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Original Article

Assessment of Allelopathic Potential of Fennel, Rue and Sage on Weed Species Hoary Cress (*Lepidium draba*)

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Abstract

The current series of experiments was conducted to assess the allelopathic potential of fennel, rue, and sage seed and plant biomass on weed species hoary cress (*Lepidium draba*). The effect of plants was evaluated through: seed cogermination in Petri dishes, effect of water extracts from fresh and dry plant biomass in two concentrations (50 and 100 g l^{-}) in Petri dishes and pots, and effect of fresh and dry plant residues in rates of 10 and 20 g kg^{-1} of soil. The cogermination of seeds affected germination and seedling length of hoary cress with fennel seeds having the highest inhibitory effect and reducing germination up to 34.9%. The water extracts in Petri dish essay had various effects, however the dry plant biomass in higher concentration reduced germination and seedlings growth for up to 100%. The applications of the extracts from fresh biomass in pots with soil differed from results in Petri dishes. Sage extract in higher concentration inhibited germination for 34.2%, and fennel extract reduced root length for 22.7%. The effect of incorporation of plant residues depended on the plant species and amount of the plant residues and was both stimulatory and inhibitory. The emergence reduction was the highest with incorporation of fresh rue residues.

Keywords: allelopathic crops, cogermination, extracts, plant residues, weed control

Introduction

Weed control with chemical herbicides is certainly an indispensable measure in modern agricultural production, but herbicide application is not possible, desirable, or sufficient in every situation. Excessive use of herbicides leads to various problems, such as weed resistance, herbicide residues, environmental pollution and risk to human health. At the same time, there is an increasing demand for organic products, thus increasing the need to reduce agrochemicals and minimize their negative effect by using a variety of alternative methods such as allelopathy (Singh *et al*., 2003; Waller, 2004). Allelopathic crops are used in different ways i.e. as surface mulch, incorporated in the soil, as cover crops or as water extracts or pure allelochemicals applied as natural herbicides (Reigosa *et al*., 2001; Singh *et al*., 2001). A number of studies reports use of different crops in weed management such as rye, wheat, buckwheat, black mustard and sunflower (Weston, 1996; Soltys *et al*., 2013), while allelopathic properties of essential oils, extracts and residues of aromatic and medicinal plants are recently being explored (Dhima *et al*., 2009; De Almeida *et al*., 2010). Poisonous and medicinal plants from the Mediterranean region represent potential and neglected source of allelochemicals (Aliotta *et al*., 2008), and among them common rue (*Ruta graveolens*), sage (*Salvia officinalis*) and fennel (*Foeniculum vulgare*) possess certain allelopathic potential on other species (Aliotta *et al*., 1996; Pirzad *et al*., 2010; Itani *et al*., 2013).

Lepidium draba, commonly known as hoary cress, is a perennial herb native to Eastern Europe and Western Asia, and invasive species in North America (Knežević, 2006; Francis and Warwick, 2008). *L. draba* produces up to 5000 seeds per plant (Hanf, 1970; Knežević, 2006), but also reproduces clonally from intact rhizomes and fragments (Miller *et al*., 1994). It is a weed in cereals, row crops, orchards and vineyards, as well as on pastures, along roadsides and on waste areas (Knežević, 2006; Francis and Warwick, 2008).

Therefore, the objective of the study was to evaluate the allelopathic effect of fennel, rue and sage seed and plant biomass on weed species hoary cress through series of experiments in Petri dishes and pots with soil.

Materials and methods

Collection of plant and seed material and extract preparation

Seeds of fennel, rue and sage used in the experiments were purchased from seed company (Migra d.o.o., Croatia, Sjemenarna d.o.o., B&H), and weed seeds of hoary cress were collected during 2014 from agricultural fields. Aboveground mass of fennel, rue and sage was harvested in the flowering stage. The proportion of biomass was oven dried at 60 °C for 48 h, cut into small pieces and ground into fine powder.

Water extracts were prepared according to Norsworthy (2003) from fresh and dry aboveground biomass. One hundred grams of the fresh or dry biomass was mixed with 1000 ml of distilled water and kept for 24 h. The mixtures were filtered through muslin cloth to remove debris and after that through

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filter paper. The obtained extracts were diluted with distilled water to give final concentrations of 5 and 10% (50 and 100 g of biomass per litre).

The experiments were conducted twice during 2014, and were laid out as a completely randomized design with four replications.

Petri dish experiments

The effect of cogermination of aromatic and medicinal crops and weed seeds was investigated according to Đikić (2005) in the first experiment. Each treatment consisted of 30 seeds of aromatic crop and 30 seeds of weed species germinating together in Petri dishes on top of filter paper soaked in distilled water. The control treatment consisted of 30 weed seeds per dish.

In the second experiment the effect of water extracts from fresh and dry biomass in two concentrations on hoary cress was evaluated. Thirty weed seeds were placed in sterilized Petri dishes on top of filter paper. In each Petri dish the equal amount of certain extract was added, while distilled water was used in the control.

Pot experiments

In the third experiment, the effect of water extracts from fresh biomass of plants in two concentrations was evaluated in pots with soil. In the pots filled with commercial substrate 30 weed seeds were sown and treated in rate of 60 ml of extract per 100 g of soil. Distilled water was used in the control treatment. Thereafter, all treatments were equally watered.

In the fourth experiment the effect of incorporated fresh and dry residues was determined, according to the modified method of Norsworthy (2003). Fresh or dry plant residues in rates of 10 and 20 g per kg of the soil were mixed with commercial substrate. Thirty hoary cress seeds were sown in the pots filled with soil. The control treatment consisted of thirty weed seeds sown in the soil without residues.

Data collection and statistical analysis

All experiments were kept at 22 ± 2 °C temperature and lasted 9 (Petri dish) and 14 days (pot experiment). Germination percentage was calculated using the formula G (%) = (Germinated seed/Total seed) x 100 in Petri dishes experiments. Emerged seedlings from the pot experiments were counted and percentage of emergence was calculated for each replication using the formula E (%) = (Emerged seed/Total seed) x 100. The seedlings were uprooted and the length of roots and shoots (cm) and fresh weight (g) were determined.

All collected data was analysed statistically with ANOVA using STATISTICA 7 statistical software (StatSoft, Inc. Tulsa, OK, USA) and differences between treatment means were compared using the LSD-test at probability level of 0.05.

Results and Discussion

Seed cogermination

The cogermination of weed and aromatic and medicinal crop seeds affected germination and seedling length, but had no effect on fresh weight of hoary cress (Table 1.). Germination reduction ranged from 21.2 to 34.9% and fennel seeds had the highest inhibitory effect. Root length was inhibited significantly only with fennel, and shoot length with rue for 9.3%.

Cogermination of weed and crop seeds can variously affect weed germination and growth and is dependent on both weed and crop species. Seeds of aromatic and medicinal crops from *Apiaceae* family in other studies showed considerable allelopathic effect. Đikić (2005) found that caraway (*Carum carvi*), dill (*Anethum graveolens*) and coriander (*Coriandrum sativum*) seeds have inhibitory effect on germination of hoary cress up to 65%, and as well as fennel seeds reduce germination of other weed species such as redroot pigweed (*Amaranthus retroflexus*), smallflower (*Galinsoga parviflora*) and creeping thistle (*Cirsium arvense*). The results of the study are also consistent with the results of Ravlić *et al.* (2013) who demonstrated that coriander and lovage (*Levisticum officinale*) have negative effect on germination and root length of hoary cress. Plant seeds contain wide range of chemical inhibitors, such as nonprotein amino acids, phenolics (Ketring, 1973; Friedman and Waller, 1983; Rashid *et al.*, 2005) and essential oils (Olle and Bender, 2010) which may negatively affect seed germination. Essential oil components such as thymol, carvon, carvacrol and limonene showed high inhibition against weed seed germination even at low concentrations (Azirak and Karaman, 2008), while *trans*-anethole, linalool, fenchone, thujone and eugonol exert high to intermediate phytotoxic effect on rigid ryegrass (*Lolium rigidum*) (Vasilakoglou *et al.*, 2013). Principal constituents of fennel seed volatile oil include anethole and fenchone (Galambosi, 1994) which may have contributed to inhibitory potential of fennel seeds.

Effect of water extracts in Petri dishes

The extracts prepared from the fresh and dry plant biomass had various effects on germination and growth of hoary cress (Table 2). Germination reduction ranged from 3.5 to 100%, with the extracts from dry biomass in higher concentration showing complete germination inhibition. On average, the fresh biomass extracts reduced germination for up to 17%, while the dry biomass extracts for over 90%. Fennel dry biomass had the highest allelopathic potential.

Both root and shoot length and fresh weight of hoary cress were under positive and negative effect of the extracts applied (Table 2.). Fresh water extracts generally stimulated seedling growth and root length was greater for 4.3 to 56.5% compared to the control. The exception was fennel extract which reduced root length for 32.9 and 59.1%. Fresh weight was reduced significantly only with higher concentrations of fennel and sage fresh extracts. The extracts from dry biomass reduced seedlings length for over 80%, as well as seedlings fresh weight, especially with the higher concentrations.

Table 1. Effect of cogermination of aromatic and medicinal plants and hoary cress seeds on germination and growth of weed

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)
Control	89.3 a	3.4 a	2.1 ab	0.0175 a
Fennel	58.1 c	2.6 b	2.2 a	0.0176 a
Rue	67.8 bc	3.1 ab	1.9 c	0.0184 a
Sage	70.4 b	3.4 a	2.0 bc	0.0173 a

Means followed by the same letter within the column are not significantly different at $p < 0.05$.

Table 2. Effect of aromatic and medicinal plant water extracts on germination and seedling growth of hoary cress on filter paper in Petri dishes

Treatments		Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)
Control		86.2 a	3.2 c	2.2 bc	0.0163 b
Fresh biomass					
Fennel	50 g l^{-1}	88.7 a	2.1 d	2.6 a	0.0175 ab
	100 g l^{-1}	83.2 a	1.3 e	2.4 ab	0.0135 c
Rue	50 g l^{-1}	62.4 bc	5.0 a	2.7 a	0.0189 a
	100 g l^{-1}	60.7 c	3.9 b	2.3 bc	0.0161 b
Sage	50 g l^{-1}	66.9 b	3.9 b	2.3 bc	0.0157 bc
	100 g l^{-1}	65.9 bc	3.3 c	2.1 c	0.0138 c
Dry biomass					
Fennel	50 g l^{-1}	1.8 f	0.1 g	0.1 e	0.0001 e
	100 g l^{-1}	0.0 f	0.0 g	0.0 e	0.0000 e
Rue	50 g l^{-1}	12.3 e	0.2 fg	0.4 d	0.0026 d
	100 g l^{-1}	0.0 f	0.0 g	0.0 e	0.0000 e
Sage	50 g l^{-1}	36.7 d	0.3 f	0.3 de	0.0021 de
	100 g l^{-1}	0.0 f	0.0 g	0.0 e	0.0000 e

Means followed by the same letter within the column are not significantly different at $p < 0.05$.

Table 3. Effect of water extracts from fresh plant biomass on emergence and growth of hoary cress in soil

Treatments		Emergence (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)
Control		61.1 bc	2.8 ab	3.4 d	0.0149 c
Fresh biomass					
Fennel	50 g l^{-1}	88.2 a	2.8 ab	4.5 a	0.0217 a
	100 g l^{-1}	95.6 a	2.2 c	4.4 a	0.0213 a
Rue	50 g l^{-1}	54.1 cd	2.7 b	3.5 cd	0.0166 bc
	100 g l^{-1}	75.5 b	2.9 ab	3.6 cd	0.0159 c
Sage	50 g l^{-1}	69.7 bc	3.2 a	3.8 bc	0.0181 b
	100 g l^{-1}	40.2 d	2.9 ab	3.9 b	0.0173 b

Means followed by the same letter within the column are not significantly different at $p < 0.05$.

Extracts from medicinal and aromatic plants show different effects against weeds species. Dhima *et al.* (2009) reported that extracts from dry plant biomass of fennel, coriander and anise (*Pimpinella anisum*) show considerable negative effect and suppression of germination, root length and fresh weight of barnyardgrass (*Echinochloa crus-galli*). Inhibitory potential of sage extract on common purslane (*Portulaca oleracea*), velvetleaf (*Abutilon theophrasti*), redroot pigweed and wild oat (*Avena sterilis*) was reported by Pirzad *et al.* (2010) and Kadioğlu and Yanar (2004). Negative effect of rue was recorded on weed species such as redroot pigweed, common lambsquarter (*Chenopodium album*), purple nutsedge (*Cyperus rotundus*), common purslane and flixweed (*Descurainia sophia*) (Aliotta *et al.*, 2000; Makizadeh *et al.*, 2009). Aliotta *et al.* (1996) argued that rue infusions and its isolated allelochemicals delayed the onset and decreased the germination and produced damage on the radicals of purslane seedlings. Medicinal plants may contain various bioactive compounds, and volatile terpenes and essential oils can be applied as weed growth regulators (Arminante *et al.*, 2006). Sage oil principal components include 1,8-cineole, α-thujone and β-pinene (Mirjalili *et al.*, 2006). Furanocoumarins 5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP), and the quinolone alkaloid graveoline isolated from rue extracts cause growth reduction through cell leakage and inhibition of cell division (Hale *et al.*, 2004). Ethanolic and aqueous extracts of fennel seed contain a high phenolic content (Oktay *et al.*, 2003), while major phenolic compounds of methanolic extract of fennel plant material include caffeoylquinic, chlorogenic, dicaffeoylquinic, and rosmarinic acids (Križman *et al.*, 2007).

Generally, the extracts from the fresh plant biomass showed lower inhibitory effect than extracts from the dry plant biomass, and even promoted root and shoot length. The differences among extracts prepared from fresh or dry biomass may be due to the different concentration of active substances extracted from the fresh and dry plant tissues (Marinov-Serafimov, 2010). Lower concentrations of allelochemicals generally have lesser or stimulatory effect on the plant growth, while negative effect increases with the increase in concentration (Dhima *et al.*, 2009; Konstantinović *et al.*, 2014). However, Qasem (1995) indicates that even dry biomass may exhibit lower inhibitory potential than fresh since harmful effect could be greatly reduced during drying process. Greater reduction in germination and growth of hoary cress seedlings were observed by Baličević *et al.* (2014) and Ravlić *et al.* (2014) when extracts from dry biomass of marigold (*Calendula officinalis* L.) and parsley (*Petroselinum crispum*) were used compared to extracts from fresh biomass. The pH and osmotic potential of the extracts as well as higher concentration of mineral nutrients and other organic compounds may result in reduced water uptake and exert greater negative effect on seed germination and seedlings development (Qasem, 2010).

Effect of water extracts in pots with soil

The application of extracts from the fresh biomass caused reduction in weed seedlings emergence only in the treatment with sage extract in higher concentration and amounted up to 34.2% compared to the control (Table 3). On the other hand, fennel extract in both concentrations greatly stimulated germination for 44.3 and 56.4%. Significant reduction of root length was recorded only in the treatment with 10% fennel extract for 22.7%. Shoot length and fresh weight of hoary cress seedlings were stimulated with all extracts applied and fennel extracts promoted shoot length and fresh weight up to 33.7 and 46.3%, respectively.

Results obtained from the experiments with extracts from the fresh plant biomass somewhat differed considering whether Petri dishes or pots with soil were used. For example, seedling emergence in the treatments with fennel was highly promoted in the soil, while in Petri dish assay germination was not affected. On the other hand, sage extract in lower concentration promoted seedling emergence oppose to the germination inhibition in Petri dishes. Likewise, stimulatory effect was sometimes more pronounced on the filter paper than in the soil and conversely.

The differences between results could be due to higher amount of extract applied to the soil which enhanced positive or negative effect (Ravlić *et al.*, 2014), or because allelochemicals diffused and degraded in the soil (Vidal *et al.*, 1998) while seeds on filter paper had direct contact with the extracts.

Table 4. Effect of aromatic and medicinal plant residues on germination and seedling length of hoary cress

Treatments		Emergence (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)
Control		63.3 bc	2.9 a	4.2 abc	0.0221 cd
Fresh residues					
Fennel	10 g kg^{-1}	47.9 cd	1.9 dc	3.5 d	0.0177 ef
	20 g kg^{-1}	73.7 b	1.5 d	3.8 cd	0.0182 def
Rue	10 g kg^{-1}	46.9 cd	3.0 a	3.8 cd	0.0163 f
	20 g kg^{-1}	40.6 d	3.0 a	3.9 bcd	0.0189 def
Sage	10 g kg^{-1}	51.2 cd	2.9 a	4.1 abc	0.0215 cde
	20 g kg^{-1}	77.5 b	3.0 a	4.5 a	0.0249 bc
Dry residues					
Fennel	10 g kg^{-1}	75.6 b	1.7 d	4.1 abc	0.0217 cde
	20 g kg^{-1}	93.2 a	2.0 dc	4.2 abc	0.0266 b
Rue	10 g kg^{-1}	58.4 bcd	2.3 bc	4.5 a	0.0268 b
	20 g kg^{-1}	61.8 bcd	1.7 d	4.2 abc	0.0322 a
Sage	10 g kg^{-1}	62.2 bcd	2.9 a	4.4 ab	0.0279 ab
	20 g kg^{-1}	69.3 bc	2.7 ab	4.2 abc	0.0280 ab

Means followed by the same letter within the column are not significantly different at $p < 0.05$.

Effect of plant residues in pots with soil

The emergence of weed seedlings was not significantly affected when plant residues were incorporated, except in the treatments with fresh rue residues in 20 g kg^{-1} which had inhibitory and dry fennel residues in 20 g kg^{-1} which had stimulatory effect (Table 4). However, higher residue rates had higher effect. Reduction of root length ranged from 8.2 to 46.6% with fresh and dry fennel residues and dry rue residues showing the highest negative potential. Shoot length on the other hand was significantly reduced only with fennel fresh residues in lower rate. Fresh weight of seedlings was inhibited with lower rates of fresh fennel and rue residues for 20.1 and 26.4%, respectively. All dry residues, except for fennel in 20 g kg^{-1} rate, had significant stimulatory effect on weed fresh weight, up to 45.9%. On average, fresh plant residues had higher inhibitory effect on emergence percent, shoot length and fresh weight of hoary cress.

Incorporation of plant residues in soil can be both stimulatory and inhibitory and the results clearly indicate that differences in the allelopathic potential depended on the plant species, amount and condition of residues. Oliva *et al.* (2002) reported severe reduction in emergence of crops when rue leaves were incorporated or used as mulch. Results indicated that soil with rue leaves had higher values of water-soluble phenolics, electrical conductivity and nitrate, and lower pH values compared to the untreated soil. Similarly, plants of fennel, parsley, and dill according to Dhima *et al.* (2009) incorporated as green manure reduced plant number of barnyardgrass, common purslane and common lambsquarters. On the other hand, Baličević *et al.* (2014) stated that marigold fresh and dry residues promoted emergence and growth of hoary cress. Aromatic plants with the capacity to produce phytotoxic essential oils could play an important role for weed suppression in sustainable agriculture systems (Dhima *et al.*, 2009).

Conclusions

The fennel, the rue and the sage seed and the plant biomass have both positive and negative allelopathic effect on weed species hoary cress. Negative allelopathic effect depended on the aromatic and medicinal plant species, concentration or whether the fresh or dry biomass was used. Certain extracts from the fresh biomass showed weed inhibition both in Petri dish and pots assays. The results also indicate that the fresh residues of plants and their incorporation could be used to suppress weed germination and fresh weight. Allelopathic effect was different depending on the media used which shows the need for conducting experiments both on the filter paper and in the soil.

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