

A Case Study of Allelopathic Effect on Weeds in Wheat

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Abstract. Most powerful and effective method of weed control is by chemical substances called herbicides. In recent years, they were published quite data on different side effects of herbicides on humans, animals, crops and the environment as a whole. Therefore, the increased interest for biological weed control lately is reasonable, since its improvement and expansion will contribute to limiting excessive use of herbicides, respectively their harmful effects and will support the successful implementation of complex weed control. The purpose of this study was to investigate the effect of selected plant species, containing allelopathic active substances, on germination, growth and biomass of some widespread weeds in wheat. Experiments were carried out at laboratory conditions using seeds of wheat (*Triticum aestivum* L., sort Sadovo 1) and most common weeds therein: Johnson grass (*Sorghum halepense* (L) Pers), white pigweed (*Chenopodium album* L.), twitch (*Cynodon dactylon* L.) and curly dock (*Rumex crispus* L.). Allelopathic substances were extracted with distilled water from flowers of lavender (*Lavandula angustifolia* Mill.), leaves of basil (*Ocimum basilicum* L.), leaves of spearmint (*Mentha longifolia* (L) Huds.), and leaves of peppermint (*Mentha piperita* L.). Of the tested active allelopathic plants, the most negative impact on germination of all weeds seeds (including wheat), as well as on the development of plants exhibited the water extract of lavender. Lavender and basil had a stronger negative effect on white pigweed and twitch compared with both mint species. A significant inhibitory effect of spearmint even at low concentrations was recorded on the germination of all weed species tested while the wheat was slightly affected, which manifests this plant as a potential effective species in strategies for weed control management.

Keywords: allelopathy, weed control, wheat, laboratory experiment.

Introduction

Weeds are one of the most serious problems in agricultural production. They are volunteer plants from the wild or semi culture species that are found in food crops despite the will of the people and harm reducing yields. Today, some 30 000 species of weeds, i.e. repeated more than crops and in quantity, size and distribution are second group after natural vegetation. According to the FAO, from the total losses worldwide

caused by the crop pests, the weeds account for 35% of losses in wheat, 28% in vegetables, 29% in fruit species and vineyards, 37% in tobacco, etc.

In modern "organic farming" the problem of weed control is increasing and refusal of chemical resources of protection from them is usually accompanied by a sharp decrease in yields. Solution to this problem could be found in the development of integrated systems for weed control,

including the advantages of chemical, biological, mechanical and preventive methods to combat in minimizing their negative sides. Integrated weed control in most respects the principle of greening and environmental protection simultaneously with increased weed control and saving energy (BARNES & PUTNAM, 1986; STOIMENOVA *et al.*, 2008).

In the last decades there were a huge number of publications concerning allelopathy (RICE, 1974, 1995; REIGOSA *et al.*, 1999; KADIOGLU *et al.*, 2005; DIMITROVA & SERAFIMOV, 2007), and recently it was included in the sustainable agriculture, which is defined as organic, alternative, restorative, biodynamic, low costing and preserving resources (DIMITROVA, 2008). Despite the attention paid to allelopathy by ecologists, biologists and herbologists, complicated relationship "competition - allelopathy" in the system "weed - crop plant" is not fully understood. Interaction between weeds and crops is simultaneously and/or sequentially, with direct or indirect effect of one plant species to another, through the synthesis of various chemical compounds - allelochemicals, that are released into the environment and affect (inhibit and/or stimulate) the germination of seeds and the development of a number of weeds and crop (REIGOSA *et al.*, 1999; KOSTADINOVA *et al.*, 2002; KADIOGLU *et al.*, 2005). Results are contradictory, confirming in varying degrees the inhibitory or stimulatory effect of various weeds on germination of seeds (KADIOGLU *et al.*, 2005; SERAFIMOV *et al.*, 2005; ALEKSIEVA & SERAFIMOV, 2008). Difficulties in this regard, from one hand are due to the lack of specific unifying experimental methods to model the laboratory and field experiments, and from the other - to make a distinction between allelopathy and competition in plant communities (STEVEN *et al.*, 1984; STOIMENOVA *et al.*, 2004a,b; SERAFIMOV *et al.*, 2008).

Due to the selective nature of allelopathy, it should not expect that it alone could destroy all weeds in a typical agricultural environment, so it could function as an element of an integrated

strategy for weed control. Integrated control is recognized as a preferred strategy in the program of the United Nations Conference on Environment and Development. Its advantages are its complexity, in full destruction of weeds and in the lower risk of environmental pollution. This requires more detailed laboratory studies on allelochemical interactions aimed at demand and supply opportunities for practical application of allelopathy in weed control in order to reduce the use of chemicals.

Most commonly used method of proving allelopathic interference in plant communities or in the "weed - crop plant" is establishing stimulating or inhibitory effect of extracted plant material on the test plants or study the effect of plant residues and their application in quartz sand and/or soil made in the laboratory (STREIBIG *et al.*, 1993; KALINOVA *et al.*, 2012; KAWORU *et al.*, 2001; PENEVA, 2006). So, in this study we aimed to investigate ex-situ the effect of selected plant species, containing allelopathic active substances, on germination, growth and biomass of some widespread weeds in wheat (*Triticum aestivum* L.).

Material and Methods

Allelopathic plant species. Based on a literature review performed as active allelopathic plant species were chosen:

- Lavender (*Lavandula angustifolia* Mill.) - Flowers

- Basil (*Ocimum basilicum* L.) - Leaves

- Spearmint (*Mentha longifolia* (L) Huds.) - Leaves

- Peppermint (*Mentha piperita* L.) - Leaves

Dried plant material of lavender, basil, spearmint and peppermint was purchased from a certified drug manufacturer.

Aqueous extracts of allelopathic plants were prepared using the following scheme: 50 g of dry plant material were crushed in a mortar with quartz sand and quantitatively transferred into a flask with 1000 ml of distilled water. After standing in the dark for 24 hours, the solution was filtered and a 5% aqueous extract was prepared (TENEVA *et al.*, 2007). By dilution with distilled water thereof were prepared test solutions with concentrations 1.25%; 2.5% and 3.75% aqueous extract.

Test species. For the purpose of this study we used seeds of wheat (*Triticum aestivum* L. sort Sadovo 1) and most common weeds therein:

- Johnson grass (*Sorghum halepense* (L) Pers)
- White pigweed (*Chenopodium album* L.)
- Twitch (*Cynodon dactylon* L.)
- Curly dock (*Rumex crispus* L.)

Seeds of wheat were purchased from a certified manufacturer and these of weeds were collected on the field in the autumn of 2013, in the period after their maturation. Viability of the collected weed seeds was tested before starting the allelopathic experiment, and the percentage of germination for the four species was above 85%.

Experimental setup. For each experimental variant, 50 seeds of each weed (Johnson grass, white goose-foot, twitch, and curly dock) and wheat, respectively, were put into containers with quartz sand and 100 ml of different test solutions. Simultaneously with each essay were set control samples with 50 seeds of the same plant species and 100 ml of distilled water (VELCHEVA *et al.*, 2013).

Laboratory experiments were conducted in June-July 2014; the exposure time was 21 days at 24-28°C and 12/12 hours light period. Periodically was adding a quantity of the solution in containers with cultures in order to maintain a constant level. All test variants were carried out in three replications.

Effect assessment. For assessing the results of the experiments were used the following parameters: 1. Quantitative parameters: number of germinated seeds in each test variant; percent of germination in each test variant (%); percent of germination comparing to the control (%); 2. Biometric parameters: length of the young plants, cm; biomass of the young plants, g. Length was measured using graph paper and the weight was recorded on an analytical balance.

Statistical evaluation of the results obtained was performed by descriptive statistical analysis and t-test ($p < 0.05$) using software Statistica 7.0 (STATSOFT INC., 2004).

Results and Discussion

From the four studies allelopathic active plants, the strongest negative effect on

weeds and wheat exhibited the aqueous extracts of lavender flowers (Table 1). Even at the lowest test concentration (1.25%) we recorded strong inhibition of germination of seeds of white pigweed and Johnson grass, and at the next concentration (2.5%) it was completely suppressed. About 35% of the seeds of twitch and curly dock germinated at the concentration of 2.5%, but with worsened biometric parameters. At the concentrations 3.75% and 5% it has been observed no germination in all plant species. Significant reducing the length of the plant with increasing concentration of the aqueous extract of lavender was found in wheat and curly dock ($p < 0.05$).

Aqueous extract of basil leaves had a strong depressing effect on germination of seeds of white pigweed, reaching up to 92% at the lowest test concentration (Table 2). Twitch also proved as highly sensitive - inhibition of germination was 77%. Germination of wheat was affected slightly from the lowest test concentration of aqueous extract of basil, but at the next one the germination of wheat was reduced by half. Relatively resistant to the effects of basil were the seeds of curly dock, which remained viable in the presence of 3 times larger quantities of extracts of basil in the grow medium. Statistical processing of the data showed that with increasing concentration of the basil extract the germination, growing up and biomass production in the Johnson grass, curly dock and wheat significantly decreased ($p < 0.05$).

Impact of the aqueous extract of spearmint leaves on the test seeds was generally negative (Table 3), more pronounced in the seeds of white pigweed and Johnson grass. Almost complete inhibition of germination was recorded there even in low concentrations of aqueous extract, while for the twitch this effect was found when increasing the dose. Germination of seeds of curly dock and development of young plants proved to be less influenced at concentration 1.25%. They showed high values in both quantitative and biometric parameters and at a concentration of 2.5%, where the germination was above 50%.

Table 1. Effect of aqueous extract of the lavender flowers on quantitative and biometric parameters

Plant species	Concentration of aqueous extract, %	Germination of test seeds, %	Germination compared to the control, %	Length, cm	Weight, g
Wheat (<i>Triticum aestivum</i> L.)	0%	86%	-	19.7 cm	118.2 g
	1.25%	53%*	62%	7.9* cm	45.1* g
	2.5%	12%*	14%	2.3* cm	11.6* g
	3.75%	0%*	-	-	-
	5%	0%	-	-	-
Johnson grass (<i>Sorghum halepense</i> (L) Pers)	0%	88%	-	4.1 cm	35.7 g
	1.25%	22%	25%	1.9 cm	12.2 g
	2.5%	0%	-	-	-
	3.75%	0%	-	-	-
	5%	0%	-	-	-
Twitch (<i>Cynodon dactylon</i> L.)	0%	93%	-	3.6 cm	29.4 g
	1.25%	71%	76%	3.1 cm	22.2 g
	2.5%	34%	37%	2.4 cm	11.8 g
	3.75%	0%	-	-	-
	5%	0%	-	-	-
White pigweed (<i>Chenopodium album</i> L.)	0%	85%	-	3.5 cm	26.0 g
	1.25%	11%	13%	1.1 cm	8.3 g
	2.5%	0%	-	-	-
	3.75%	0%	-	-	-
	5%	0%	-	-	-
Curly dock (<i>Rumex crispus</i> L.)	0%	98%	-	6.8* cm	66.5 g
	1.25%	60%*	61%	4.4* cm	33.8* g
	2.5%	32%*	33%	2.7* cm	14.9* g
	3.75%	1%*	1%	1.4* cm	2.2* g
	5%	0%*	-	-	-

* Significant difference with the control at p<0.05

Table 2. Effect of aqueous extract of the basil leaves on quantitative and biometric parameters

Plant species	Concentration of aqueous extract, %	Germination of test seeds, %	Germination compared to the control, %	Length, cm	Weight, g
Wheat (<i>Triticum aestivum</i> L.)	0%	86%	-	19.7 cm	118.2 g
	1.25%	75%*	87%	11.3* cm	99.1* g
	2.5%	43%*	50%	5.5* cm	43.3* g
	3.75%	2%*	3%	1.4* cm	6.8* g
	5%	0%*	-	-	-
Johnson grass (<i>Sorghum halepense</i> (L) Pers)	0%	88%	-	4.1 cm	35.7 g
	1.25%	61%*	69%	3.5* cm	22.9* g
	2.5%	27%*	31%	2.2* cm	15.5* g
	3.75%	5%*	6%	1.4* cm	2.8* g
	5%	0%*	-	-	-
Twitch (<i>Cynodon dactylon</i> L.)	0%	93%	-	3.6 cm	29.4 g
	1.25%	21%	23%	2.0 cm	6.3 g
	2.5%	0%	-	-	-
	3.75%	0%	-	-	-
	5%	0%	-	-	-
White pigweed (<i>Chenopodium album</i> L.)	0%	85%	-	3.5 cm	26.0 g
	1.25%	7%	8%	0.4 cm	3.5 g
	2.5%	0%	-	-	-
	3.75%	0%	-	-	-
	5%	0%	-	-	-
Curly dock (<i>Rumex crispus</i> L.)	0%	98%	-	6.8 cm	66.5 g
	1.25%	91%	93%	5.5* cm	58.8 g
	2.5%	56%*	57%	4.2* cm	31.0* g
	3.75%	23%*	24%	2.5* cm	19.1* g
	5%	0%*	-	-	-

* Significant difference with the control at p<0.05

Significant negative effect with increasing concentration of the aqueous extract of spearmint leaves was found both on the germination of all the tested seeds, and the development of plants ($p < 0.05$).

Wheat proved relatively resistant to the effects of spearmint and maintained good parameters for the three lower concentrations. This result is promising and it is necessary to carry out more detailed laboratory and field experiments to determine the potential of spearmint in integrated strategy for weed management.

Strongest negative impact of the aqueous extract of peppermint leaves we reported in Johnson grass seeds, followed by wheat seeds (Table 4). Concentration of 1.25% aqueous extract proved less active in terms of germination of all test seeds and stronger inhibitory effect occurs with increasing dose. It was also established that peppermint has a significant negative impact on growth. For wheat, twitch, Johnson grass and white pigweed the length of young plant under effect of 1.25% aqueous extract was reduced by half compared with the control.

Considering displayed strong sensitivity of seeds and young plants of wheat we can point out that peppermint is not suitable for allelopathic active species in wheat crops.

Chenopodium album L. is a common weed of wheat and other arable crops and orchards. Cases of resistance of *C. album* to herbicides like atrazine, metribuzin and linuron have been reported in many European countries and the USA since 1980 (ELEFTHEROHORINOS *et al.*, 2000). This has promoted research for alternative eco-friendly methods for its control. Preliminary studies of ANJUM & BAJWA (2007) have shown the susceptibility of broadleaved weeds like *C. album* to sunflower extracts. These authors tested the potential of sunflower leaf extract in weed management of *C. album*. In comparison with synthetic herbicides the crude extract failed to eradicate this weed completely, but the highest tested concentration successfully killed the weed and overcame weed crop competition and consequently increased

wheat yield significantly. In our study, *C. album* seeds were found as very sensible to both basil and lavender aqueous extracts – the germination was almost fully suppressed at lowest test concentration of 1.25% extract. Extracts from spearmint and peppermint leaves also exhibited negative effect at seed germination but at concentrations above 3.75%.

Cynodon dactylon and *Sorghum halepense* are C4 perennial grasses that are considered to be among the world's worst weeds (HOLM *et al.*, 1977). *Cynodon dactylon* propagates mainly vegetatively, through stolon and rhizome fragmentation, but *Sorghum halepense* reproduces both by seed and by rhizomes (HOROWITZ, 1973). Rhizomes of both weeds are the main reserves of carbohydrates and dormant buds for over-wintering. A single plant of *Sorghum halepense* can produce 40 to 90 m of rhizomes per growing season while *Cynodon dactylon* fresh weight of subterranean parts down to 45 cm can range from 420 to 780 g m^{-2} during one year (HOROWITZ, 1972, 1973). Also, rhizomes are the primary means of *Cynodon dactylon* and *Sorghum halepense* dispersal in the field because mechanical tillage of weed-infested fields produces fragmentation and dispersal of rhizomes propagules, from which new ramets can be formed (FERNANDEZ, 2003; MITSKAS *et al.*, 2003).

Allelopathic potential of residues of some brassica species, which are round white radish (*Raphanus sativus* L.), garden radish (*R. sativus* L.), black radish (*R. sativus* L. var. *niger*), little radish (*R. sativus* L. var. *radicula*), turnip (*Brassica campestris* L. subsp. *rapa*) and rapeseed (*Brassica napus* L. *oleifera* DC.) on Johnson grass were investigated under both laboratory and field conditions by UREMIS *et al.* (2009). All these species suppressed Johnson grass in field and laboratory conditions. In our research, the most effective plant species against Johnson grass was found to be lavender and peppermint which reduce the germination of seeds up to four times even at 1.25% aqueous leaf extract, and completely suppressed it in higher doses. Basil and spearmint leaf extracts also inhibited seed

Table 3. Effect of aqueous extract of the spearmint leaves on quantitative and biometric parameters

Plant species	Concentration of aqueous extract, %	Germination of test seeds, %	Germination compared to the control, %	Length, cm	Weight, g
Wheat (<i>Triticum aestivum</i> L.)	0%	86%	-	19.7 cm	118.2 g
	1.25%	78%	91%	12.7* cm	98.3 g
	2.5%	54%*	63%	6.5* cm	43.4* g
	3.75%	19%*	22%	2.7* cm	23.2* g
	5%	0%*	-	-	-
Johnson grass (<i>Sorghum halepense</i> (L) Pers)	0%	88%	-	4.1 cm	35.7 g
	1.25%	46%*	52%	2.3* cm	22.9* g
	2.5%	8%*	9%	1.7* cm	6.5* g
	3.75%	1%*	1%	1.1* cm	2.3* g
	5%	0%*	-	-	-
Twitch (<i>Cynodon dactylon</i> L.)	0%	93%	-	3.6 cm	29.4 g
	1.25%	68%*	73%	2.6* cm	19.7* g
	2.5%	22%*	24%	1.9* cm	10.2* g
	3.75%	4%*	4%	1.1* cm	3.9* g
	5%	0%*	-	-	-
White pigweed (<i>Chenopodium album</i> L.)	0%	85%	-	3.5 cm	26.0 g
	1.25%	25%*	29%	1.2* cm	9.9* g
	2.5%	3%*	4%	0.4* cm	1.8* g
	3.75%	0%*	-	-	-
	5%	0%*	-	-	-
Curly dock (<i>Rumex crispus</i> L.)	0%	98%	-	6.8 cm	66.5 g
	1.25%	90%	92%	6.1 cm	60.9 g
	2.5%	55%*	56%	4.8* cm	42.4* g
	3.75%	15%*	15	2.2* cm	21.0* g
	5%	0%*	-	-	-

* Significant difference with the control at p<0.05

Table 4. Effect of aqueous extract of the peppermint leaves on quantitative and biometric parameters

Plant species	Concentration of aqueous extract, %	Germination of test seeds, %	Germination compared to the control, %	Length, cm	Weight, g
Wheat (<i>Triticum aestivum</i> L.)	0%	86%	-	19.7 cm	118.2 g
	1.25%	41%*	48%	9.4* cm	75.1* g
	2.5%	9%*	10%	1.8* cm	11.6* g
	3.75%	10%*	11%	1.6* cm	11.4* g
	5%	0%*	-	-	-
Johnson grass (<i>Sorghum halepense</i> (L) Pers)	0%	88%	-	4.1 cm	35.7 g
	1.25%	24%*	27%	1.8* cm	10.4* g
	2.5%	2%*	2%	0.9* cm	2.6* g
	3.75%	0%*	-	-	-
	5%	0%*	-	-	-
Twitch (<i>Cynodon dactylon</i> L.)	0%	93%	-	3.6 cm	29.4 g
	1.25%	72%*	77%	2.9* cm	20.5* g
	2.5%	31%*	33%	1.8* cm	11.2* g
	3.75%	1%*	1%	0.6* cm	1.6* g
	5%	0%*	-	-	-
White pigweed (<i>Chenopodium album</i> L.)	0%	85%	-	3.5 cm	26.0 g
	1.25%	66%*	78%	2.4* cm	19.1* g
	2.5%	18%*	21%	1.5* cm	9.2* g
	3.75%	3%*	4%	0.8* cm	2.7* g
	5%	0%*	-	-	-
Curly dock (<i>Rumex crispus</i> L.)	0%	98%	-	6.8 cm	66.5 g
	1.25%	80%*	82%	5.8* cm	51.3* g
	2.5%	52%*	53%	3.3* cm	29.9* g
	3.75%	19%*	20%	1.6* cm	17.3* g
	5%	0%*	-	-	-

* Significant difference with the control at p<0.05

germination but at concentrations above 3.75%.

Cynodon dactylon is the most damaging weed in world scale and takes the fourth place among weeds in production of allelopathic compounds (AL-SAADAWI *et al.*, 1990). Allelopathic effect is not just decreasing germination but also delaying the time of germination which could affect plant competition (ESCUDERO *et al.*, 2000; EL-KHATIB *et al.*, 2004). CHAVES & ESCUDERO (1997) found that the exudate secreted by *Cistus ladanifer* leaves inhibited the seed germination of *Cynodon dactylon* and *Rumex crispus*. Allelopathic species used in our study demonstrated significant negative effect on seed germination of *C. dactylon* on concentrations above 3.75% aqueous extract, most pronounced in basil and peppermint.

Rumex crispus L. (curled dock) and *Rumex obtusifolius* L. (broad-leaved dock) are among the most often studied weed species worldwide, the latter is also considered as one of the five most widely distributed non-cultivated plant species in the world (ALLARD, 1965). These *Rumex* species are of agricultural significance because they compete with sown or native pasture species or arable crops and occupy area which could be utilized by more palatable crop species. For Central Europe, it is estimated that more than 80% of all herbicides used in conventional grassland farming are used to control these species (GALLER, 1989). The fear of an infestation of grassland and arable land by *R. crispus* and *R. obtusifolius* is also among the most important obstacles for farmers to switch from conventional to organic farming in Central Europe (DIERAUER & STOPPLER-ZIMMER, 1994). Extracts or natural chemicals derived from various plant species have been shown to reduce germination and shoot and root development of *Rumex* seedlings (ZALLER, 2004). REIGOSA *et al.* (1999) have tested the effect of six phenolic compound derived from *Capsicum annuum* leaves on the germination and growth of *Rumex crispus* and other 5 weeds. They found that highest concentrations and combined mixture of phenolic substances inhibited the germination, but lower

concentrations had no effect or were stimulatory. Our results were similar as we also found that low doses of tested allelopathic active plants could not influence the seed germination of this weed. Most pronounced was the effect of lavender flowers aqueous extract which suppressed germination at 3.75% concentration.

Conclusions

From the allelopathic active plant species used in this study, the aqueous extract of lavender flowers exhibited most negative impact on germination of all test seeds and on the development of young plants. Significant inhibitory effect of spearmint leaves even at low concentrations was recorded on the germination of weed species tested, while the wheat seeds were slightly affected.

So, we can point out that due to proven strong negative effect of lavender both on weed and on wheat, it is unsuitable for mixed plantings. In terms of spearmint, there was demonstrated inhibitory effect on weeds while the wheat was found to be resistible. These results indicate the potential for integration of spearmint in sustainable agriculture and organic farming, but for this purpose it is necessary to continue research in the field.

Potential allelochemicals must be characterized as they can provide new and cheap synthetic analogues of natural products having greater selectivity, stability and efficacy to control weeds and pests. They should also undergo toxicity testing to confirm their safety on non-target species. A logical progression in this research is required to develop agrochemicals based on natural products.

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