	25.7b	0.39b
5	70c	4.3c	3.7b	6c	15.6c	0.23c
10	22d	1.0d	4.4a	2d	3.4d	0.17d
MS	3661.1**	35.8**	2.65**	419.0**	1707.9**	0.06**

In each column, means which have similar letters do not have significant difference based on multiple-range test at 5% probability level. **: significant at the 1% probability level.
GP, GR, ATG, RL, CL and SDM: germination percentage, germination rate, average time of germination, coleoptiles length and seedling dry matter, respectively.

Allelopathic effect of wild mustard on crops germination

The germination rates and embryo growth indicators such as radicle/coleoptiles lengths of all examined crops were influenced by wild mustard aqueous

extracts although their responses were somehow different (Table 2). The radicle length, coleoptiles length, germination percentage, and average time of germination of wheat were significantly affected by the aqueous extracts of wild mustard (Table 2). Increasing in concentration of aqueous extract of wild mustard caused a remarkable reduction in all measured characteristics compared to the control treatment. The wheat germination percentages were significantly reduced by 57, 85, and 100 percent at concentration of 2.5, 5, and 10 respectively (Fig 2). The same reduction pattern were occurred in radicle and coleoptile length; the embryo failed to grow when the aqueous extract with 10 w/v concentration were applied. Earlier work has also indicated marked reductions in growth of wheat following the application of aqueous extracts of a range of *Brassica* species (Manson-Sedum *et al*., 1986).

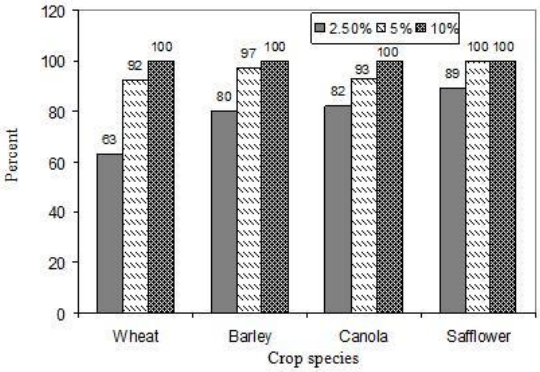

Fig . 1. Seed germination reduction of wild mustard at different crop species extract concentrations .

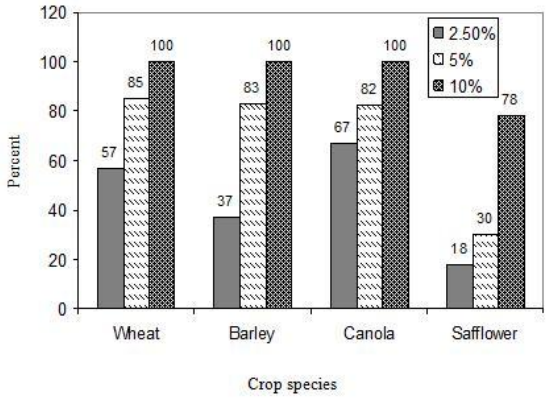

Fig. 2. Seed germination reduction of crop species at different wild mustardextract concentrations.

Aqueous extracts of wild mustard significantly (α = 0.01) reduced embryonic characteristics and germination related factors in barely (Table 2). Gradual increase in concentration of the extracts cause a decrease in germination percentage by 37, 83 and 100 percent compared to control which had 100% germination rate (Fig 2). The germination rate also reduced from 8.4 at control treatment to 0.8 at concentration of 5w/v (Table 2). Serious reductions were observed in lengths of radicles and coleoptiles; the embryo growth was terminated by concentration of 10 w/v. Other studies showed the same results in reduction of germination in barely after applying allelopathic substances extracted from *Brassicaceae* family (Shaji *et al*., 2007).

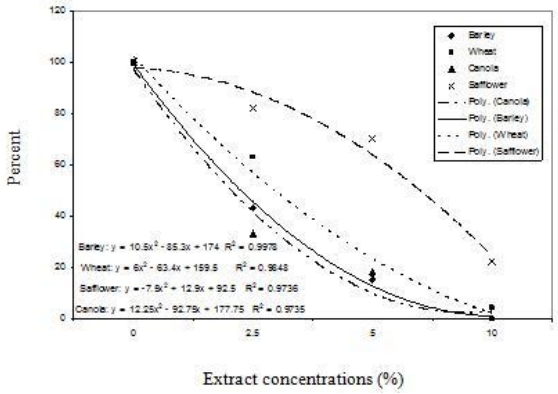

Fig. 3. Trend of regression changes of crop species germination at different wild mustard extract concentrations.

Embryo growth and germination rate of canola and safflower were significantly affected by aqueous extracts of wild mustard (Table 2). In the preset study, germination rate was entirely failed in canola at 10% w/v of wild mustard aqueous extracts while safflower germination were more resistant as it could geminate even at the highest concentration of aqueous extracts by 22%; however, it was a dramatic reduction compared to the control treatment (Table 2). Canola embryo growth reductions affected by extracts were not significant between 2.5 and 5% of extracts. Hadadchi and Masoodi Khorasani (2006) stated that physiological characteristics and growth indicators of canola embryo were significantly reduced by allelopathic effects of wild mustard. The lowest length of safflower radicles and coleoptiles were achieved by 2.0 and 3.4 mm at 10w/v concentration of wild mustard extracts.

The investigated characteristics of crops exhibit that the variation of seed germination and embryo growth were less affected in safflower than canola, barley and wheat meaning that safflower is less susceptible to the allelochemicals produced by neighbouring plants (Fig 3). In all plants, the radicles showed more sensitivity than the coleoptiles to allelopathic substances although both had dramatic reductions. Studied showed that radicles is more susceptible than coleoptiles to the growth inhibitors (Burgos and Talbert, 2000).The influential allelopathic effect of wild mustard can be justified due to the existence of glocosinolates especially isotyocianates which can be found in *Brassicaceae* family (Yamane *et al.*, 1992 and Peterson *et al.*, 2001).

Conclusion

In general, the study of germination and embryo growth of economical crops affected by different concentrations of wild mustard aqueous extracts and *vice versa* showed that factors such as species and genetic variations can be involved in allelopathic effects (Wu *et al.*, 1999) since it can be seen safflower which is more resistant among the other crops. It has been reported that seed germination of different species such as wheat, barley and canola response differently to the allelopathic effects of *Nepeta meyeri* aqueous (Mutlu and Atici, 2009). In addition to the better performance of safflower against alleclochemicals, the allelopathic effects of this species are positively more effective on germination and embryo growth of wild mustard. Thus, the aqueous extract of safflower can be suggested as a biological control agent in field management methods. It can be further suggested to use this species in crop rotations due to its better performance against allelopathic effects. The future researches can be considered to find the exact components of allelochemicals in safflower and the target cells on which these substrates may influence. These findings may help researchers to have better understanding the mechanism of plant-environment interactions to find a new weed control method for this problematic weed and protect the environment while the applications of chemical agents which are hardly decomposable will be reduced.

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