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Full Length Research Paper

Allelopathic effects of *Amaranthus retroflexus* and *Amaranthus cruentus* extracts on germination of garden cress

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The objective of the present study was to analyze and compare the allelopathic activities of weedy and grain amaranths. For this purpose, the seeds of garden cress (*Lepidium sativum* L.) were germinated on filter paper moistened with aqueous extracts of *Amaranthus retroflexus* L. and *Amaranthus cruentus* L. cv. 'G6'. The extracts were prepared from fresh roots, stems, leaves, and inflorescence with seeds (1: 2 w/v in water) and used, either undiluted (100%) or at varying concentrations (75, 50, and 25%). Although, all the extracts delayed germination, the leaf extracts of both species and the inflorescence extracts of grain amaranth, proved the more powerful. The root and stem extracts of grain amaranth reduced germination significantly only when used undiluted, whereas none of the extracts at 25% concentration, except that of the pigweed leaves, affected root elongation. However, in some cases, root elongation was even stimulated. Compared to the pigweed amaranth, the grain species exerted a stronger inhibitory effect on the germination process, and root elongation.

Key words: Allelopathy, germination rate, germination speed, grain amaranth, *Lepidium sativum*, pigweed, root elongation.

INTRODUCTION

Many species of weeds, as well as crop plants, are known to be allelopathic. Allelochemicals are secondary metabolites present as soluble compounds or in a volatile state in different plant organs, including leaves, flowers, fruits, and buds (Rice, 1984), which may substantially differ in allelopathic activity (Ciarka et al., 2009). Haig (2008) classified allelochemicals into several categories, such as glucosinolates, phenolic compounds, terpenoids, alkaloids, hydroxamic acids, and other compounds (flavonoids, quinones, polyacetylenes). Many of such natural compounds have been shown to be promising prospects for natural pesticides' development (Dayan et al., 2009; Ma et al., 2011).

According to studies on systematics, the genus *Amaranthus* includes 60 species (Sauer, 1967; Brenner et al., 2000), or 87 species (Jacobsen and Mujica, 2003), most of which are cosmopolitan weeds known to make cultivation difficult when the initial tilling brings the buried seeds to the surface and exposes them to light, whereas, cultivated amaranth species can be used, not only as a source of edible seeds, leafy vegetables, and forage, but also as ornamentals. According to Sauer's taxonomic key (Sauer, 1967), three species are important in seed production, namely; *Amaranthus cruentus* L., *Amaranthus hypochondriacus* L., and *Amaranthus caudatus* L. All these three originate from South America and belong to a group of cereal-like grain crops or pseudocereals.

Given its several advantages as a crop, its unique nutritional properties, and its use as food and feed (Ayo, 2001; Berghofer and Schoenlechner, 2002; Kabuage et al., 2002; Jacobsen et al., 2003; Bavec and Bavec, 2006; Muyonga et al., 2008; Grobelnik et al., 2009a, 2009b; Rosa et al. 2009), grain amaranth is receiving increasing attention as an alternative crop worldwide. Although, the

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Table 1. Analysis of variance (F-ratio) of surveyed germination indices (total germination, Gt; speed of germination, S; speed of accumulated germination, As), and traits of garden cress.

Source of variation	Degree of freedom	Gt(arcsin)	S(arcsin)	As(square root)	Root length	Seedling fresh weight
Species (S)	1	4.07*	46.08**	55.40**	69.77**	3.91*
Plant part (P)	3	226.30**	324.64**	456.47**	63.31**	3.36^{ns}
Concentration (C)	3	79.54**	170.56**	197.68**	202.47**	0.58^{ns}
S×P	3	76.64**	92.94**	134.83**	81.06**	1.76^{ns}
S×C	3	12.57**	15.61**	18.38**	7.68**	5.00**
P×C	9	22.09**	13.48**	30.29**	4.22**	1.42^{ns}
S×P×C	9	10.17**	5.44**	7.29**	7.14**	4.66**
Residual [a]	124	86.65	47.16	0.01	13.09	1.89

[a] Mean square of residual; **, * Significant at $P < 0.01$ and $P < 0.05$, respectively; ns = not significant.

allelopathic effects of pigweed (*Amaranthus retroflexus* L.) are well documented (Quasem, 1995; Costea et al., 2004; Rezaie and Yarnia, 2009), while those of cultivated (grain) amaranth are not, the available literature being scarce and inadequate (Machado, 2007; Mathiassen et al., 2008; Prinslo and Du Plooy, 2010). The present study is an attempt to contribute to filling this gap: as amaranth is becoming more widely grown as a component of crop rotation, and is being introduced into more countries, its susceptibility to allelochemicals and its own allelopathic activity need to be studied in more detail.

MATERIALS AND METHODS

The plants of pigweed (*A. retroflexus*) were collected at an organic farm near Maribor, Slovenia (46°30'N, 13°30'E; elevation 275 m), and those of grain amaranth (*A. cruentus* cv. 'G6'), also known as red amaranth, at an organic farm near Kranj Slovenia (46°14'N, 14°21'E; elevation 388 m). Both species were collected during September, 2010, when the plants were close to maturity. The sampled plants were separated into roots, stems, leaves, and the inflorescences with seeds and chopped into 2 cm length. 200 g lots were then soaked in 400 mL of distilled water for 60 h at ambient temperature (24 ± 2°C). The water, containing the leachates, was first filtered through cheesecloth in order to remove any fibres and other debris, and then through the graded filter paper (Oemolk elite, Vienna, Austria) used for filtering milk. The filtrate was used in the bioassay as the standard full-concentrated (100%) stock or further diluted to concentrations of 75, 50, or 25%, and stored in dark bottles for one month at 6°C, prior to use.

Seeds of garden cress (*L. sativum* L. cv. 'Gladkolistna'), with a guaranteed germination level of 90%, were bought at the market. Twenty seeds were evenly placed within each sterile Petri dish (10 cm in diameter) lined with filter paper (No. 1 Whatman International, Maidstone, UK). The filter paper was moistened with 5 mL of the extracts or with distilled water, which served as a control. The sealed Petri dishes were arranged in a completely randomized design with five replications and was incubated at 25 ± 1°C for 12 h of light alternating with 12 h of darkness. Since the preliminary germination test for the cress seedlings showed no infection, surface disinfection of the seeds was not carried out.

The treatments is comprised of three factors: amaranth species (2 levels: pigweed or grain amaranth), plant part (4 levels: roots, stems, leaves, or inflorescence with seeds), and concentration of the extracts (4 levels: undiluted (100%) or diluted to 75, 50, or 25% concentrations). Germination was recorded every 24 h for 3 days: a seed was considered to germinate when the radicle was 1 mm long. In order to assess the allelopathic effects on germination precisely, two other parameters of germination were considered in addition to the total germination (Gt), namely; the speed of germination (S) and the speed of accumulated germination (As), as recommended by Anjum and Bajwa (2005). Indexes S and As were calculated according to Equations 1 and 2, respectively:

$$S = (N_1/1) + (N_2–N_1) \times 1/2 + (N_3–N_2) \times 1/3 \quad (1)$$

$$As = N_1/1 + N_2/2 + N_3/3 \quad (2)$$

Where N is the proportion of germinated seeds obtained during the first (N_1), second (N_2), and third (N_3) day of experiment. The average fresh weight of a seedling was calculated by weighing, and the average root length was calculated by measuring the root lengths of all the seedlings in each Petri dish.

In order to improve the normality of distribution and homogeneity of variance (tested using Cochran's C and Bartlett tests), all the data describing germination were arcsin-transformed (Gt and S), or square-root-transformed (As), and subjected to analysis of variance (ANOVA), using Statgraphics Centurion XV (Statpoint Technologies Inc., Herndon, Virginia, USA) at the level of $P < 0.05$. A comparison of means was performed by Fisher's least significant difference (LSD) procedure ($\alpha = 0.05$), and by *t*-test when the extracts were compared to the control. The results are presented as the means ± standard errors (SEM) of five replications.

RESULTS AND DISCUSSION

Analysis of variance showed that all the three factors, individually and in combination, exercised significant effects on all the germination indices and the root length of the garden cress (Table 1). The garden cress seeds used in the germination tests were healthy with high scores for germination (95 to 100%), speed of germination (S, 0.85 to 1.0), speed of accumulated germination (As, 1.54 to 1.83), seedling weight (0.25 mg), and root length (14.0 to 18.7 mm). The aforementioned values apply for the control plants and represent an average of eight lots, four for each of the two tested species (Table 2 and Figure 1).

When comparing average values, the grain amaranth

Table 2. Effects of amaranth species, source of the extract (plant part), and concentration of the extract (%) on germination indices (total germination, Gt; speed of germination, S; speed of accumulated germination, As) of garden cress.

Plant part	Concentration (%)	*A. retroflexus*			*A. cruentus* cv. 'G6'		
		Gt	S	As	Gt	S	As
Root	100	92 ± 3.7a	0.85±0.03a*	1.55±0.06a*	87 ± 3.4a*	0.51±0.02d*	0.87±0.05d*
	75	95 ± 2.2a	0.77±0.05a*	1.37±0.01a*	93 ± 3.0a	0.63±0.05c*	1.08±0.10c*
	50	97 ± 1.2a	0.83±0.05a*	1.50±0.10a*	98 ± 1.2a	0.83±0.02b*	1.48±0.05b*
	25	95 ± 1.5a	0.92±0.03a*	1.67±0.06a*	96 ± 1.8a	0.96±0.02a*	1.75±0.04a*
Mean		94.75A	0.84A	1.52A	93.50A	0.73A	1.29A
Stem	100	95 ± 2.2a	0.80±0.02a*	1.43±0.04a*	89 ± 3.3b*	0.49±0.03c*	0.81±0.06c*
	75	94 ± 2.9a	0.83±0.04a*	1.49±0.07a*	100 ± 0.0a	0.86±0.02a*	1.53±0.05a*
	50	99 ± 1.0a	0.92±0.03a*	1.66±0.06a*	100 ± 0.0a	0.64±0.02b*	1.11±0.05b*
	25	96 ± 2.9a	0.87±0.05a*	1.58±0.11a*	96 ± 2.2a	0.94±0.02a*	1.71±0.04a*
Mean		96.00A	0.85A	1.54A	96.25A	0.73A	1.29A
Leaf	100	0 ± 0.0c*	0.00±0.00c*	0.00±0.00c*	14 ± 1.8b*	0.06±0.01c*	0.08±0.02c*
	75	0 ± 0.0c*	0.00±0.00c*	0.00±0.00c*	19 ± 6.4b*	0.09±0.03c*	0.12±0.04c*
	50	14 ± 6.6b*	0.07±0.03b*	0.10±0.04b*	82 ± 6.8a*	0.36±0.04b*	0.53±0.06b*
	25	89 ± 2.9a*	0.52±0.05a*	0.87±0.09a*	92 ± 4.1a	0.82±0.03a*	1.74±0.06a*
Mean		25.75C	0.15C	0.24C	51.75B	0.33B	0.55B
Inflorescence	100	91 ± 3.3a	0.53±0.05c*	0.88±0.113c*	6 ± 2.9c*	0.04±0.01c*	0.06±0.02c*
	75	92 ± 3.4a	0.74±0.09b*	1.31±0.17b*	6 ± 2.9c*	0.04±0.02c*	0.07±0.03c*
	50	91 ± 3.6a	0.75±0.03b*	1.33±0.06b*	68 ± 5.8b*	0.30±0.04b*	0.43±0.07b*
	25	95 ± 1.6a	0.94±0.02a*	1.72±0.04a*	95 ± 1.6a	0.73±0.07a*	1.29±0.14a*
Mean		92.25A	0.74A	1.31A	43.75B	0.28B	0.46B
Control	0	97.75	0.98	1.76	98.75	0.99	1.78

[a–d] Means (± SEM) followed by different letters within species and plant parts are significantly different. [A–C] Means (interaction S×P) followed by different letters are significantly different.* Values followed by an asterisk are significantly different from those in the control.

exerted a stronger inhibitory effect on the cress germination percentage (71%), speed of germination (0.52), speed of accumulated germination (0.90), and root length (9.7 mm), than pigweed amaranth (77%, 0.64, 1.15 and 14.5 mm, respectively). The leaf and inflorescence extracts of both species exerted the highest significant inhibitory effects on the germination percentage, the most severe effect being that of leaf extract from pigweed amaranth (Table 2). None of the extracts from pigweed amaranth, except leaf extract, affected the Gt as compared to that seen in the control, but those from grain amaranth showed a concentration-dependent effect on germination.

Thus, leaf extracts from pigweed amaranth in 100 and 75% concentrations suppressed germination totally, and those in 50 and 25% decreased it significantly, the corresponding Gt values being 14 and 89%. Similarly, leaf extracts from grain amaranth in concentrations above 25% reduced cress germination when compared to the control. Although, the varying concentrations of inflorescence extract from the pigweed caused no significant differences in Gt, inflorescence extracts from grain amaranth in concentrations above 25% reduced Gt drastically to 6 and 68%. The reduction was not significant at the lowest (25%) concentration of inflorescence extract for either species. As to the root and stem extracts of grain amaranth, they were effective only undiluted (100%) in reducing the germination percentage, when compared to the percentage seen in the control ($P < 0.05$). Since the grain amaranth used in the present study accumulated the highest proportion of biomass in inflorescences with seeds and in leaves; they comprised 33.4 and 30.2% of fresh plant weight, respectively (Grobelnik, 2006). *The results for allelopathy for those particular plant parts were parallel to those* reported by Ciarka et al. (2009). The authors ascribed the allelopathic potentials of different sunflower plant's parts to the allelopathic activities of particular organs, and also to the biomass partitioning pattern.

They stated that the greater the part of biomass portioned

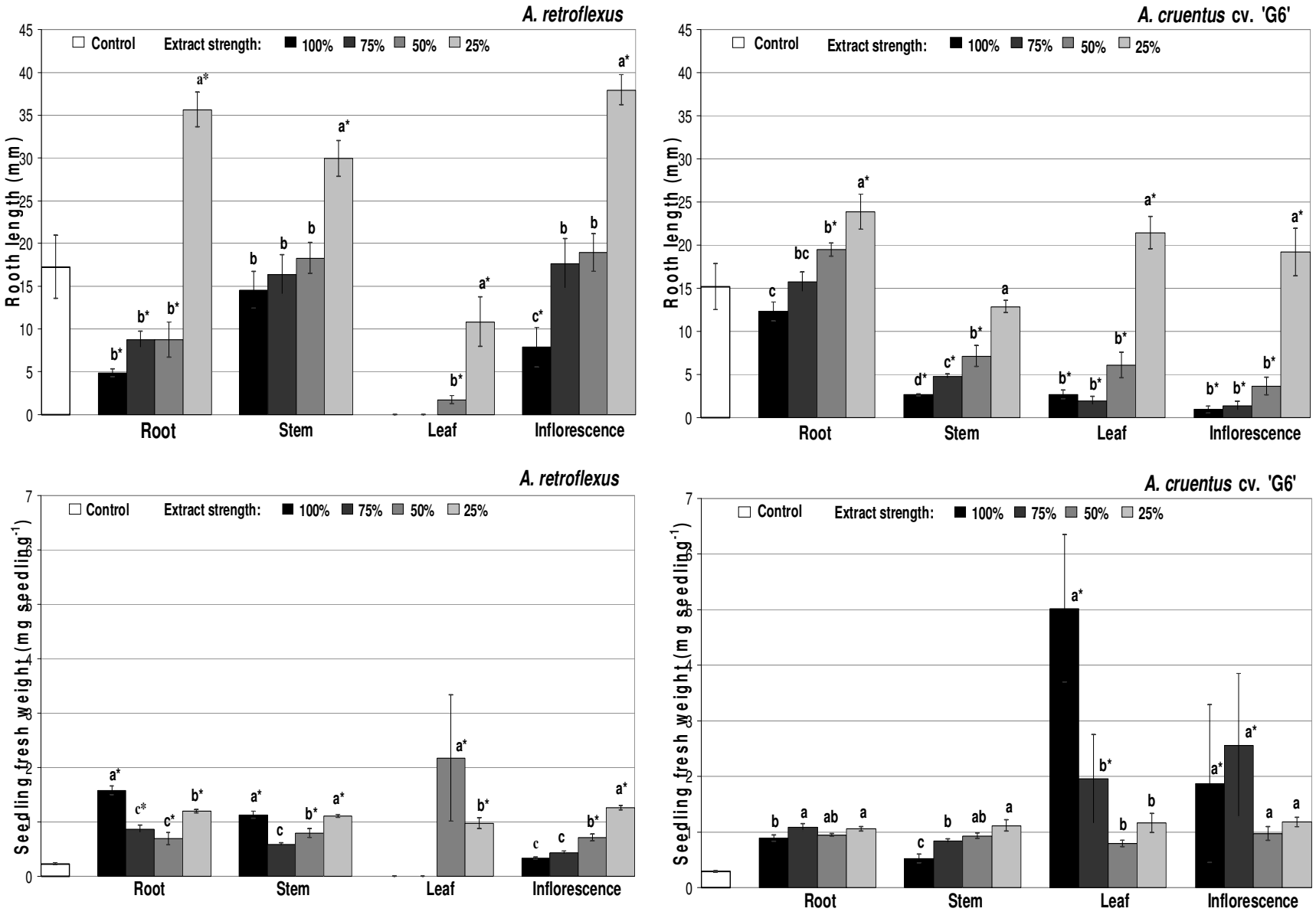

Figure 1. Effect of sources (plant parts) and concentration (%) of extracts prepared from *A. retroflexus* and *A. cruentus* cv. 'G6' on the root length and fresh weight of seedlings of garden cress. [a–d] Means followed by different letters within species and plant parts are significantly different. Error bars indicate standard error of means. * Values followed by an asterisk are significantly different from those in the control.

to plant organs of higher allelopathic potential, the greater the allelopathic activities of the biomass. Literature on biomass accumulation and its distribution in pigweed amaranth was not found, but according to known morphological features and differences between species, it could be assumed that, in comparison to grain amaranth, less biomass is distributed to inflorescence and seeds, and greater portioned to the leaves. Therefore, the presumed differences in biomass distribution might be the reason for allelopathic differences regarding the leaf and inflorescence extracts of the tested amaranth species. The extracts not only reduced the germination percentage but also delayed the germination process drastically. As also reported by Anjum and Bajwa (2005), both indexes (S and As) were found to be more sensitive to the allelopathic activity of the extracts, than Gt: all the treatments resulted in significantly lower values for germination speed when compared to the values recorded in the control, namely; S of 0.99 and As of 1.77 (Table 2). The extracts also affected root growth (Figure 1): the roots were significantly longer as much as 35.7, 29.9, and 37.9 mm in seedlings treated respectively with root, stem, and inflorescence extracts from pigweed at the lowest (25%) concentration; the average root length in the control was only 17.3 mm. The leaf extracts from pigweed were detrimental: the treated roots were significantly shorter, being 6.4 and 15.5 mm when exposed to leaf extracts of 25 and 50% concentrations, respectively. In the grain amaranth, the stimulatory effect on root length was confined to the root, leaf, and inflorescence extracts of 25% concentration (23.9, 21.4, and 19.2 mm, respectively) as compared to those in the control, in which the average length was only 15.2 mm. The stem extract from grain amaranth at a concentration 25% failed to stimulate root elongation and, at higher concentrations, resulted in significantly shorter roots. The stimulatory effect of leaf extract from grain amaranth was only confined to the lowest concentration; whilst higher concentrations led to markedly shorter roots, as did those of the inflorescence extracts of grain amaranth.

Lastly, the extracts also affected seedling vigour significantly, as expressed in terms of the fresh weight of seedlings, but the effect was only confined to the species and interactions that involved the species (Table 1). The seedlings of the cress treated with grain amaranth extracts were on average, heavier by 0.57 mg, than those treated with pigweed amaranth extracts. The extremely high mass of these seedlings (2.1 to 9.1 mg seedling^{-1}) could be ascribed to the fact that amaranth is a rich source of nutrients, which was also reflected in the extracts. However, it should be pointed out that the heaviest seedlings appeared when the cress was treated with undiluted (100%) leaf extract from grain amaranth that is, in the treatment where only 14% of seeds could germinate. The seeds that germinated were perhaps resistant or tolerant to the allelochemicals available in the extract. The extracts of pigweed amaranth also led to seedlings being heavier, on average, when compared to those in the control (Figure 1).

Pigweed has been shown to be sensitive to plant extracts, previous plants in a rotation, and to mulches and residues of various crops (Costea et al., 2004; Yarnia et al., 2009). Likewise, the negative impact of pigweed extracts and its residues on the germination and growth of a wide-variety of field crops and vegetables has also been documented (Quasem, 1995; Costea et al., 2004; Rezaie and Yarnia, 2009). Quasem (1995) investigated the allelopathic effects of extracts from both fresh and dry tissues at different concentrations, and of the residues of three weedy amaranth species, on durum wheat (*Triticum durum*). The authors found that the allelopathic effects, or phytotoxicity of the three species of amaranths evident in the reduced germination and growth of wheat seedlings was concentration-dependent, and that the effects of *A. retroflexus* were consistent, irrespective of whether observed in the laboratory, glasshouse, or under field conditions. The same experiment also reported that shoot extracts were more detrimental than root extracts (Quasem, 1995). In contrast, Rezaie and Yarnia (2009) found both root and shoot extracts to be equally harmful to safflower (*Carthamus tinctorius* L.): safflower seeds failed to germinate when treated with extracts of dried *A. retroflexus*, either as roots or as shoots. However, in terms of its effect on plant height under irrigation, root extract was less effective than shoot extract.

Literature on the allelopathic effects of cultivated amaranth is scarce and inadequate. Mathiassen et al. (2008) showed that cultivated amaranths possess allopathic properties: germinating seeds reduced root and shoot lengths by up to 50% in green field speedwell (*Veronica agrestis* L.), and annual meadow grass (*Poa anua* L.); however, no allelopathic effect was observed on germination percentage and the early growth of perennial ryegrass (*Lolium perenne* L.). Prinslo and Du Plooy (2010) mentioned that vegetable amaranths inhibited the germination of vegetables and weed seeds. However, results obtained in the present study accord well with those of Machado (2007), who screened the potential allelopathic effects of various plant species on the germination of downy brome (*Bromus tectorium* L.), and found that a 5% aqueous extract of dried shoots of grain amaranth (*A. cruentus*) injurious to downy brome: germination had been reduced to 5% (it was 89% in the control); root length to 0.2 mm (36.9 mm in the control); and shoot length to 0.1 mm (12.5 mm in the control). Root extract was less phytotoxic with the corresponding values being 63(92%), 4.3(33.6 mm), and 2.8(13.6 mm), respectively.

Conclusion

This particular study was suggested by field observations carried out on yield and seed quality in amaranth sole

cropping, and a maize–amaranth intercropping system conducted over many years, and presents the results of our first study of grain amaranth allelopathy. It was found that aqueous extracts of tested grain amaranth exert allelopathic activity, but their effects on germination and early growth of garden cress were concentration-dependent, and depended strongly on the plant parts' assayed. Since those results derived from laboratory experiments, which often differ from those obtained during field experiments (Quasem, 2010), further research to test the phytotoxicity of grain amaranth extracts using soil as a growth medium in controlled environments, and crop residues under field conditions is under way.

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