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Full Length Research Paper

Phytotoxicity effects of *Rumex crispus* L. grounded biomass on spring barley grain germination

Vytautas Pilipavičius[1]*, Kęstutis Romaneckas[1], Egidijus Šarauskis[2], Edvardas Vaiciukevičius[2] and Paulius Kerpauskas[2]

[1]Department of Soil Management, Faculty of Agronomy, Aleksandras Stulginskis University, Studentu street 11, LT-53361 Akademija, Kaunas r., Lithuania.
[2]Faculty of Agricultural Engineering, Aleksandras Stulginskis University, Studentu street 15, LT-53362 Akademija, Kaunas r., Lithuania.

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Common agro ecosystem weed with allelopathic activities Curled dock *Rumex crispus* L. was used to investigate its allelopathic-phytotoxic effects on spring barley germination in laboratory experiments. The experiment of allelopathic-phytotoxic effect was carried out in three stages. Grounded seeds, roots and over-ground part of *Rumex crispus* L. (0 g; 0.01 g; 0.05 g; 0.1 g; 0.25 g and 0.5 g respectively) effected germination of spring barley. *R. crispus* allelopathic-phytotoxic substances (grounded seed, root and over-ground biomass) of low concentration (0.01 to 0.1 g per Petri dish) initiated spring barley germination, and regularly smothered germination as *R. crispus* concentration increased till 0.25 to 0.5 g per Petri dish. The higher the concentration of *R. crispus*, the stronger was the inhibitory effect. This regularity was confirmed by the correlation-regression analysis. Increased concentration of recipient plant (5, 10 and 15 grain of spring barley per Petri dish) did not compensate phytotoxical inhibitory effect of *R. crispus* grounded biomass. *R. crispus* over-ground biomass mostly suppressed germination of spring barley as compared to *R. crispus* grounded root applications. *R. crispus* grounded seed biomass had significant stimulation effect on spring barley grain germination. The period of treatment for spring barley grain germination increasing it from 7 till 14 days had negative effect.

Key words: Allelopathy, curled dock, germination, grounded seed and root and over-ground biomass, *Hordeum vulgare* L., phytotoxicity, *Rumex crispus* L., spring barley.

INTRODUCTION

Plant competition in a crop can start at a very early stage of development even at initial germination. From the scientific and agronomical point of view, it is important to identify how weed biological active substances effect on cultural plants. Curled dock *Rumex crispus* L. belongs to Polygonaceae family. It grows in soils of normal humidity, neutral pH or slightly acid light or sandy loams. *R. crispus* is spread in mostly all Europe, Asia (till China and Japan), America, North Africa, and New Zealand. *R. crispus* belongs to the flora elements of Eurasia; however, nowadays it is spread beyond its early habitat boundaries. Seeds and roots of *R. crispus* are used as medicinal raw material (Aleksandraviciute et al., 1961). In all crops of agricultural plants or agrophytocenoses continuous competition takes place between the crop plants and the weeds. Weeds and agricultural plants are biologically adapted to use the same nutritional elements, consequently, just after sprouting plants act for survival (Pilipavičius, 2005, 2007), that slightly becomes competition for jointly used nutritions in the soil (Lazauskas, 1990). In organic farming dock species, in particular *R. crispus* and *Rumex obtusifolius* are very common and noxious weeds on arable fields and in grassland (Pekrun et al., 2002). Whereas, *R. crispus* near the soil surface

*Corresponding author: E-mail: vytautas.pilipavicius@lzuu.lt.

form broad leaves, regarding that, *R. crispus* became very competitive weed that decrease other plant species abundance (Niggli et al., 1993). Their thrifty plants shade soil surface leveled air-temperature changes and herewith inhibits seed germination from soil top layer (Cavers and Harper, 1964; Pekrun et al., 2002). Recently, another aspect of plant interference has met a growing interest: allelopathy (Delabays et al., 2004). Nearly 240 weeds are known to possess allelopathic properties that further make them competitively stronger (Qasem and Foy, 2001). Chemicals with allelopathic activity are present in many plants and in many organs, including leaves, flowers, fruits and buds (Putman and Tang, 1986). Under certain conditions, these compounds are released into the environment, either as exudates from living tissues or by decomposition of plant residues in sufficient quantities to affect neighbouring plants (Einhellig, 1996). As a plant-plant mechanism, allelopathy has been studied intensively, with focus on aspects such as inhibition of seed germination, shoot and root growth, or changes in physiological parameters and biomass allocation (Ninkovic, 2003; Reigosa et al., 1999). Alellopathy involves both inhibitory and stimulatory biochemical interactions between plants (Uludag et al., 2006). Weed interference has two components: competition and allelopathy. Both components can not be differentiated in the field (Radosevich et al., 1997) however; it is possible to show allelopathic effects of weeds with *in vitro* studies (Uludag et al., 2006). This difficulty reflects the complexity of allelopathic interactions. Allelopathic effects are often modified by additional biotic and abiotic stress factors, uncertain meteorological events, or physical, chemical, and biological soil factors, all of which can influence the residence time, persistence, concentration, and fate of allelopathic compounds in the environment (Inderjit and Keating, 1999). Instead of phytotoxic allelochemicals seed germination also depend on precipitations and soil moisture (Baskin and Baskin, 1987; Roberts and Potter, 1980), variation of temperatures (Baskin and Baskin, 1987), light (Kuht and Ries, 1999; Roeb, 1977), harvester ants (Baraibar et al., 2011), seed pests and pathogens (Brust and House, 1988). All those factors as well seed germination depends on seed position and depth in the soil (Mohler and Galford, 1997; Sauerborn et al., 1988), osmotic potential (Beres and Sardi, 2000; Torma and Hodi, 2002), pH (Pilipavičius et al., 2006; Zaller, 2006) and other conditions of growing media (Lazauskas, 1990; Morton and Buchele, 1960; Pilipavičius, 2007). However, barley *Hordeum vulgare* L. are salt tolerance at germination and the seedling stage (Flowers and Flowers, 2005; Mano and Takeda, 1997). In addition many allelochemicals can directly affect epigaeic or soil microorganisms and the fauna and successively induce further interactions (Malkomes, 2006).

The seeds, roots and over-ground part of Curled dock *R. crispus* L. exclude biologically active, phytotoxic substances, which influence recipient plant growth. It is proved, that *Rumex* spp. release allelochemicals by leaf extracts (Einhellig and Rasmussen, 1973; Zaller, 2006), root exudates (Strom, 1998), many species by decomposing plant residues (Bonanomi et al., 2006). Field observations of vegetation patterning suggested that *R. crispus* might be allelopathic (Einhellig and Rasmussen, 1973). The aim of this work was to evaluate allelopathic-phytotoxic influence of *R. crispus* L. grounded seed, root and over-ground biomass on two-row spring barley *H. vulgare* L. germination under laboratorial conditions.

MATERIALS AND METHODS

The study was carried out at the Soil Management Department of the Lithuanian University of Agriculture in the period of 2005-2006. The seeds, roots and over-ground part of Curled dock *R. crispus* L. were collected at the Research Station of the Lithuanian University of Agriculture in the summer and autumn of 2005. The seeds were cleaned and stored in darkness at room temperature until use. The roots and over-ground biomass were air-dried and stored in darkness at room temperature until use as well. In laboratory experiments the allelopathic-phytotoxic influence of grounded *R. crispus* seed, root and over-ground biomass on spring barley variety *Aura* grain germination was investigated. *R. crispus* seed, root and over-ground biomass was grounded on purpose to get as possible complete accessibility of phytotoxic allelochemicals allocated in its seeds, roots and over-ground biomass. Particle size of grounded *R. crispus* biomass was approximately 0.4 mm. The experimental criteria were: number of spring barley grains (5, 10 and 15 grains); *R. crispus* grounded seed, root and over-ground air-dry biomass (0.0, 0.01, 0.05, 0.1, 0.25, 0.5 g); and period for spring barley grain germination (7 and 14 days). The experiment each treatment combination of spring barley grain number and separately applied *R. crispus* grounded seed, root and over-ground air-dry biomass weight effect on barley germination was conducted in four replications. Spring barley grains and different air-dry biomass of grounded *R. crispus* seeds, roots or over-ground part were put on filter paper in sterilized Petri dishes of 10 cm diameter and watered by 10 ml of distilled water. The germination was induced keeping humidity on filter paper. The data were subjected to two-factorial analysis of variance ANOVA applying Fisher *LSD* tests at the 0.05 level of significance on statistical package “Selekcija” (Tarakanovas, 1997). The correlation-regression analyses by SigmaPlot 8.0 programmes (SPSS Science, 2000) were applied evaluating degrees and directions of interdependent phenomena.

RESULTS AND DISCUSSION

In laboratorial trial allelopathic-phytotoxic influence of Curled dock *Rumex crispus* L. grounded biomass (seed, root and over-ground air-dry biomass) was investigated on spring barley variety *Aura* seed germination. At the first stage of experiment (Figure 1) increasing *R. crispus* grounded root biomass from 0 to 0.01 g, in Petri dish, increased spring barley seed germination during 7 days period of sprouting. Later, after 14 days of sprouting, increasing *R. crispus* grounded root biomass to 0.1 gram in *Petri* dish, the number of germinated spring barley grains increased as compared to the control treatment (0 g of *R. crispus* root biomass). This regularity remained changing spring barley grain number from 5 to 15. Further increasing *R. crispus* grounded root biomass to 0.25

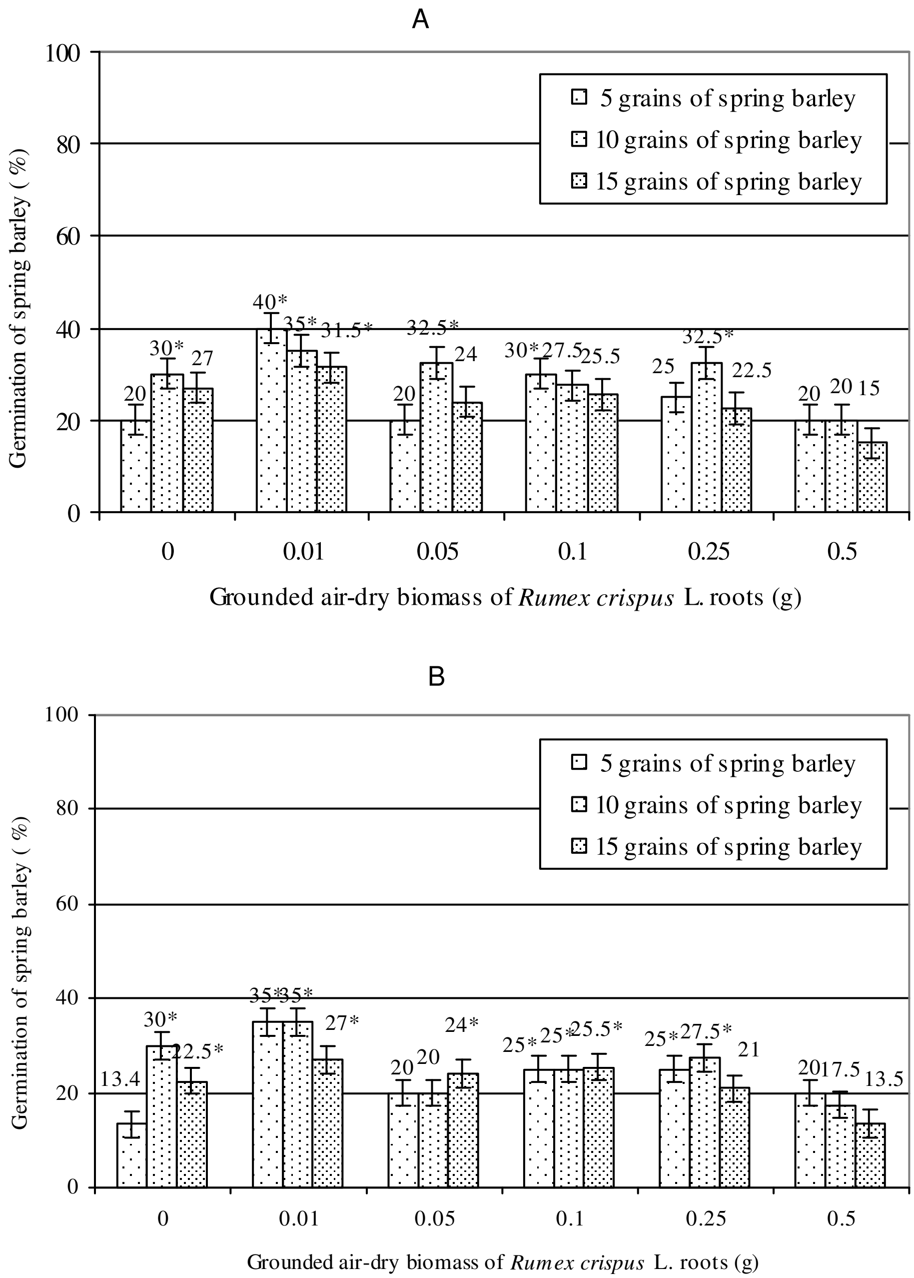

Figure 1. Phytotoxicity effect of *Rumex crispus* L. grounded root air-dry biomass on spring barley germination [%] during 7 A and 14 B days of sprouting (⌶ - Standard error, $LSD_{0.05}$ – least significant difference at 95% level of probability, * - significant difference at 95% level of probability from control treatment – *Petri* dish with 5 grains of spring barley and 0 g of *R. crispus* biomass). (a) Germination after 7 days of sprouting, LSD0.05=9.44. (b) Germination after 14 days of sprouting, LSD0.05=8.01.

and 0.5 g per Petri dish, the germination of spring barley grain decreased (Figure 1).

Correlation-regression analysis showed negative linear relationships: the germination of spring barley grain directly depended on *R. crispus* grounded root, over-ground and seed air-dry biomass (Table 1). According to regression equations, increase of *R. crispus* grounded root air-dry biomass by 0.1 g per *Petri* dish inhibited spring barley grain germination by 15 to 26% and 4 to22% depending on number of grains and sprouting days. The influence of *R. crispus* grounded root biomass show strong regularity to inhibit grain germination

Table 1. Dependence of *R. crispus* grounded seed, root and over-ground air-dry biomass on spring barley grain germination under laboratorial conditions.

R. cispus air-dry biomass	Spring barley grain number	Regression equation	Correlation coefficient, *r*	*P*
		After 7 days of sprouting		
	5	y = 28.151 – 15.278 x	- 0.369	0.472
Root	10	y = 32.884 – 21.759 x	- 0.788	0.063
	15	y = 28.204 – 26.071 x	- 0.920	0.009
	5	y = 47.503 – 71.448 x	- 0.846	0.034
Over-ground part	10	y = 27.353 – 26.503 x	-0.521	0.289
	15	y = 25.412 – 15.902 x	- 0.576	0.231
	5	y = 45.877 + 21.693 x	0.349	0.497
Seed	10	y = 56.264 – 22.071 x	- 0.482	0.333
	15	y = 41.968 – 8.031 x	- 0.178	0.737
		After 14 days of sprouting		
	5	y = 23.789 – 4.761 x	- 0.127	0.810
Root	10	y = 29.103 – 21.559 x	- 0.646	0.166
	15	y = 25.677 – 22.597 x	-0.914	0.011
	5	y = 40.966 – 83.296 x	- 0.935	0.006
Over-ground part	10	y = 26.452 – 26.058 x	- 0.555	0.253
	15	y = 24.076 – 20.285 x	- 0.720	0.107
	5	y = 44.156 + 22.049 x	0.283	0.587
Seed	10	y = 54.236 – 27.929 x	- 0.521	0.289
	15	y = 39.616 – 13.951 x	- 0.289	0.579

increasing spring barley grain number from 5 to 15 per Petri dish (Table 1). At the second stage of experiment (Figure 2) allelopathic effect of *R. crispus* grounded over-ground part air-dry biomass on spring barley germination during 7 and 14 days of sprouting was evaluated. Analysing the period of germination was observed that the number of germinated spring barley grains after 14 days was marginally lower than after seven days. However, during both germination periods correlation analysis proved medium to strong dependences of *R. crispus* grounded over-ground part air-dry biomass increase on spring barley grain germination decrease. For 7 days sprouting period correlation coefficients formatives 0.521 to 0.846 and for 14 days sprouting period – 0.555 to 0.935 (Table 1). It was found that increasing *R. crispus* grounded over-ground part air-dry biomass of all concentrations inhibited germination of spring barley grains in treatment with 5 grains. Germination was inhibited by 36% as compared to control treatment (0 g) with highest *R. crispus* over-ground biomass concentration (0.5 g) after 7 days of sprouting. After 14 days of sprouting analogous comparison compounded 9 times (Figure 2). In treatments with 10 and 15 grains of spring barley *R. crispus* over-ground biomass initiated spring barley seed germination reaching maximum at 0.05 to 0.1 g per Petri dish; the germination was by 1.6 to 2.5 times higher than in control treatment (0 g). Further increasing *R. crispus* grounded over-ground part air-dry biomass to 0.5 g per Petri dish the seed number of germinated spring barley decreased from 15 to 20% till 75% depending on barley grain number (treatment of 10 and 15 grains) in Petri dish compared with control treatment (0 g) (Figure 2).

At the third stage of experiment (Figure 3) allelopathic-phytotoxic effect of *R. crispus* grounded seed biomass on spring barely *Aura* germination was evaluated. The number of germinated spring barely grains after 7 days of sprouting was similar or a bit higher than after 14 days of sprouting. Increasing concentration of *R. crispus* grounded seed air-dry biomass per Petri dish from 0 till 0.01, 0.05 and 0.1 g, the spring barley grain germination increased till 1.9 to 2.2 times sprouting 5 barley grains, till 1.4 times sprouting 10 grains of barley and till 1.6 times sprouting 15 barley grains. This tendency was proven by the correlation-regression analysis too. Sprouting 5 grains of spring barley per Petri dish was established increase of grain germination increasing *R. crispus* grounded seed biomass (Table 1). Contrariwise, analogous

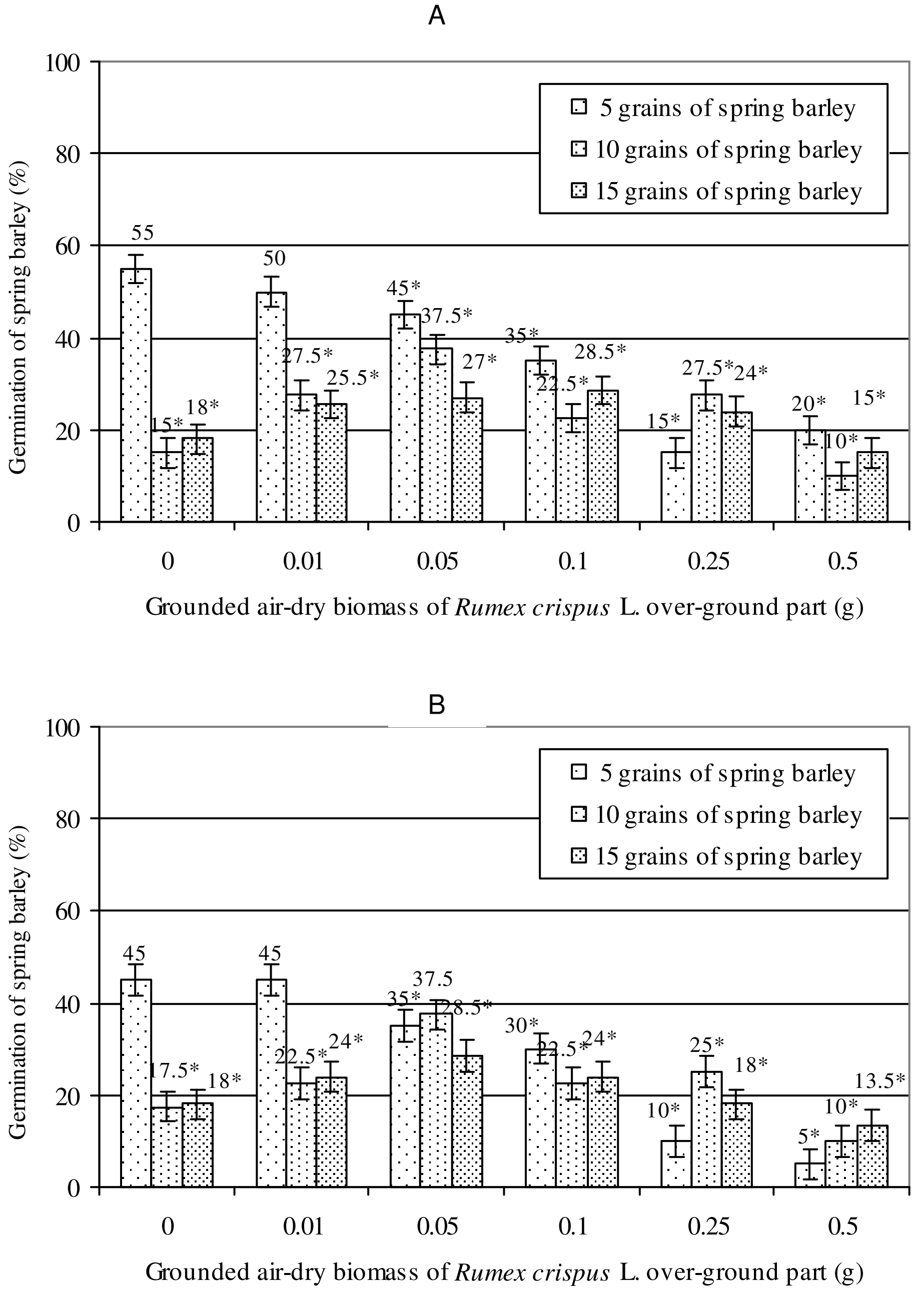

Figure 2. Phytotoxicity effect of *Rumex crispus* L. grounded over-ground part air-dry biomass on spring barley germination [%] during 7 A and 14B days of sprouting (- Standard error, $LSD_{0.05}$ – least significant difference at 95% level of probability, * - significant difference at 95% level of probability from control treatment – *Petri* dish with 5 grains of spring barley and 0 gramm of *R.crispus* biomass). (a) Germination after 7 days of sprouting, LSD0.05=8.89. (b) Germination after 14 days of sprouting, LSD0.05=9.54.

experiments with *Cirsium arvense* grounded seed biomass did not show tendency to stimulate spring barley germination. *C. arvense* allelopathic substances (grounded seed biomass) already at the lowest concentration influencing plants have been inhibited spring barley germination, and regularly smothered germination as *C. arvense* concentration increased (Pilipavičius, 2008). Continuously increasing *R. crispus* grounded seed biomass per Petri dish till 0.5 g, the spring barley grain germination was inhibited, however, it was left a bit higher

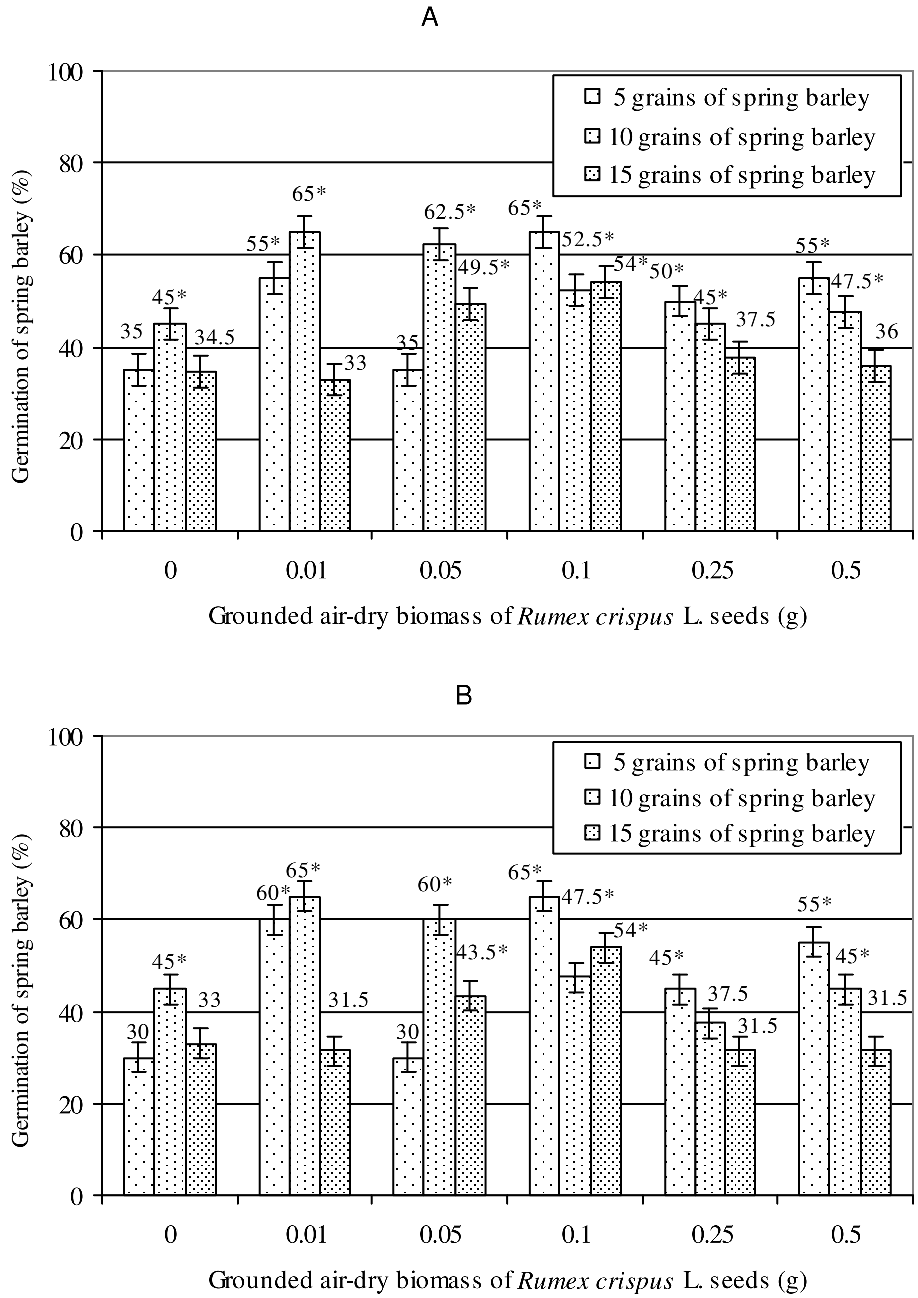

Figure 3. Phytotoxicity effect of *Rumex crispus* L. grounded seed air-dry biomass on spring barley germination [%] during 7 A and 14 B days of sprouting (- Standard error, *LSD*0.05 – least significant difference at 95% level of probability, * - significant difference at 95% level of probability from control treatment – *Petri* dish with 5 grains of spring barley and 0 gram of *R.crispus* biomass). (a) Germination after 7 days of sprouting, LSD0.05=9.73. (b) Germination after 14 days of sprouting, LSD0.05=9.24.

higher or at least at the control treatment level where *R. crispus* grounded seed biomass was not applied (Figure 3). The influence of time period for spring barley germination 7 or 14 days has the analogous tendency of weak to very strong correlation. Therefore, there were established similar tendencies comparing spring barley grains germination results after 7 and 14 days of experiment increasing *R. crispus* root, over-ground part and seed grounded air-dry biomass per *Petri* dish (Table 1).

Summarised results of this study showed that *R. crispus* grounded seed, root and over-ground biomass containing allelopathic-phytotoxic substances applied in low concentrations (0.01-0.1 g per Petri dish) initiated spring barley germination, and regularly smothered germination (except seed biomass) as *R. crispus* concentration increased (0.25 to 0.5 g per Petri dish). The higher the concentration of *R. crispus*, the stronger is the inhibitory effect. This regularity was confirmed by the correlation-regression analysis. Increased concentration of recipient plant (spring barley grains) did not compensate phytotoxical inhibitory effect of *R. crispus* grounded biomass. *R. crispus* over-ground biomass mostly suppressed germination of spring barley as compared to *R. crispus* grounded root applications. *R. crispus* grounded seed biomass had significant stimulation effect on spring barley grain germination. The period of treatment for spring barley grain germination has negative effect increasing it from 7 till 14 days.

Future investigations are necessary to establish effects under field conditions of *R.crispus* seeds, roots and over-ground part on spring barley germination. Probably the most difficult step in this process is to ensure that the extracted compound is the one released under field conditions, and determine if these amounts are sufficient to essentially affect plant germination and growth (Azirak and Karaman, 2008).

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