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Allelopathic effect of some essential oils on seed germination of *Lathyrus annuus* and *Vicia villosa*

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Abstract

It has been shown that chemicals found in extracts or essential oils of plants have allelopathic effects on seed germination. Laboratory bioassay was laid out in order to study the inhibitory effects of some essential oils on seed germination of two weeds (*Lathyrus annuus* and *Vicia villosa*). The treatments included control (distilled water), ethanol 9.6% and different concentrations (200, 400, 600 and 800 ppm supplement with 9.6% ethanol at all) of wild mint (*Mentha longifolia*), coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare*), green cumin (*Cuminum cyminum* L.) and caraway (*Carum carvi* L.) essential oils. Results showed that both ethanol and essential oils prevented seed germination of *Vicia villosa*, and Inconsiderable rates of germination (2.2 – 4.4 %) were observed only by ethanol and low concentrations of essential oils. Also all essential oils and ethanol decreased *Lathyrus annuus* germination compared with control. All essential oils except for the fennel essential oil were more effective on inhibiting *Lathyrus annuus* germination than ethanol. Moreover, radicle growth was stopped after 7 days and seedlings were did not appear at treated seed for both species. The germination percentage at untreated seeds of *Vica villosa* and *Lathyrus annus* was 75.55% and 80%, respectively.

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Introduction

Weeds are a serious pest that damages most of the crops and are eternal problem for agriculture. Also in fields, weeds compete with crops for growth factors such as light, moisture and nutrient elements. So, a weed impresses crops in all directions and reduces the quality and quantity of the agricultural product, as well as increase the cost of production (Samad *et al.,* 2008). Weed management with chemical methods like the synthetic herbicides although is effective but their continuous and excessive use will be associated with resistance of weeds to herbicides (Bhowmik and Inderjit, 2003; Ismail *et al.,* 2012). Moreover, the risk of residual herbicides in agricultural products and foods and contamination of soil and groundwater with herbicides are serious threat to the environment and human health (Jamil *et al.,* 2009; Kordali *et al.,* 2007).

Therefore, seeking to obtain suitable natural compounds as a safe alternative source is essential for weeds management and for circumvention of the bio-environmental problems induced by synthetic herbicides. One of the possible solutions is allelopathy. Allelopathy is an interference mechanism which plants release chemical compounds and they have effect on other plants (Gholami *et al.,* 2011). The role of allelopathy as interactions of plant to plant and especially its potential for weed management in agricultere are consedriable (Kolahi *et al.,* 2009).

Essential oils which are one of the natural compounds from a number of higher plants are used as medicinal, antimicrobial, antioxidant substances, etc. (Omidbaigi, 2005). Recent experiments show that secondary metabolites and chemical compounds existing in extracts or essential oils of some plants have allelopathic effects on seed germination and seedling growth in tested plants (Romagni *et al.,* 2000; De Feo *et al.,* 2002; Abrahim *et al.,* 2003; Kordali *et al.,* 2007; Paudel and Gupta, 2008; Ramezani *et al.,* 2009; Brito *et al.,* 2010; Ismail *et al.,* 2012; Rassaeifar *et al.,* 2013). Because of possible use of essential oils as natural herbicide in weed controlling, they may be considered as valuable alternatives in organic farming. In order to achieve efficient and reliable plant sources in weed controlling, the allelopathic potential of different plants or essential oils should be tested on various plant species. In this area, the available data about some weeds including *Lathyrus annuus* and *Vicia villosa* are scarce.

Thus, in this context we have investigated the allelopathic effects of wild mint **(***Mentha longifolia***),** coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare*), caraway (*Carum carvi*) and green cumin (*Cuminum cyminum* L.) essential oils against germination of *Lathyrus annuus* and *Vicia villosa* with a view to explore them as a bioherbicide for weed management.

Material and methods

Plant material

A laboratory experiment was conducted at Islamic Azad University, Sanandaj Branch, Kurdistan, Iran. Seeds of *Lathyrus annuus* and *Vicia villosa* were obtained from a Sanandaj landrace in Kurdistan, Iran. The essential oils were extracted from a fresh leaves of wild mint and dry seeds of coriander**,** fennel, green cumin and caraway by hydro-distillation using the Clevenger.

Treatment setting

The treatments included control (distilled water), ethanol 9.6 % and concentrations of 200, 400, 600 and 800ppm of wild mint, coriander, fennel, caraway and green cumin essential oils. Essential oils were dissolved in ethanol and were prepared at foresaid concentrations with 9.6 % ethanol at all concentrations.

Germination Test

Fifteen seeds of each species were placed on a filter paper in Petri dishes. Twenty milliliters from each test solution (distilled water or ethanol 9.6 % or essential oils) separately were applied to each

replicate for each treatment. All experiments were carried out at 20±1ºC, relative humidity of 55-65% and 16-h light/8-h dark photoperiod in incubator condition. The number of germinated seeds was recorded every day for the time necessary to achieve the final number of germinated seeds. Seeds were observed daily and considered germinated when the radicle was approximately 1 mm long or more and the percentage of germination was calculated as follows:

$$GP=100\frac{n}{N}$$

Where: GP = germination percentage, n = number of seeds germinated, N = total number of seeds.

Statistical analysis

The experiment was established in a factorial lay out based on completely randomized design with three replications. Data were analyzed using SAS software (2001) and the comparison of means were done by Duncan test ($P<0.05$).

Results

Vicia villosa

The obtained results indicated that *Vicia villosa* germination effectively affected by essential oils and ethanol. All essential oils and ethanol significantly inhibited seed germination at *Vicia villosa* (Fig. 1). Fennel essential oil completely suppressed *Vicia villosa* germination and inconsiderable germination (2.2 – 4.4 %) was observed only by ethanol and low concentrations of other essential oils (200 and 400ppm of green cumin and 200ppm of wild mint, caraway and coriander). In high concentrations of essential oils there was not any germinated seed, while the highest germination (75.55%) was obtained by control (Table 1). Maximum radicle length was 4 millimeter and its growth was stopped after 7 days. Moreover seedlings were not appeared at any one of the treated seeds, while at control treatment both radicle and seedling growth occur normally (data not shown).

Lathyrus annuus

As shown in Fig. 2, our results showed that both ethanol and essential oils had inhibitory effects on seed germination of the *Lathyrus annuus* and decreased germination significantly than control. The highest inhibitory effect of essential oils was obtained by wild mint and subsequently by caraway essential oil; its germination percentage also was 17.72% and 17.78% respectively. Among of all treatments, the highest germination was obtained by control (80%) and in treated seeds it was obtained by ethanol (42.22%). Except for fennel essential oil, that had no significant differences with ethanol, other essential oils significantly reduced *Lathyrus annuus* germination than ethanol (Fig. 2). In this case, more reviews showed that concentrations of 400, 600 and 800ppm at wild mint; 600 and 800ppm at caraway and coriander and 800ppm at fennel and green cumin were more effective on controlling *Lathyrus annuus* germination compared with ethanol (Table 1).

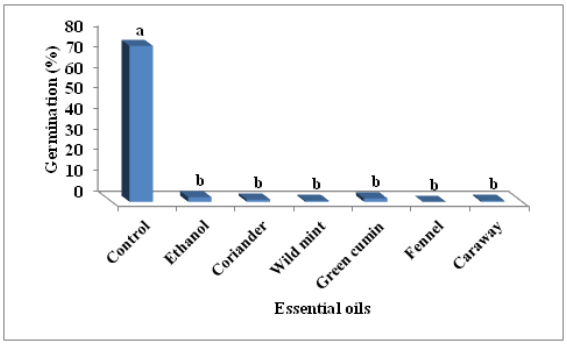

Fig. 1. *Vicia villosa* germinated seeds subjected to some essential oils and ethanol. Means followed by the same letter are not significantly different at 5% by Duncan.

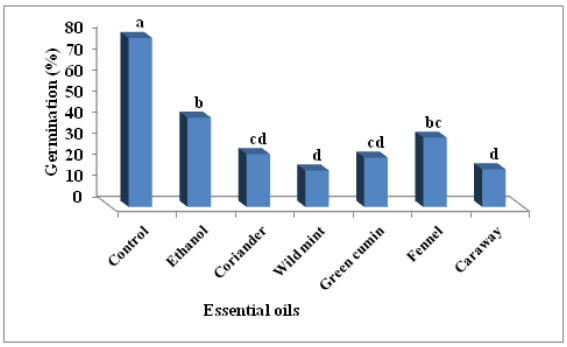

Fig. 2. *Lathyrus annuus* germinated seeds subjected to some essential oils and ethanol. Means followed

by the same letter are not significantly different at 5% by Duncan.

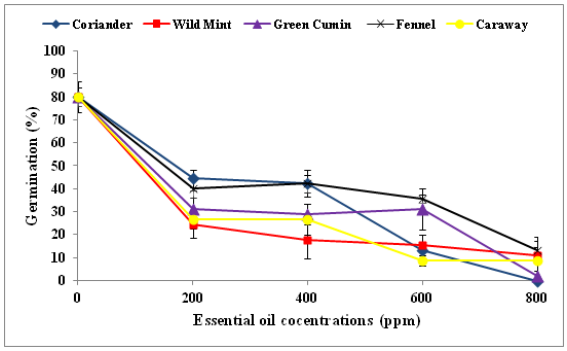

Fig. 3. *Lathyrus annuus* germinated seeds subjected to defferent concentration of essential oils. The vertical bars represent the SE of the mean

With increasing the concentration of essential oils, the germination of *Lathyrus annuus* was decreased, but it varied in different treatments. Although overall, wild mint and caraway essential oils had a greater impact on inhibiting germination in *Lathyrus annuus*, but there was not any significant difference among of its used concentrations (Table 1 and Fig. 3). At fennel and green cumin, with increasing of concentration to 800ppm, at coriander to 600 or 800ppm inhibitory effect on germination significantly were increased than other concentrations. Also 800ppm of coriander essentials oil completely inhibited *Lathyrus annuus* germination (Table 1 and Fig. 3).

Like *Vica villosa*, also in *Lathyrus annuus* radicle and seedling growth was observed only in untreated seeds (distillated water). Treated seeds with both ethanol and essential oils were destroyed and radicle growth completely stopped (data not shown).

Table 1. *Lathyrus annuus* and *Vicia villosa* germinated seeds number subjected to some essential oils and ethanol.

Treatments	Concentration (ppm)	*Lathyrus annus*	*Vicia villosa*
Caraway	200	26.66 ^{bcde}	2.22 ^{b}
	400	26.66 ^{bcde}	0 ^{b}
	600	8.89 ^{efg}	0 ^{b}
	800	8.89 ^{efg}	0 ^{b}
Fennel	200	40 ^{bc}	0 ^{b}
	400	42.22 ^{bc}	0 ^{b}
	600	35.55 ^{bc}	0 ^{b}
	800	13.33 ^{defg}	0 ^{b}
Coriander	200	44.44 ^{b}	4.44 ^{b}
	400	42.22 ^{bc}	0 ^{b}
	600	13.33 ^{defg}	0 ^{b}
	800	0 ^{g}	0 ^{b}
Green Cumin	200	31.11 ^{bcd}	4.44 ^{b}
	400	28.88 ^{bcd}	2.22 ^{b}
	600	31.11 ^{bcd}	0 ^{b}
	800	2.22 ^{fg}	0 ^{b}
Wild Mint	200	24.44 ^{cde}	2.22 ^{b}
	400	17.77 ^{def}	0 ^{b}
	600	15.55 ^{defg}	0 ^{b}
	800	11.11 ^{efg}	0 ^{b}
Ethanol	9.6%	42.22 ^{bc}	2.22 ^{b}
Control	Pure Water	80 ^{a}	75.55 ^{a}

Means followed by the same letter are not significantly different at 5% by Duncan.

Discussion

In order to evaluate the allelopathic effects of some essential oils on germination of two weeds (*Lathyrus annuus* and *Vicia villosa*), we dissolved essential oils in ethanol to use in germination testing. Due to the presence of ethanol in essential oils solution, we compared the effects of essential oils with both control (distillated water) and ethanol 9.6%.

Considering the foregoing results, seed germination of two weeds was significantly affected by ethanol and essential oils. The degrees of inhibitory effects of

essential oils compared with ethanol were different depending on weed species. *Vicia villosa* were more influenced by treatments and all of them highly significant suppressed the seed germination, also due to the strong inhibitory effect of ethanol and the same effect with essential oils we could not correctly evaluate the essential oil effects than ethanol. Unlike, at *Lathyrus annuus* all essential oils concentration-dependent reduced seed germination compared with ethanol. About of ethanol effects on germination, Salehi *et al* (2008) cleared that seed treatment with 3% and 0.1-0.2% ethanol increased germination percentage in *Cynodon*, *Festuca* and *Lolium*, respectively. They also showed that soaking the seeds in 10% ethanol significantly decreased germination at all tested plants and reported that high concentrations of ethanol may cause embryo damage or death.

The results revealed that the essential oils showed a good allelopathic especially against *Lathyrus annuus*. This finding is in agreement with that of Paudel and Gupta (2008) who reported inhibitory effects of eucalyptus, camphor, and lemongrass essential oils on seed germination and seedling growth in *Parthenium hysterophorus*. Azizi *et al* (2006) showed that *Bunium persicum* and *Cuminum cyminum* essential oils suppressed the germination of *Bromus tectorum, Centura ovina* and *Descurainia sophia* especially at high concentration. They also reported that *Bunium persicum* had a more inhibitory effect than *Cuminum cyminum* essential oil. Also, the allelopathic effects of some Apiaceae seeds such as fennel, cumin, caraway, celery, dill, anise and coriander reported by Lamoureux and Koning (1998) at bioassay testing on Lettuce seed germination.

Allelopathic activity in our study may be may related to presence of aromatic compounds, especially monoterpenes in essential oils. Essential oils contain natural flavours and fragrances grouped as monoterpenes, sesquiterpenes and aliphatic compounds and monoterpenes are as major components at essential oils (Kordali *et al.,* 2007; Yoshimura *et al.,* 2011). The allelopathic activities of some essential oils and their monoterpenes on seeds germination or seedling growth at several species have been shown in previous studies (Dudai *et al.,* 1999; Abrahim *et al.,* 2000; Tworkoski, 2002; Singh *et al.,* 2004; Kordali and Atabeyoglu, 2006; Kordali *et al.,* 2007)

The exact mechanisms of alleophatic activity of essential oils on seed germination are not completely understood for us. However, according to previous reports some of the interactions of essential oils or monoterpenes are the following;
Inhibition of cell proliferation and DNA synthesis (Nishida *et al.,* 2005; Yoshimura *et al.,* 2011), lipid oxidation (Zunino and Zyagadlo, 2004), interfere with cuticular waxes and enhance transpiration (Schulz *et al.,* 2007), inhibition of starch synthesis (Yoshimura *et al.,* 2011), inhibition of mitochondrial ATP production (Abrahim *et al.,* 2003), Inhibition of mitosis (Romagni *et al.,* 2000).

Conclusion

A notable finding of the present study are that ethanol and essential oil significantly inhibited germination of two weeds in laboratory condition, but there were not statistical effects between ethanol and essential oils on *Vicia villosa* germination, while at *Lathyrus annuus* all essential oils dose-dependent reduced seed germination compared with ethanol. Therefore, the essential oil as a safe bio-herbicide and environment-friendly could be potentially used to develop new methods for weed management under organic farming systems.

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