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EFFECT OF MARIGOLD (*CALENDULA OFFICINALIS* L.) COGERMINATION, EXTRACTS AND RESIDUES ON WEED SPECIES HOARY CRESS (*CARDARIA DRABA* (L.) DESV.)

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Abstract

The aim of the study was to examine allelopathic effect of marigold (*Calendula officinalis* L.) on germination and growth parameters of weed species hoary cress (*Cardaria draba* (L.) Desv.). In total, four experiments were conducted. In Petri dishes, cogermination of hoary cress and marigold seeds, and extracts from fresh and dry marigold biomass in concentrations of 5 and 10% (50 and 100 g per litre of distilled water) were evaluated. In pots with soil, effect of extracts from fresh marigold biomass in aforementioned concentrations and effects of fresh and dry marigold residues in two rates (10 and 20 g/kg of soil) were examined. Cogermination of hoary cress and marigold seeds promoted germination and growth of weed. Extracts from fresh and dry marigold biomass, in Petri dish assay, reduced germination of hoary cress on average for 11.9 and 96.9%. Extracts from fresh biomass promoted shoot length of weed seedlings. On the other hand, extracts from dry biomass strongly inhibited all measured parameters. Higher concentration had greater inhibitory effect. Extracts from fresh marigold biomass applied in pots with soil, had stimulatory effect on weed growth, except for root growth which was inhibited with higher concentration (for 6.6%). Fresh marigold residues incorporated in soil had stimulatory effect on germination and seedling growth of hoary cress. Dry residues also had positive effect, but root length was inhibited for 15.4% with lower and 10.2% with higher rate.

Keywords: allelopathy, cogermination, water extracts, plant residues, marigold (*Calendula officinalis* L.), hoary cress (*Cardaria draba* (L.) Desv.)

Introduction

Although weed management in modern agriculture primarily relies on use of chemical herbicides, their excessive and irrational application

leads to various problems in weed control (weed resistance, herbicides residues), environmental pollution and adverse effects on human and animal health (Macías *et al.*, 2003, Singh *et al.*, 2003, Baretto *et al.*, 2000). It is therefore necessary to reduce the amount of chemicals and minimize their negative impact with use of alternative non-chemical and environmentally friendly methods, such as allelopathy. Allelopathy represents the influence on one organism on the other, whether it is harmful or beneficial, through production of allelochemicals (Rice, 1984).

Allelopathically active crops can be utilized in different ways against weeds i.e. as water extracts and surface mulch, incorporated in the soil, as cover crops, in crop rotation (Singh *et al.*, 2003, Reigosa *et al.*, 2001). Allelopathic potential of medicinal and aromatic crops and their use as water extracts, essential oils and soil incorporated green manure has been increasingly explored (Đikić *et al.*, 2005b, Dudai *et al.*, 1999, Dhima *et al.*, 2009).

Marigold (*Calendula officinalis* L.) is an annual or biennial plant, with yellow or orange large and numerous flowerheads, belonging to the Asteraceae family (Parađiković *et al.*, 2013, Erhatić *et al.*, 2014). It is grown as medicinal and ornamental plant and used in cosmetic and pharmaceutical industry (Šilješ *et al.*, 1992, Cromack and Smith, 1998). Marigold grown in consociation reduces pest occurrence in cabbage (Jankowska *et al.*, 2009) and soil amended with marigold tissue reduces the incidence of *Meloidogyne incognita* nematodes (Prakash and Rao, 1997). It also shows allelopathic properties against other plant species. According to Ruszkowski *et al.* (2004) marigold secretes 3-O-monoglucoside through its roots and reduces the growth of other susceptible plants such as lettuce, while water extracts from shoots and plant residues show negative allelopathic effect on basil (Szabo and Prazna, 1996). Đikić (2004, 2005b) reported both positive and negative effects of marigold extracts on weed species.

The objective of the study was to determine the allelopathic effect of marigold (*C. officinalis*) on hoary cress (*Cardaria draba* (L.) Desv.) through cogermination, use of water extracts and plant residues.

Materials and methods

The experiments were conducted in 2013 in the Laboratory of Phytopharmacy at the Faculty of Agriculture in Osijek.

Seeds of marigold (*C. officinalis*) were purchased from seed company Sjemenarna Zagreb d.o.o., and weed seeds of hoary cress (*C. draba*) were collected during 2013 from agricultural fields in Osijek-Baranja County. The weed seeds were surface-sterilized for 20 min with 1% NaO-

Cl (4% NaOCl commercial bleach), then rinsed three times with distilled water (Siddiqui *et al.*, 2009).

Marigold aboveground mass was harvested in full flowering stage (phenological stage 6/65 (Hack *et al.*, 1992)). Fresh biomass was shade dried, cut into small pieces and ground with electronic grinder into fine powder. Water extracts were prepared according to Norsworthy (2003) from fresh and dry aboveground mass of marigold. One hundred grams of fresh or dry biomass was mixed with 1000 ml of distilled water and kept for 24 h at room temperature. The mixtures were filtered through muslin cloth to remove debris and after that through filter paper. The obtained extracts were diluted with distilled water to give final concentrations 5 and 10% (50 and 100 g of biomass per litre). Commercial substrate was used in experiments with soil medium.

In total, four experiments were conducted using Petri dishes with filter paper and pots with soil.

In the first experiment the effect of cogermination of marigold and weed seeds was investigated according to Đikić (2005a). The treatment consisted of thirty seeds of both marigold and hoary cress germinating together in Petri dishes (9 cm diameter) on top of filter paper soaked in distilled water. Control treatment consisted of 30 weed seeds per dish.

In the second experiment the effect of marigold water extracts from fresh and dry biomass on hoary cress was evaluated on filter paper. Thirty seeds were placed in sterilized Petri dishes (9 cm in diameter) on top of filter paper. In each Petri dish 2 ml of extract was added, while distilled water was used in control. Additional extract/water was added to each as needed.

In the third experiment the effect of water extracts from marigold fresh biomass in soil medium was evaluated. Thirty weed seeds were sown in pots filled with commercial substrate and treated with 30 ml of extract or water in control treatment. Thereafter, all treatments were equally watered.

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

In the first and second experiment Petri dishes were kept at room temperature (22 °C ± 2) for 9 days, while in third and fourth experiment pots with soil for 14 days. All treatments had four replications and all experiments were conducted twice.

Germination percentage was calculated for each replication using the formula: G = (Germinated seed/Total seed) x 100. At the end of each experiment, seedling root length (cm), shoot length (cm) and fresh weight (mg) were determined. The collected data were analysed statistically with ANOVA and differences between treatment means were compared using the LSD-test at probability level of 0.05.

Results and discussion

Cogermination of marigold and hoary cress seeds showed no significant effect on germination, root length and fresh weight of weed seedlings (Table 1), although all measured parameters were slightly higher than in control. Contrary, shoot length was significantly stimulated in cogermination.

Table 1. Effect of cogermination on germination and seedling growth of hoary cress

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)
Control	77.5 a	2.72 a	1.90 b	0.014 a
Cogermination (marigold + hoary cress)	78.3 a	3.12 a	2.05 a	0.034 a

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

Cogermination of weed and crop seeds can affect weed germination and growth variously, and depends on donor and recipient species. According to Ravlić *et al.* (2013) basil and coriander reduced germination of hoary cress, but promoted shoot length, while basil also promoted root length. Đikić (2005a) found that caraway, dill and coriander have inhibitory effect on germination and fresh weight of hoary cress, while dill had stimulatory effect on germination of quackgrass.

In Petri dish assay, water extracts from fresh and dry biomass of marigold showed negative allelopathic effect on hoary cress germination (Table 2). Lower concentration of fresh biomass extract showed no significant effect, while higher concentration reduced germination for 17.6%. Extracts from dry marigold biomass had greater effect and both concentrations reduced germination for 93.8 and 100%, respectively. Extracts from fresh biomass showed no significant positive or negative effect on weed seedlings fresh weight and root length. However, they significantly promoted shoot length, especially the lower concentration, for 23.8%. Ex-

tracts from dry biomass, on the other hand, had significant inhibitory effect on root and shoot length and reduction amounted up to 100% with the higher concentration. On average, with the increase of concentration of biomass in extracts, all measured parameters decreased. However, there was no statistical difference in inhibitory potential between 50 and 100 g/l biomass on fresh weight, root and shoot length. Extracts from fresh marigold biomass had lower inhibitory effect on germination and growth of weed seedlings than extracts from dry marigold biomass.

Table 2. Effect of marigold water extracts on germination and seedling growth of hoary cress on filter paper

Marigold biomass in water, g/l	Germination (%)			Fresh weight (g)		
	Fresh biomass	Dry biomass	Average	Fresh biomass	Dry biomass	Average
0	80.8 a	80.8 a	80.8 a	0.012 a	0.012 a	0.012 a
50	75.7 a	4.99 b	40.4 b	0.014 a	0.006 ab	0.010 ab
100	66.6 b	0.00 b	33.3 c	0.012 a	0.000 b	0.006 b
Average	74.4 A	28.6 B		0.013 A	0.006 B	
Marigold biomass in water, g/l	Root length (cm)			Shoot length (cm)		
	Fresh biomass	Dry biomass	Average	Fresh biomass	Dry biomass	Average
0	2.49 a	2.49 a	2.49 a	2.35 c	2.35 a	2.35 a
50	2.48 a	0.08 b	1.28 b	2.91 a	0.01 b	1.46 b
100	2.56 a	0.00 b	1.28 b	2.63 b	0.00 b	1.31 b
Average	2.51 A	0.86 B		2.63 A	0.79 B	

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

Germination, shoot length and fresh weight of hoary cress sown in soil were all stimulated significantly when extracts from fresh marigold biomass in both concentrations were applied (Table 3). Germination was stimulated over 100%, and shoot length and fresh weight for up to 26.6 and 46.1%, respectively. Root length of hoary cress seedlings was stimulated with lower (for 6.2%), and inhibited with higher concentration (for 6.6%), but there were no differences in compare with control treatment.

Extracts from medicinal and aromatic plants show different allelopathic effects against weeds. Szabo and Prazna (1996) reported negative allelopathic effect of marigold shoot extract on germination of basil seeds. According to Đikić (2004, 2005b) marigold water extracts from fresh biomass (200 g/l) inhibited germination of smallflower (*Galinsoga parviflora* Cav.), but had stimulatory effects on germination of hoary cress, yellow

foxtail (*Setaria glauca* (L.) PB.) and common lambsquarters (*Chenopodium album*) in experiments in Petri dishes.

Table 3. Effect of marigold water extracts (fresh biomass) on germination and seedling growth of hoary cress in soil

Marigold biomass in water, g/l	Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)
Control	34.2 b	2.74 a	3.12 b	0.013 b
50	68.7 a	2.91 a	3.89 a	0.019 a
100	71.6 a	2.56 a	3.95 a	0.018 a

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

Results obtained from the experiments with extracts from fresh marigold biomass somewhat differed considering whether Petri dishes or pots with soil were used. Germination was inhibited in experiment with Petri dishes, and highly stimulated when extracts were applied to soil. However, shoot length was stimulated in both experiments, while root length was not affected significantly. Differences between germination results could be due to higher amount of extract applied to soil (30 ml) or direct contact of seed with extract on filter paper.

Incorporated fresh and dry marigold residues stimulated germination and seedling growth of hoary cress, except for root length which was inhibited when dry residues were applied (Table 4). Germination was stimulated from 38.8 to up to 52.0% compared to the control treatment. The highest measured shoot length was in treatment with 20 g/kg of fresh residues, and was for 33.2% higher compared to the control treatment. Inhibition of root length with dry residues was for 15.4% in treatment with lower and 10.2% in treatment with higher rate. Except for root length, with the increase of residue content, germination, shoot growth and fresh weight increased. Fresh residues had higher impact on root growth, while all other parameters were equally affected regardless whether the residues were fresh or dry.

Incorporation of plant residues in soil can be both stimulatory and inhibitory. Szabo and Prazna (1996) reported negative allelopathic effect of marigold residues incorporated into the soil on basil, while Đikić (2007) found that residues can be both stimulatory and inhibitory. In her study, mugworth stimulated, while tomato, potato and barley residues inhibited germination and sprouting of quackgrass (*Agropyron repens* (L.) PB.).

Table 4. Effect of marigold residues on germination and seedling length of hoary cress

Marigold residues, g/kg	Germination (%)			Fresh weight (g)		
	Fresh biomass	Dry biomass	Average	Fresh biomass	Dry biomass	Average
0	39.4 b	39.4 b	39.4 b	0.014 b	0.014 b	0.014 b
10	56.9 a	54.7 a	55.8 a	0.019 a	0.018 a	0.017 a
20	59.9 a	59.1 a	59.5 a	0.013 b	0.017 a	0.018 a
Average	52.1 A	51.1 A		0.016 A	0.016 A	
Marigold residues, g/kg	Root length (cm)			Shoot length (cm)		
	Fresh biomass	Dry biomass	Average	Fresh biomass	Dry biomass	Average
0	2.85 b	2.85 a	2.85 ab	3.25 c	3.25 b	3.25 b
10	2.85 b	2.41 b	2.63 b	3.82 b	3.79 a	3.81 a
20	3.33 a	2.56 ab	2.94 a	4.33 a	3.66 a	3.99 a
Average	3.01 A	2.61 B		3.80 A	3.57 A	

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

Conclusions

Results of conducted experiments showed that marigold has allelopathic effect on hoary cress, which is in most cases stimulatory. Cogermination of marigold and weed seeds stimulated germination and growth of weed seedlings, although not significantly. Water extracts from fresh marigold biomass in Petri dish and pot experiment had positive effect on hoary cress, except for germination in Petri dish assay. Extracts from dry marigold biomass had inhibitory effect on hoary cress, but since experiments in pots with soil were not conducted, high inhibition could be due to osmotic pressure. Marigold residues, both fresh and dry, promoted hoary cress growth, except for dry residues which inhibited root length of weed seedlings.

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