COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.02
Agrovoc descriptors: allelopathy, amaranthaceae, weeds, biological competition, crops, tolerance, resistance to injurious factors, germination, growth

Agris category code: f62, f40, h60

Allelopathic effects of redroot pigweed (*Amaranthus retroflexus* L.) on germination & growth of cucumber, alfalfa, common bean and bread wheat

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Received March 09, 2015; accepted July 27, 2015.
Delo je prispelo 09. marca 2015, sprejeto 27. julija 2015.

ABSTRACT

Allelopathy is one of the important interactions among plants. Weeds can reduce crops productions in farms by their allelopathic effects. Redroot pigweed (*Amaranthus retroflexus* L.) is the most common weed in Iran with well-known allelopathic potential. In the presented experiment, the allelopathic effects of redroot pigweed on germination and growth of four important crop species including cucumber (*Cucumis sativus* L.), alfalfa (*Medicago sativa* L.), common bean (*Phaseulus vulgaris* L.) and bread wheat (*Triticum aestivum* L.) was studied. The effect of different concentrations of redroot pigweed leachate on seed germination and seedlings growth parameters of tested plants was significant, but not same in all studied species. Bread wheat and cucumber were more resistance in seed germination stage in comparison to common bean and alfalfa. Except alfalfa, all plant species showed certain rate of resistance in the most measured parameters. According to the obtained results, bread wheat and common bean were the most resistant species, cucumber was resistant at low concentration but sensitive at high concentration, and alfalfa was the most sensitive species to the redroot pigweed leachate treatments. Therefore, the cultivation of resistant plant species (such as bread wheat and common bean plants) in the regions with redroot pigweed's invasion is appropriate way in management of the farms.

Key words: allelopathy, redroot pigweed, resistance, crop species, leachate concentration

IZVLEČEK

ALELOPATIČNI UČINEK NAVADNEGA ŠČIRA (*Amaranthus retroflexus* L.) NA KALITEV IN RAST KUMAR, LUCERNE, NAVADNEGA FIŽOLA IN KRUŠNE PŠENICE

Alelopatija je ena izmed najpomembnejših interakcij med rastlinami. Pleveli lahko zmanjšajo pridelek zaradi njihovih alelopatičnih učinkov. Navadni ščir (*Amaranthus retroflexus* L.) je v Iranu najpogostejši plevel z dobro znanim alelopatičnim učinkom. V tej raziskavi smo preučevali alelopatski učinek navadnega ščira na kalitev in rast štirih pomembnih kulturnih rastlin in sicer kumar (*Cucumis sativus* L.), lucerne (*Medicago sativa* L.), navadnega fižola (*Phaseulus vulgaris* L.) in krušne pšenice (*Triticum aestivum* L.). Učinek različnih koncentracij izvlečka navadnega ščira na kalitev in rastne parameter preiskuševanih rastlin je bil značilen toda ne enak pri vseh rastlinah. Krušna pšenica in kumare so bile bolj odporne na stopnji kalitve v primerjavi s fižolom in lucerno. Z izjemo lucerne so vse preiskušene vrste pokazale določeno odpornost pri vseh merjenih parametrih. Glede na rezultate te raziskave sta se krušna pšenica in navadni fižol izkaza kot najbolj odporna, kumare so bile pri manjših koncentracijah ekstrakta navadnega ščira odporne, a občutljive pri velikih koncentracijah. Lucerna je bila najbolj občutljiva na izločke navadnega ščira pri vseh obravnavanjih. Na osnovi dobljenih rezultatov priporočamo kmetovalcem na območjih z večjim pojavljanjem navadnega ščira gojenje nanj odpornih rastlin kot sta krušna pšenica in navadni fižol.

Ključne besede: alelopatija, navadni ščir, odpornost, kmetijske rastlines, koncentracije izvlečkov

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1 INTRODUCTION

Farmers have realized long-time ago that in addition to cultivated crop species, specific plants are growing in agricultural land called weeds (Dhole et al., 2011; Salehian and Eshaghi, 2012; Modhej et al., 2013). In addition to reduction of resources available to crop species (competition), allelochemical compounds produced by weed may also affect plants growth (allelopathy) (Shahrokhi et al., 2011; Modhej et al., 2013; Konstantinović et al., 2014). Allelopathy is one of the most important interactions between plants (Amini et al., 2012; Amini, 2013; Konstantinović et al., 2014), which occurs via production of certain compounds called allelochemicals, mainly a subset of secondary metabolites (Khan et al., 2010; Amini et al., 2012; Soltys et al., 2013). Plants can release these compounds into the environment by different ways such as leaching the allelopathic materials from the shoot by rainfall, releasing volatile phytotoxic compounds from plant's green parts, releasing phytotoxic compounds from decomposed plant material and eventually, releasing phytotoxic compounds by the root exudates (Weir et al., 2004; Terji, 2008; Amini et al., 2012; Soltys et al., 2013). Almost 250 weed species that have invaded farms have been identified which have the potential to produce allelopathic compounds (Shahrokhi et al., 2011). Although many species from the genus *Amaranthus* are weeds, the redroot pigweed *(Amaranthus retroflexus* L.*)* is the most famous with the well-known allelopathic effects (Costea et al., 2004; Shahrokhi et al., 2011; Shahrokhi et al., 2012; Konstantinović et al., 2014). Moreover redroot pigweed is a common weed in Iran and can be seen frequently on agricultural lands (Shahrokhi et al., 2011; Shahrokhi et al., 2012). This plant is one of the main components of desert and semi-desert's flora (Lamonico, 2010; Duretto and Morris, 2011) and expansion from Iran in the desert belt of the world could be the reason of its high distribution in this country. Redroot pigweed is summer annual C_4 species with high biological potential and can produce a lot of seeds (Lamonico, 2010; Duretto and Morris, 2011; Amini et al., 2012; Shahrokhi et al., 2012). It is one of the few resistant plants to several herbicides, including atrazine, simazine, imazethapyr, thifensulfuron, and linuron (Costea et al., 2004; Sarabi et al., 2011).

Many researchers have reported the allelopathic effects of redroot pigweed on different crops (Shahrokhi et al., 2011; Tejeda-Sartorius et al., 2011; Amini et al., 2012; Dogaru et al., 2012; Mlakar et al., 2012; Namdari et al., 2012; Shahrokhi et al., 2012; Konstantinović et al., 2014). Nodaway, several allelic compounds such as aldehydes, alkaloids, apocarotenoids, flavonoids, steroids, xyloids, chlorogenic acid and saponins have been identified from amaranth residues (Shahrokhi et al., 2011; Shahrokhi et al., 2012).

The recent study was carried out in order to evaluate the allelopathic effects of different leachate concentrations of redroot pigweed on germination and growth of some important and common crop species in Iran in order to determine their sensitivity and resistance to chemicals produced by redroot pigweed.

2 MATERIALS AND METHODS

2.1 Experimental design and treatments

The experiment was conducted as factorial based on completely randomized design (CRD) with 3 replications. Experimental factors were crop species at four levels including cucumber (*Cucumis sativus* 'Basmenj'), alfalfa (*Medicago sativa* 'Hamedan'), common bean (*Phaseulus vulgaris* 'Dorsa') and bread wheat (*Triticum aestivum* 'Pishgam') and different concentrations of redroot pigweed leachate (5 % and 10 %). Double distilled water was considered as control.

2.2 Sampling and plant extract preparation

Redroot pigweed fresh material including root, stem, leaf and flower was collected from crop fields of Khosroshahr (East Azerbaijan, Iran) and powdered after air drying under lab conditions. For leachate preparation, 10 grams of powdered material were suspended in100 ml double distilled water and mixed for 24 hours by a horizontal rotary shaker for producing uniform suspension (Shahrokhi et al., 2011; Mlakar et al., 2012; Shahrokhi et al., 2012). Suspension was filtered using two layers of sterile cheese cloth and this filtrate was considered as leachate. Furthermore, leachate 5 % was prepared by dilution of leachate 10 % using double distilled water.

2.3 Plant culture and bioassay tests

For evaluation of the allelopathic effect of weed extracts on germination and growth of cucumber, alfalfa, common bean and bread wheat seeds were disinfected using 1 % (v/v) sodium-hypochlorite solution for 5 minutes and washed sufficiently using sterile distilled water. Ten seeds of each species were placed in Petri dishes containing sterile filter paper and 5 ml of leachate with appropriate concentration were added. Control seeds were moistened with 5 ml of sterile double distilled water. Petri dishes were sealed with parafilm for prevention of pollution and water evaporation and transferred to darkness. After 2 days all germinated seeds were transferred to climate chambers with controlled conditions (25-30 °C, 16/8 (light/dark) photoperiod and relative humidity of 60 %). The percentage of germinated seeds was recorded daily and growth parameters like seedling length, shoot length, seminal root length, fresh and dry weight of seedlings were determined after 7 days. Relative growth rate (Equation 1) (Tomlinson et al., 2012) and seedling survival rate (Equation 2) (Kusmana, 2010) were calculated using following formulae:

Equation 1: $$RGR = \frac{\Delta y}{y \Delta t} \times 100$$

Where RGR is relative growth rate, Δy is growth amount, Δt is growth time (day) and y is the fresh/dry weight of primary tissue or organ.

Equation 2: $$SSR = \frac{A}{B} \times 100$$

Where SSR is seedling survival rate, A is number of germinates and B is the number of germinated seeds.

2.4 Data analysis

The data were analyzed using GLM procedure by SPSS software (Ver.16) and Tukey's multiple range tests was used for mean comparisons at 1 % probability level.

3 RESULTS

According to statistical evaluation (analysis of variance), the effect of different concentrations of redroot pigweed leachate on seed germination percentage; seedling, shoot and seminal root length; fresh and dry weight of seedlings and its interaction with crop species was significant ($p < 0.01$). However, the effect of different leachate concentrations on germination and growth of species was not the same (Table 1).

Table 1: Mean squares of redroot pigweed leachate's concentrations effect on germination and growth related characteristics of crop species

Source of variation	Df	Shoot Length	Seminal root Length	Seedling Length	Fresh Weight	Dry Weight	Germin. Percentage
Treatments	2	3148.0 **	16473.4 **	31332.8 **	88395.3 **	6706.9 **	5077.8 **
Species	3	2431.3 **	2949.7 **	10382.9 **	194355.7 **	74284.8 **	3425.9 **
Treatments*Species	6	852.1 **	1585.8 **	3835.2 **	19268.8 **	4578.5 **	848.2 **
Error	24	17.67	46.53	122.22	3046.29	844.98	75.0
Coefficient of variation (%)		11.67	18.94	30.70	153.31	80.74	24.05

**: significant at $p < 0.01$ by Tukey's multiple range tests, n = 3

3.1 Seed germination

Except for bread wheat plants, treatment with 5 % redroot pigweed leachate of led to decrease in seed germination. The highest reduction was recorded in alfalfa. Treatment of plants with 10 % redroot pigweed leachate led to a decrease in seed germination in all species. Bread wheat and alfalfa plants showed the lowest and the highest decrease in seed germination respectively. According to the results of germination percentage, bread wheat and cucumber were identified as the most resistant, and alfalfa and common bean were classified as the most sensitive plants among studied species (Figure 1). Daily recording of germination percentage indicated a delay phase in germination of treated plants in all species (Figure 2). The results of these records were also similar to the germination percentage. On the other hand, bread wheat plants showed the lowest delay time, whereas the highest delay time was observed in alfalfa.

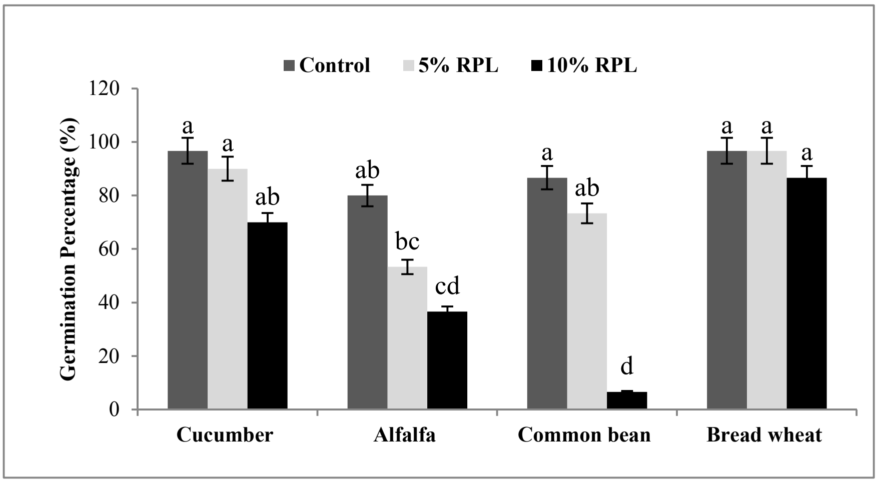
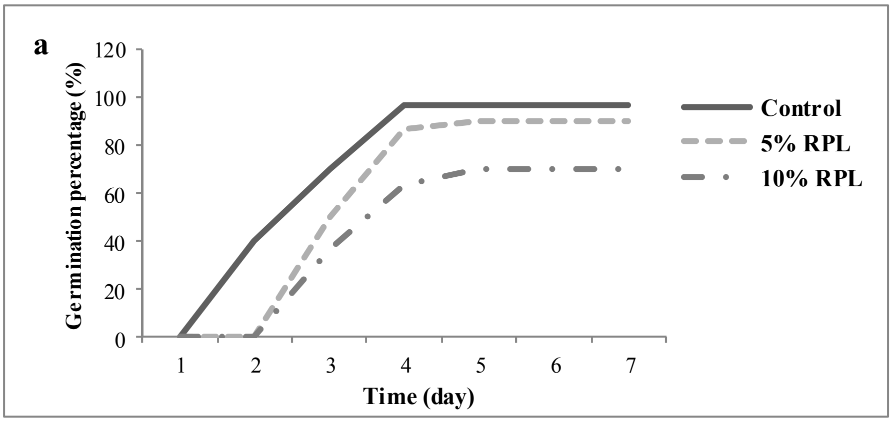


Figure 1: The effect of different concentrations (5 % and 10 %) of the redroot pigweed's leachate (RPL) on seed germination of crop species (n = 3, $p < 0.01$).



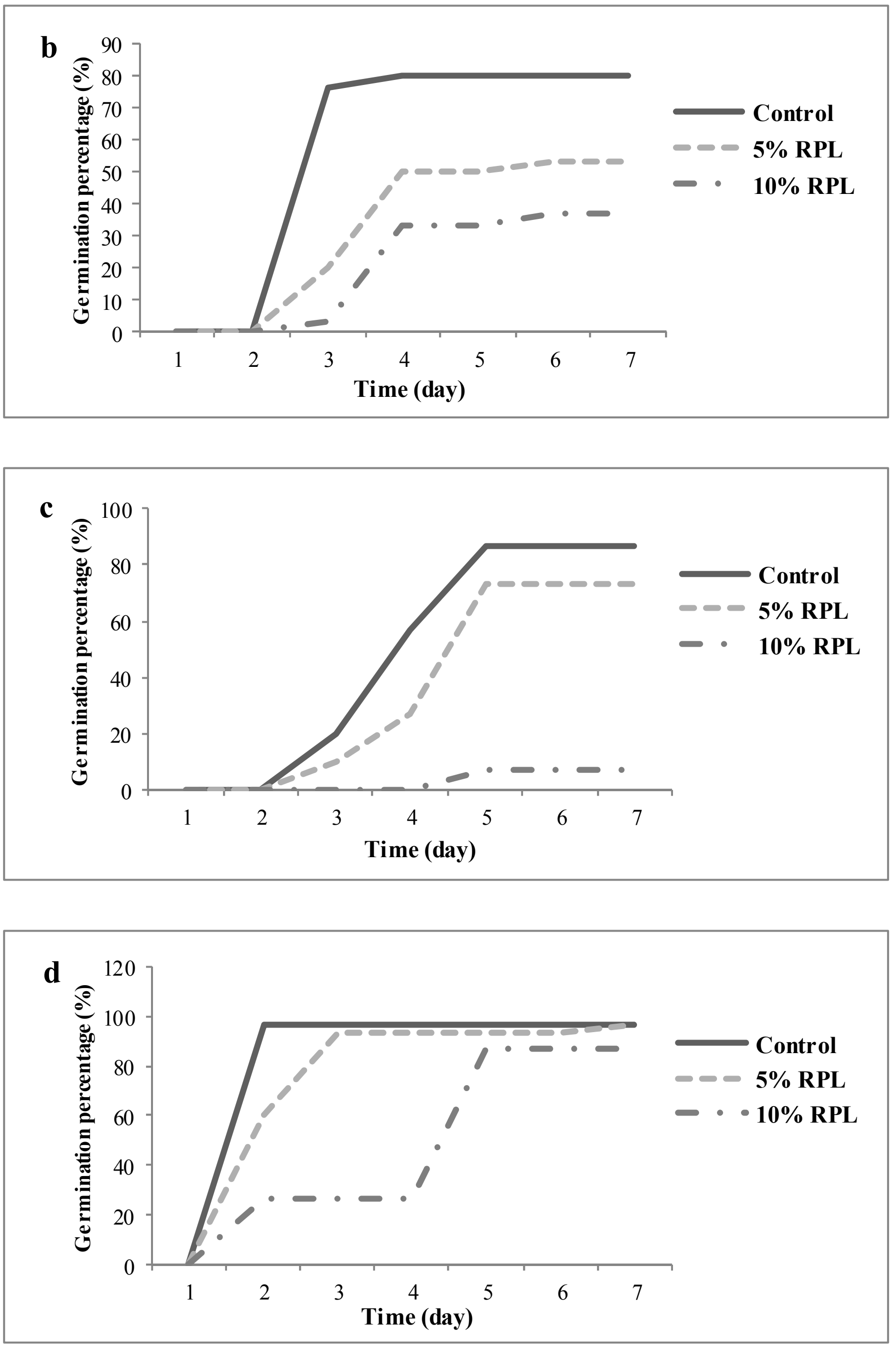


Figure 2: Effects of redroot pigweed leachate (RPL, 5 and 10 %) on seed germination of (a) cucumber, (b) alfalfa, (c) common bean, (d) bread wheat during 7 days after treatment (n = 3, $p < 0.01$).

3.2 Seedling survival rate (SSR)

Only the alfalfa seedling survival rate (SSR) significantly decreased in plants treated with 5 % redroot pigweed leachate and other species were not affected significantly ($p < 0.01$). Treatment of 10 % redroot pigweed leachate led to considerable decrease in alfalfa and cucumber seedling survival rate, while this was not notably affective on bread wheat and common bean plants. Analysing this parameter, bread wheat and common bean were the most resistant, cucumber was relatively sensitive and alfalfa was the most sensitive plants in response to the redroot pigweed leachate (Figure 3).

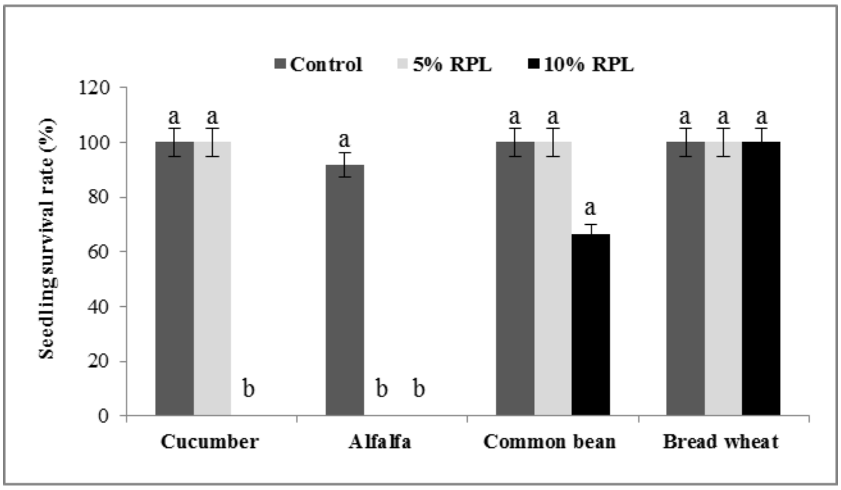


Figure 3: The effects of different concentrations (5 % and 10 %) of the redroot pigweed's leachate (RPL) on seedling survival rate of crop species (n = 3, $p < 0.01$).

3.3 Shoot, seminal root and seedling length

Shoot length of cucumber was not notably affected by treatment with 5 % of redroot pigweed leachate, but in bread wheat and alfalfa this parameter decreased significantly ($p < 0.01$). In common bean plants shoot length decreased 19.24 % in comparison with control plants, but was not significant (Table 2). Significant decrease in shoot length of bread wheat; cucumber and alfalfa were observed in plants treated by leachate 10 %. In common bean plant, this parameter decreased by 27.62 %, but the trend was not significant ($p < 0.01$) (Table 2). According to the obtained results, common bean and bread wheat were the most resistant plants; cucumber was moderately sensitive and alfalfa was the most sensitive species to the redroot pigweed leachate, respectively.

In all studied species seminal root length was significantly shorter in plants treated with 5 % and 10 % leachate of redroot pigweed plant, however, the difference among plants treated with 5 % and 10 % leachate was only significantly different in cucumber plant ($p < 0.01$) (Table 2). Therefore, according to this parameter, common beans and bread wheat were the most resistant plants; cucumber and alfalfa were the most sensitive species to redroot pigweed plant.

The results of seedling length were similar to the results of shoot and seminal root length. Common bean and bread wheat were classified as the most resistant species, cucumber was moderately susceptible and alfalfa was the most sensitive species to redroot pigweed leachate (Table 2).

Redroot pigweed leachate increased shoot/seminal root length ratio of cucumber, bread wheat and common bean plants and the ratio was higher in plants treated with 10 % leachate (Table 2). The shoot/seminal root length ratio in cucumber, common bean and bread wheat plants treated with 5 % leachate were 4.21, 2.98 and 3.71 times bigger than in control plants, respectively. In bread wheat and common bean plants treated with 10 % leachate shoot/seminal root length ratio was 6.01 and 8.154 times higher, respectively, when compared to controls. However, the effect of redroot pigweed leachate on shoot/seminal root length ratio and was not significant ($p < 0.01$). Considering this parameter, among studied species bread wheat and common bean can be classified as the most resistant, cucumber as moderately susceptible and alfalfa as the most sensitive to redroot pigweed leachate.

Table 2: The effect of different concentrations (5 and 10 %) of the redroot pigweed's leachate on shoot, seminal root and seedling length (mm) and shoot/seminal root length ratio of crop species (n = 3, $p < 0.01$).

Plant Species		Parameters			
		Shoot Length	Seminal root Length	Seedling Length	Shoot/ Seminal root
Cucumber	Control	61.07±2.99 ^{a}	123.79±11.18 ^{a}	181.09±19.04 ^{a}	0.494±0.02 ^{b}
	5 %	63.67±2.46 ^{a}	30.99±4.41 ^{b}	94.68±5.23 ^{b}	2.081±0.31 ^{a}
	10 %	0.0±0.0 ^{b}	0.0±0.0 ^{c}	0.0±0.0 ^{c}	0.0±0.0 ^{c}
Alfalfa	Control	11.46±1.10 ^{a}	30.28±4.78 ^{a}	41.75±5.35 ^{a}	0.383±0.06 ^{a}
	5 %	0.0±0.0 ^{b}	0.0±0.0 ^{b}	0.0±0.0 ^{b}	0.0±0.0 ^{b}
	10 %	0.0±0.0 ^{b}	0.0±0.0 ^{b}	0.0±0.0 ^{b}	0.0±0.0 ^{b}
Common bean	Control	19.33±0.72 ^{a}	41.24±5.93 ^{a}	60.57±6.46 ^{a}	0.474±0.06 ^{a}
	5 %	15.61±1.64 ^{a}	11.95±4.04 ^{b}	27.56±5.21 ^{b}	1.416±0.51 ^{a}
	10 %	13.99±0.83 ^{a}	6.12±3.71 ^{b}	20.12±2.88 ^{b}	2.848±1.86 ^{a}
Bread wheat	Control	54.13±10.99 ^{a}	87.65±17.76 ^{a}	141.79±28.69 ^{a}	0.617±0.01 ^{b}
	5 %	22.66±1.70 ^{b}	10.15±2.20 ^{b}	32.81±3.70 ^{b}	2.285±0.40 ^{ab}
	10 %	9.12±1.81 ^{b}	1.89±0.32 ^{b}	11.02±1.48 ^{b}	5.031±1.98 ^{a}

3.4 Fresh and dry weight of seedlings

Both 5 and 10 % leachate leads to significant decrease in seedling fresh weight of bread wheat, cucumber and alfalfa plants ($p < 0.01$). In common bean seedling fresh weigh decreased up to 34 %, but the trend was not significant ($p < 0.01$). Alfalfa growth was completely inhibited by both treatments, whereas cucumber growth was only inhibited with 10 % redroot pigweed leachate (Table 3). Therefore, according to this parameter, common beans and bread wheat were the resistant plants; cucumber and alfalfa were the sensitive species to redroot pigweed allelopathic effects.

The effect of redroot pigweed leachate on seedling dry weight of cucumber and alfalfa plants was similar to its effect on fresh weight. 5 % leachate stimulated common bean dry weight, but significant decrease occurred in plants treated with 10 % leachate in comparison with control plants ($p < 0.01$). However, the effect of leachate on bread wheat plants dry weight was in contrast and not significant. On the other hand, leachate of 5 % reduced and leachate of 10 % stimulated dry material accumulation in bread wheat plants (Table 3). Considering this parameter, bread wheat is the most resistance and alfalfa is the most sensitive species to redroot pigweed leachate.

3.5 Related growth rate (RGR)

Relative growth rate (RGR) varied between studied species and redroot pigweed leachate treatments (Table 3). In cucumber, RGR was considerably decreased, while in bread wheat plants it was slightly stimulated. Common bean was differently affected by redroot pigweed leachate. In this plant RGR was significantly increased ($p < 0.01$) by 5 % leachate treatment, whereas 10 % leachate led to considerable reduction. Considering the results of this parameter, bread wheat and common bean are the most resistance, cucumber is moderately sensitive and alfalfa is the most sensitive species to redroot pigweed leachate.

Table 3: The effect of different concentrations (5 and 10 %) of the redroot pigweed's leachate on fresh and dry weight of seedlings (mg) and relative growth rate (RGR, $1.day^{-1}$) of crop species in 7 day after treatment (n = 3, $p < 0.01$).

Plant Species	Treatments	Parameters		
		Fresh Weight	Dry Weight	RGR
Cucumber	Control	320.12±31.86 [a]	31.72±5.44 [a]	10.05±2.11 [a]
	5 %	239.27±36.76 [b]	24.94±3.08 [a]	7.89±0.98 [a]
	10 %	0.0±0.0 [c]	0.0±0.0 [b]	0.0±0.0 [b]
Alfalfa	Control	25.08±2.69 [a]	0.64±0.05 [a]	4.52±0.36 [a]
	5 %	0.0±0.0 [b]	0.0±0.0 [b]	0.0±0.0 [b]
	10 %	0.0±0.0 [b]	0.0±0.0 [b]	0.0±0.0 [b]
Common bean	Control	438±32.94 [a]	233.15±16.93 [ab]	13.22±0.96 [ab]
	5 %	425.44±6.11 [a]	251.16±25.97 [a]	14.27±1.47 [a]
	10 %	288.75±86.62 [a]	155.01±46.41 [b]	8.79±2.64 [b]
Bread wheat	Control	142.33±37.37 [a]	27.44±1.32 [a]	9.55±0.46 [a]
	5 %	74.82±10.60 [b]	26.70±5.77 [a]	9.29±2.01 [a]
	10 %	67.64±2.44 [b]	31.22±1.76 [a]	10.87±0.62 [a]

4 DISCUSSIONS

In the present study, different concentrations of redroot pigweed's leachate caused 6.90-100 % reduction in seed germination of studied species. Allelopathic inhibitory potential of redroot pigweed was frequently reported in the literatures (Shahrokhi et al., 2011; Tejeda-Sartorius et al., 2011; Amini et al., 2012; Dogaru et al., 2012; Mlakar et al., 2012; Namdari et al., 2012; Shahrokhi et al., 2012; Konstantinović et al., 2014). Control of redroot pigweed is not necessary in natural environments because its populations have disturbed distribution and displaced by other plants after several years over natural succession (Costea et al., 2004). Most control methods documented for this weed are specific to agricultural systems (Costea et al., 2004; Rosskopf et al., 2005; Zhang and Mu, 2008; Dogaru et al., 2012). The use of herbicides as a thin layer on the surface of soil is one of these methods (Sarabi et al., 2011; Sodaei zadeh and Hosseini, 2012). The development of resistant varieties from redroot pigweed by using herbicides showed that these methods were not effective in long time periods (Costea et al., 2004; Rosskopf et al., 2005; Sarabi et al., 2011). Also, biological control of redroot pigweed using insects was ineffective (Costea et al., 2004; Rosskopf et al., 2005); however, there are reports on different pigweed species control by fungi *Phomopsis amaranthicola* Rosskopf, Charud, Shabana & Benny. Sp. nov. in field conditions (Rosskopf et al., 2005). Nowadays, only cultivation of crop species which are resistant to redroot pigweed allelochemicals in fields aggressed with this plant appears to be promising. The results of this study clearly showed that seed germination and seedling growth of studied species were differently affected by redroot pigweed allelochemicals. According to recorded parameters, common bean was sensitive in seed germination but resistant in seedling growth stages. The exactly contrast responses were observed in cucumber. However, bread wheat plant was relatively resistance species in both germination and subsequent seedling growth phases. Although alfalfa slightly germinated in the presence of redroot pigweed allelochemical but its seedling growth and development was completely inhibited. Therefore, this species was the most sensitive species. The result of germination rate clearly showed that redroot pigweed can reduce crop plants seed germination, but the effective time is different in species. While bread wheat seeds germination started in first day, in other species germination showed a delay phase up to 3 days. Furthermore, seeds germination rate in treated bread wheat plants was lower in comparison to control, but the germination rate increased over time and finally reached to control. Weaker compensation ability was observed in other species. Therefore, the allopathic effect of redroot pigweed on the seeds germination of crops is higher in short time and compensation capability was dependent on plant species and leachate concentration. The effect of redroot pigweed leachate on seminal root length was higher than that in shoot lenght. Measurements of other growth parameters and studying of mechanisms involved in resistance of these organs can be useful in fine evaluation of this finding. Also conducting of this experiment using soil, perlite or hydroponic culture of plants could lead to reliable results for evaluation of allopathic effects of redroot pigweed on crops.

5 CONCLUSIONS

According to the results of presented study, bread wheat and common bean were the most resistant species, cucumbers was resistant species at low redroot pigweed leachate concentration but sensitive at high concentration, and alfalfa was the most sensitive species to the redroot pigweed leachate. Therefore, among the four studied crop species the cultivation of bread wheat and common bean plants in the regions with redroot pigweed's invasion is affordable, and avoidance of alfalfa cultivation in these regions is essential because this species is quite sensitive to compounds produce by redroot pigweed.

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