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Articles

Biological features of Amaranthus blitum L. and A. retroflexus L. invading potato plantings

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The paper addressed the study of some biological features of two weed species of *Amaranthus* L. – a widespread species *A. retroflexus* L. confined to Siberia and a rare species *Amaranthus blitum* L. Plants of different vital status were found in populations of two species invading potato plantations. The paper reports the morphometric parameters of normally developed and small plants of both species. The features of seed germination and the degree of seedling development were revealed. Laboratory experiments were performed to find out the effect of aqueous extracts of the herb *Melilotus officinalis* (L.) Pallas on germination and development of seedlings of two *Amaranthus* species. The root elongation bioassay of *Amaranthus blitum* seedlings showed a higher phytotoxic effect of the *Melilotus officinalis* extract compared to *Helianthus annuus* L. and *Helianthus tuberosus* L. extracts.

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Keywords

Amaranthus blitum L., *A. retroflexus* L., weeds, allelopathy, bioassay

Introduction

The status of all species of the genus *Amaranthus* L. is listed as adventitious (alien) to the Russian Federation and Siberia; some species are included in the list of invasive and potentially invasive plants of the regional floras of Russia (Vinogradova et al. 2010; Black book… 2016). At present, 5 species of *Amaranthus* L. have been recorded in synanthropic (segetal and ruderal) habitats of Western Siberia: *Amaranthus albus* L. (white amaranth), *A. blitoides* S. Watson (prostrate amaranth), *A. blitum* L. (purple amaranth) or *A. lividus* L. (livid amaranth), *A. cruentus* L. (red amaranth), *A. powelii* S. Watson (Powell amaranth), *A. retroflexus* L. (green amaranth) (Ebel 2012).

Amaranthus retroflexus is one of the economically significant weeds distributed in agrocenoses of agricultural crops in Western Siberia (Ebel 2012; Terekhina et al. 2021), *A. blitum* is known only for single localities in the Tobolsk, Kurgan, Kemerovo, Tomsk and Novosibirsk regions, and Altai Krai. This species is most often confined to private gardens (Ebel, 2012; Ebel et al. 2015; Zykova 2019).

Numerous factors, primarily competition for water, light, and nutrients, make amaranth harmful to crops (Costea and Tardif, 2003). Moreover, the allelopathic effect of plant residues and root exudates cannot be ignored (Souza et al. 2011).

Amaranthus species are noxious weeds in field crops in many countries; therefore, there is an urgent need for more effective weed management (Vasilakoglou et al. 2013; Gerasimova and Mitova 2020; Kalkhoran et al. 2021). One of the causes for the insufficient effectiveness of chemical protection agents is the resistance developed in weeds (Heap 2022). Resistance developed to various herbicides, including acetolactate synthase inhibitors (atrazine and paraquat),has been reported for *A. blitum* biotypes in North America, Europe, and Asia (Itoh et al. 1992; Manley et al. 1996; Heap 2022).

Numerous experiments of Russian and foreign scientists indicate a high potential of plant extracts of various plant species that have a phytotoxic effect on weed amaranth. Most of the studies analyzed the most common species *A. retroflexus*. The spectrum of plants showing high allelopathic activity against *A. retroflexus* in laboratory experiments is very diverse. These include widely known medicinal species (Kondratiev et al. 2020; Azizi and Fuji 2006), agricultural crops: buckwheat (Gfeller et al. 2018), sunflower (Ghorbani et al. 2008), topinambour (Tesio et al. 2010), and many others. Few studies focus on the effect of plant extracts on *A. blitum* in *in vitro* experiments (Ghimire et al. 2019).

The aim of this study was to conduct a comparative analysis of some biological features of two weed species, *A. retroflexus* and *A. blitum*, which invade potato plantations in the Tomsk region.

Materials and methods

The study of the ecological and biological features of *A. blitum* and *A. retroflexus* was conducted in the Tomsk region: Tomsk, Timiryazevo settlement, private garden plot, potato plantations (coordinates 56°29'23"N and 84°52'40"E).

To perform morphometric measurements and study seed germination characteristics, plants of both species were collected on September 15, 2021. Morphometric parameters were measured in generative plants in the fruiting phase. A weight method was employed to determine fresh weight.

The viability of freshly harvested amaranth seeds and seeds stored for 9 months was estimated by germination in laboratory conditions. For germination, 9-cm Petri dishes were used. A total of 25 seeds were placed on a bed of filter paper saturated in distilled water. Fourfold repetition. Germination energy was recorded on day 3, germination occurred on day 5. Morphometric parameters (length of root and hypocotyl) were measured in five-day-old amaranth seedlings.

The allelopathic activity of plant extracts was assessed by bioassay. The conventional method for determining the allelopathic activity of plants by bioassay of seedlings was employed (Grodzinsky 1991). Aqueous extracts of plants were prepared by hot extraction (Bukharov et al. 2015).

Bioassay of allelopathic activity of *Melilotus officinalis* for seed germination of two amaranth species

A comparative analysis of allelopathic sensitivity of two amaranth species was performed by bioassay. For bioassay, we used an aqueous extract (1% and 0.25%) of *Melilotus officinalis* (L.) Pallas, early ripening Omsk variety. The experiment employed seeds of *A. blitum* and *A. retroflexus*, which came out of dormancy after 9 months of storage. In Petri dishes, 25 seeds were placed on a bed of filter paper saturated in an aqueous extract of sweet clover or distilled water (in the control). Fourfold repetition. Germination was performed at 25 °C. Plant emergence was estimated on day 5. The length of the root and hypocotyl was measured in five-day-old seedlings.

Bioassay of bioherbicidal activity of plant extracts on *A. blitum* seedlings

The study employed the root elongation bioassay (Araniti et al. 2015). A total of 10 pre-germinated seeds of *A. blitum* with a root length of 3–5 mm were placed in Petri dishes saturated in aqueous extracts of various plants. The raw material used for extract preparation was as follows: grass of sweet clover *Melilotus officinalis* (L.) Pallas, early ripening Omsk variety; leaves of sunflower *Helianthus annuus* L., Yenisei variety; leaves of topinambour *Helianthus tuberosus* L., Nakhodka variety. Raw materials were collected in the flowering phase and air-shadow dried. The initial 2% aqueous extract was prepared by hot extraction. An extract of a weaker concentration (0.5%, 1%) was obtained by successive dilution. In the control, seedlings were germinated in distilled water. After 48 hours of germination at 25 °C, the length of the seedling roots was measured.

The toxicity index of plant extracts was calculated by the formula (1):

TI, %=(Lc – Lex) / Lc×100, (1)

where TI – the toxicity index, %; L_c – the length of the root or hypocotyl in the control, mm; L_{ex} – the length of the root or hypocotyl in the experiment, mm.

Statistical processing of the results was peformed using Microsoft Office Excel 2013 and Statistica 8.0 software. The tables present arithmetic mean values as “mean ± error of mean”.

Results

In one of the plots in the vicinity of Tomsk (Timiryazevo settlement, summer cottage), the *A. blitum* population has been monitored since 2016. The seeds of this species were apparently brought to the plot with humus for planting potatoes in 2012 from Zorkaltsevo settlement, Tomsk region. Since that time, *A. blitum* plants have been abundantly self-seeded annually in this area.

Despite the agrotechnical measures taken to regulate the population growth, new shoots could be observed regularly. During the observation period (mid-September), we recorded the presence of fruit-bearing individuals of different sizes (large, medium, small) (Figs 1–2).

Analysis of the data presented in Tables 1, 2 shows significant differences in size and weight indicators for large and small individuals of *A. blitum* and *A. retroflexus*. The largest individuals of *A. blitum* exhibited the shoot height of 66 cm, the root length of 35 cm, the number of lateral branches of 13, and the shoot fresh weight of 121 g.

Figure 1. *Large individuals A. blitum.*

Figure 2. *Medium and small individuals A. blitum.*

Parameters	Large individuals	Small individuals
Shoot height, cm	46.2 ± 1.8	7.9 ± 0.6
Number of shoot metamers, pcs.	15.4 ± 0.5	8.5 ± 0.5
Leaf length with petiole, cm	12.7 ± 0.5	3.7 ± 0.2
Leaf blade length, cm	7.1 ± 0.3	2.0 ± 0.1
Leaf blade width, cm	4.6 ± 0.1	1.3 ± 0.01
Root length, cm	23.3 ± 1.1	14.7 ± 1.0
Plant fresh biomass, g	48.9 ± 2.6	1.4 ± 0.1
Shoot fresh weight, g	42.8 ± 2.9	1.1 ± 0.1
Root fresh weight, g	6.1 ± 0.4	0.3 ± 0.002

Table 1. *Morphometric parameters of Amaranthus blitum in potato plantations*

Parameters	Large individuals	Small individuals
Shoot height, cm	108.5 ± 2.6	10.8 ± 0.7
Number of shoot metamers, pcs.	17.8 ± 0.4	9.2 ± 0.3
Leaf length with petiole, cm	16.9 ± 0.8	9.1 ± 0.3
Leaf blade length, cm	9.4 ± 0.9	3.9 ± 0.3
Leaf blade width, cm	6.4 ± 0.4	1.9 ± 0.08
Root length, cm	29.0 ± 1.9	19.4 ± 1.2
Plant fresh biomass, g	178.0 ± 10.9	1.9 ± 0.1
Shoot fresh weight, g	164.9 ± 11.6	1.5 ± 0.05
Root fresh weight, g	13.1 ± 0.7	0.4 ± 0.001

Table 2. *Morphometric parameters of Amaranthus retroflexus in potato plantations*

Despite a twofold reduction in the number of metamers, small representatives of *A. blitum* completed its development cycle and produced full seeds. The studied population exhibited regular fruiting of *A. blitum* and *A. retroflexus* and full seed formation. It should be noted that no data on the features of seed germination of *A. blitum* are available in domestic literature.

According to our data, freshly harvested seeds of both species are in a state of physiological dormancy (B_1), and after several months of dry storage, they came out of dormancy. The seeds of *A. retroflexus* are characterized by a deeper dormancy compared to the seeds of *A. blitum*, which germinate more evenly after dormancy. The dynamics of seed emergence and germination of this species is similar to that of cultivated *Amaranthus* species grown in the Siberian Botanical Garden (Tomsk), in particular, *A. paniculatus* variety Cherginsky.

A comparative study of the germination and morphology features of seedlings of weed species *A. blitum* and *A. retroflexus* is of relevance. Table 3 summarizes data on the seedling germination and morphology obtained in laboratory conditions for both species.

Parameter	Statistical characteristics	Amaranthus blitum	Amaranthus retroflexus
Germination energy	X ± m, %	98.0 ± 1.0	62.7 ± 5.4
	min–max, %	96–100	56–76
	CV, %	2.0	15.0
Germination	X ± m, %	98.0 ± 1.0	73.3 ± 3.9
	min–max, %	96–100	64–80
	CV, %	2.0	9.3
Root length	X ± m, mm	25.2 ± 0.4	42.5 ± 1.1
	min–max, mm	16–33	25–58
	CV, %	13.8	19.4
Hypocotyl length	X ± m, mm	13.3 ± 0.2	24.5 ± 0.6
	min–max, mm	7–20	16–35
	CV, %	18.1	19.2

Table 3. *Seed germination and seedling morphology of two amaranth species after 9 months of storage*

The study of the *A. blitum* population showed its high stability. Annual fruiting and high seed viability contribute to the regular renewal and formation of a dense population. Apparently, a high soil seed bank of this species ensures the population growth, despite a regular control of its spread in potato plantations. This is evidenced by the differentiated size of *A. blitum* generative individuals and the simultaneous presence of individuals of different sizes within the population.

Aqueous extracts of sweet clover affected the laboratory germination of seeds of both species. Thus, 0.25% extract decreased seed germination of *A. blitum* from 98% to 95%, whereas 1% extract decreased its germination to 66%.

The seeds of *A.retroflexus* exhibited high sensitivity. At a low concentration of sweet clover extract, its seed germination decreased to 53%, while 1% extract did not induce germination (Tables 4, 5).

Experiment	Number of measurements	Root length, mm		
		X mx	lim (min–max)	Toxicity index, %
Amaranthus blitum				
Control	98	25.2 ± 0.4	16–33	0
0.25 %	95	4.5 ± 0.2	2–8	82.1
1.0 %	66	2.1± 0.01	1–3	91.7

Amaranthus retroflexus				
Control	73	42.5 ± 1.1	25–58	0
0.25 %	53	3.7 ± 0.2	2–5	91.3
1.0 %	0	0	0	100.0

Table 4. *Effect of aqueous extracts of sweet clover on the root length of amaranth seedlings*

Experiment	Number of measurements	Hypocotyl length, mm		
		Xmx	lim (min–max)	Toxicity index, %
Amaranthus blitum				
Control	98	13.3 ± 0.2	7–20	0
0.25 %	95	12.0 ± 0.3	7–16	9.8
1.0 %	50	1.4± 0.02	1–2	89.5
Amaranthus retroflexus				
Control	73	24.5 ± 0.6	16–35	0
0.25 %	53	10.1 ± 0.6	7–13	58.8
1.0 %	0	0	0	100.0

Table 5. *Effect of aqueous extracts of sweet clover on the hypocotyl length of amaranth seedlings*

For pre-germinated seeds of *A. blitum*, the inhibitory effect of aqueous extracts of sweet clover and sunflower increased as their concentration increased (Table 6). The root length of *A. blitum* decreased by 70%, 81% and 82% under the effect of 0.5%, 1% and 2% aqueous extracts of sweet clover, respectively. Aqueous extracts of sunflower insignificantly decreased the root length of *A. blitum*. Aqueous extracts of topinambour at a concentration of 0.5 and 1% stimulated root growth, while 2% extracts had a moderate inhibitory effect.

Experiment	Root length, mm	
	X mx	Toxicity index, %
Control	20.1 ± 1.0	0
Sweet clover		
2 %	3.6 ± 0.3	82.1
1 %	3.9 ± 0.2	80.6
0.5 %	6.0 ± 0.4	70.1
Sunflower		
2 %	5.4 ± 0.3	73.1
1 %	9.8 ± 0.5	51.2
0.5 %	10.3 ± 0.6	48.8
Topinambour		
2 %	16.1 ± 0.6	19.9
1 %	26.8 ± 1.3	-33.3
0.5 %	29.6 ± 0.8	-47.3

Table 6. *Effect of aqueous extracts on the root length of A. blitum seedlings*

Our data on the phytotoxic effect of plant extracts on amaranth seedlings are consistent with the data obtained earlier for *Amaranthus paniculatus* L. seedlings exposed to synthetic herbicides. The roots of amaranth seedlings are most sensitive to the content of herbicides in solution (Spivak et al. 2003).

Conclusion

The presence of generative individuals of different size groups in the populations of two weed species *Amaranthus retroflexus* and *Amaranthus blitum* invading potato plantations indicates a regular emergence of weed seedlings during the growing season. Small individuals capable of forming full seeds complicate weed management, since they can hardly be seen. The agrotechnical measures (weeding) imply the removal of large plants, while periodically appearing seedlings and smaller individuals cause harm forming full seeds and thereby providing a permanent soil seed bank in the plot.

The obtained results indicate a high allelopathic activity of aqueous extracts of *Melilotus officinalis* against germination of both amaranth species. Our data on the alleopathic activity of aqueous extracts of different plants confirmed a high bioherbicidal activity of sweet clover (Автор, 2022). It was found that *A. retroflexus* seeds exhibit a higher allelopathic sensitivity compared to that of *A. blitum*. With regard to a more even germination of *A.blitum* seeds after dormancy and a uniform development of seedlings, the seeds of this species can be used as test plants to assess the toxic effect of plant extracts and chemical toxicants.

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