



Research Article

The Allelopathic Effects of Turkish Hulled Wheat Lines on Germination of *Amaranthus retroflexus* L. and *Lolium perenne* L. Seeds

Yasin Emre Kitis¹ , Mehmet Tekin² , Taner Akar^{2*} 

¹Department of Plant Protection, Faculty of Agriculture, Akdeniz University, Antalya, Turkey

²Department of Field Crops., Faculty of Agriculture, Akdeniz University, Antalya, Turkey

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Abstract. The aim of this study was to determine allelopathic effects of some emmer (*Triticum dicoccum* Schrank) and einkorn (*Triticum monococcum* L.) wheat lines on germination of redroot pigweed (*Amaranthus retroflexus* L.) and perennial ryegrass (*Lolium perenne* L.). For this purpose, forty-nine emmer and thirty-six einkorn wheat lines with two commercial durum wheat cultivars (cvs. Svevo and Saragolla) were sown in experimental field of Akdeniz University. Leaves of emmer and einkorn lines were cut at the end of tillering stage (Z29) to obtain sufficient plant extracts. Afterwards, germination rates of weed species were recorded by carrying out petri experiments. Many of the emmer and einkorn wheat lines highly inhibited the germination of two weed species compared to durum wheat cultivars but some lines were found very efficient for inhibition of both species. Four lines of emmer and eight lines of einkorn wheat inhibited germination of redroot pigweed over 90% while one emmer line and six lines of einkorn inhibited germination of ryegrass over 80%. According to average germination values, it was revealed that while ten lines of emmer and seventeen lines of einkorn reduced germination of both weed species by more than 50%, effect of commercial wheat cultivars remained at the rate of 35% and 18%, respectively. As a result, there was a clear evidence that some lines of emmer and einkorn wheat had a suppressive effect on germination of two important weed species. This is the first report about the allelopathic potential of the emmer and einkorn wheats. However, further researches are needed to test effectiveness of these wheats on allelopathy under both greenhouse and field conditions in detail.

***Corresponding author**

tanarakar@akdeniz.edu.tr

Türk Kavuzlu Buğday Hatlarının *Amaranthus retroflexus* L. ve *Lolium perenne* L. Tohumlarının Çimlenmesi Üzerine Allelopatik Etkileri

Anahtar kelimeler:

Kavuzlu buğdaylar, siyez, gernik, yabancı ot, inhibisyon etkisi

Özet. Bu çalışma ile bazı gernik (*Triticum dicoccum* Schrank) ve siyez buğdayı (*Triticum monococcum* L.) hatlarının horozibiği (*Amaranthus retroflexus* L.) ve çok yıllık çim (*Lolium perenne* L.) tohumlarının çimlenmeleri üzerine etkilerinin belirlenmesi amaçlanmıştır. Bu amaçla 49 gernik ve 36 siyez hattı, 2 ticari makarnalık buğday çeşidi (Svevo ve Saragolla) ile birlikte Akdeniz Üniversitesi araştırma alanına ekilmiştir. Gernik ve siyez hatlarının yaprakları kardeşlenme dönemi sonunda (Z29) bitki ekstraktlarını elde etmek amacıyla kesilmiştir. Yürütülen Petri denemeleriyle yabancı ot türlerinin çimlenme oranları kaydedilmiştir. Birçok gernik ve siyez hattı makarnalık buğdaya kıyasla iki yabancı ot türünün çimlenmesini yüksek oranda inhibe etmiştir fakat bazı hatlar inhibisyon konusunda oldukça başarılı bulunmuştur. 1 gernik hattı ile 6 siyez hattı çok yıllık çim türünün tohumlarının çimlenmesini %80'in üzerinde inhibe ederken 4 gernik hattı ile 8 siyez hattı horozibiği tohumlarının çimlenmesini %90'ın üzerinde inhibe etmiştir. Ortalama çimlenme değerlerine göre, 10 gernik hattı ile 17 siyez hattı her iki yabancı ot türünün de çimlenmesini %50'nin üzerinde geriletirken ticari buğday çeşitlerinin etkilerinin sırasıyla %35 ve %18 oranlarında kaldığı belirlenmiştir. Sonuç olarak, bazı gernik ve siyez hatlarının iki önemli yabancı ot türünün çimlenmesi üzerine baskılayıcı bir etki gösterdiği ortadadır. Bu çalışma, gernik ve siyez buğdaylarının allelopatik potansiyellerinin ortaya konduğu ilk rapordur. Fakat bundan sonraki çalışmalarla bu buğday türlerinin sera ve arazi koşullarında allelopati üzerine etkinliğinin daha detaylı bir şekilde araştırılması gereklidir.

INTRODUCTION

The hulled wheats, einkorn and emmer, are among the first domesticated species and also transitional forms between wild and modern wheat species. These species were widely cultivated in the past, but later on, they were neglected due to cultivation of high-yielding modern wheat varieties. However, the hulled wheat species have been attracting interest of farmers especially in developed countries for both high nutritional potentials (Shewry, 2018; Tekin *et al.*, 2018) and suitability to low-input and organic farming (Konvalina *et al.*, 2014) compared to modern wheats. Moreover, they are claimed to be efficient in weed competitiveness, too.

Weeds are one of the major constraints limiting crop yield in agricultural systems, especially in organic farming. They compete with cultivated crops for light, moisture, space and nutrients and therefore, the growth of weeds must be controlled for a successful cultivation (Bashir *et al.*, 2018). In conventional farming systems, weeds are routinely controlled by synthetic inputs (herbicides), but continuous use of herbicides has created some challenges including economic, environmental and human health concerns in these days (Bertholdsson, 2005; Narwal, 2010). More natural approaches such as use of allelopathic relationships in farming system can be chosen instead of routine use herbicides. Allelopathy is an important mechanism in weed-crop competition and weed-crop interference. Plants may have inhibitory or rarely stimulatory effects on germination and growth of other plants caused by the release of plant-produced secondary metabolites or decomposition products of microorganisms to the aerial or soil environment (Aslam *et al.*, 2017). Numerous crops have been studied more or less properly for their allelopathic effects against weeds or other crops. Main effect on weed, possibly mediated by the release of secondary metabolites (allelochemicals) has been studied for many crop species such as wheat, barley, alfalfa, clovers, vetches, oats, rice, cotton and maize (Kruse *et al.*, 2000; Arif *et al.*, 2015; Kitis *et al.*, 2016). Allelopathic crops can be used for multiple approaches including crop rotations, cover crops and residue mulches, intercropping, phytotoxic varieties and tree farming in addition to water extract applications which act as natural herbicide (Narwal, 2010). Wheat (*Triticum* spp.), as one of these allelopathic crops, is one of the important cereals extensively investigated and also used as a cover crop. Many chemicals are released from the wheat living plants and decomposing residues which are responsible for wheat allelopathy (Aslam *et al.*, 2017). Additionally, many studies have been conducted on wheat for intercropping, cover crops and residue mulches (Bertholdsson, 2005). Ma (2005) also reported that the aqueous extraction of wheat residues is allelopathic to a number of weed species. Common or bread (*Triticum aestivum* L.) wheat was generally used as genetic material in these studies but there were few studies cultivated wheat species such as durum (*T. turgidum* var. *durum*) and hulled (einkorn and emmer) wheats (Dong *et al.*, 2013).

Emmer wheat is claimed to be efficient in weed competitiveness due to its early vigour/high plant height especially in early development stages (Konvalina *et al.*, 2012) and einkorn is also very competitive with high tillering capacity (Nakhforoosh *et al.*, 2014). They are obviously the two most suitable wheat species that can be preferred for cultivation under organic or low input conditions but there is no comprehensive scientific study to reveal allelopathic effects of these species. In this study, we aimed to determine allelopathic effects of some einkorn and emmer wheat lines developed during the intensive selection studies on germination of weed species.

MATERIAL AND METHOD

Preparation of Aqueous Extracts

Forty-nine emmer and thirty-six einkorn wheat lines with two commercial durum wheat cultivars as check (Saragolla - Svevo cvs.) and two important weed species, redroot pigweed (*Amaranthus retroflexus* L.) and perennial ryegrass (*Lolium perenne* L.), were used in this study. The hulled wheat population were collected from different provinces of Turkey (Kaplan *et al.*, 2014) and hulled wheat lines (Coskun *et al.*, 2019) were developed from population in a project funded by TUBITAK (The Scientific and Technological Research Council of Turkey, Project No: 214O401).

The hulled wheat lines with these two checks were sown as two rows in experimental area of Akdeniz University (Antalya, Turkey). Any herbicides were not applied to experiment until sampling the genotypes. The fresh leaves of all wheat lines and cultivars were cut at the end of the tillering stage (Z29) and then leaf samples were transferred to weed science laboratory. 100 g leaf samples from each genotype were weighed and washed under tap water. The samples were subsequently washed in distilled water. After that, all samples were kept in glass jars containing 300 mL pure water for 24 h at room temperature. Later on, the solutions were filtered and final extracts were applied to petri dishes without delay.

Bioassay

Aqueous extracts of wheat lines were tested on germination of redroot pigweed (*A. retroflexus*) and perennial ryegrass (*L. perenne*). The seeds were surface sterilized with 1.5% (v/v) sodium hypochlorite solution for 1 min and washed twice with distilled water. One hundred seeds of each weed species were placed on double layer Whatman No. 1 filter papers in 9 cm diameter sterile plastic petri dishes. Ten mL of extract was applied without dilution on the petri dishes in three replications per wheat line. The same amount of purified water was applied to control group. To reduce moisture loss and avoid contamination, the petri dishes were put in plastic bags. While petri dishes containing redroot pigweed seeds were put in the incubator at 30 ± 2 °C, other petri dishes with perennial ryegrass seeds were kept at 20.5 ± 1.5 °C for germination. After 7 days, seeds with 0.5 cm radicle length were counted as germinated ones.

Statistical Analysis

The germination rate of weed seeds was determined by the following Eq. (1):

$$\text{Germination rate (\%)} = (S/S_0) \times 100 \quad (1)$$

where S_0 is number of sown seeds while S represents number of germinated seeds for each treatment.

The allelopathic effect of aqueous extracts on germination of the weed species was actually determined by inhibition rate over control defined by the following Eq. (2):

$$\text{Inhibition (\%)} \text{ over control} = (1 - N/N_0) \times 100 \quad (2)$$

where N_0 and N represent germinated seed numbers in the control and treatments, respectively.

Descriptive statistics such as mean and standard error of mean were calculated to reveal a range of variation for germination and inhibition rates. Additionally, variance analysis was applied by using XLSTAT statistical software (Addinsoft Co.) and differences between means of aqueous extracts were analyzed by least significant difference (LSD) at $p < 0.05$.

RESULTS AND DISCUSSION

The allelopathic effects of emmer and einkorn wheat lines on the germination of perennial ryegrass and redroot pigweed were given in Table 1 and Table 2. The maximum germination rates in emmer wheat experiments for redroot pigweed and perennial ryegrass were 80.3% and 91% for the control applications, respectively (Table 1). The most promising inhibition rates were 93.1%, 93.6%, 91.4% and 89.9% for emmer wheat lines 7, 33, 34, 35 on redroot pigweed and 82.5% and 76.6% for emmer wheat lines 1 and 60 on perennial ryegrass, respectively (Table 1). Variance analysis showed that statistically significant ($p < 0.01$) differences were found among the emmer wheat lines in terms of inhibiting the germination of redroot pigweed and perennial ryegrass seeds (Table 1). Inderjit *et al.* (2001) reported that the root length of perennial ryegrass was extremely affected by wheat depending on the density of wheat seeds but the shoot growth of the ryegrass was not changed whether in presence or absence of wheat. Aqueous leaf extracts of emmer wheat lines were not able to sufficiently suppress germination of perennial ryegrass seeds as indicated by Inderjit *et al.* (2001). This difference can be attributable to different applications and wheat species used in this study.

Table 1. Effects of emmer lines on germination and inhibition rates of the weed species.

Çizelge 1. Gernik hatlarının yabancı ot türlerinin çimlenme ve inhibisyon oranları üzerine etkisi.

Emmer wheat Line/Cultivars	Redroot pigweed		Perennial ryegrass	
	Germination rate (%)	Inhibition rate (%)	Germination rate (%)	Inhibition rate (%)
Line 1	28.3	64.3	16.0	82.5
Line 2	79.3	0.9	30.3	66.8
Line 3	71.7	10.7	51.7	43.3
Line 4	37.0	54.5	42.3	53.7
Line 5	74.7	7.0	49.7	45.6
Line 6	73.0	8.9	37.7	58.6
Line 7	5.3	93.1	37.3	59.1
Line 9	78.3	2.0	41.7	54.5
Line 11	48.7	39.0	60.0	34.3

Table 1. Continue.

Çizelge 1. Devami.

Emmer wheat Line/Cultivars	Redroot pigweed		Perennial ryegrass	
	Germination rate (%)	Inhibition rate (%)	Germination rate (%)	Inhibition rate (%)
Line 13	66.7	16.6	41.3	54.7
Line 14	69.3	13.3	66.0	27.7
Line 15	52.7	33.8	46.7	49.0
Line 16	57.0	28.5	46.0	49.6
Line 18	75.0	6.4	55.0	39.7
Line 18	47.7	40.4	52.3	42.7
Line 19	50.3	37.5	61.0	33.3
Line 20	26.3	67.3	43.3	52.5
Line 21	50.0	37.9	39.3	56.9
Line 22	30.7	61.5	34.3	62.4
Line 23	51.0	37.0	50.0	45.2
Line 24	70.0	12.5	64.7	29.1
Line 25	68.3	14.7	55.3	39.5
Line 29	48.7	39.4	35.3	61.3
Line 32	75.7	5.0	43.0	52.9
Line 33	5.0	93.6	50.0	45.3
Line 34	6.7	91.4	48.7	46.8
Line 35	8.0	89.9	46.0	49.6
Line 37	58.0	27.8	64.0	29.8
Line 38	68.3	14.3	66.3	27.2
Line 39	51.0	36.8	69.3	24.1
Line 40	76.0	5.2	64.0	29.9
Line 41	45.3	43.4	53.3	41.5
Line 42	44.0	44.1	50.0	45.1
Line 43	70.7	12.0	57.7	36.6
Line 44	25.3	68.2	57.3	37.2
Line 45	71.7	10.4	53.0	42.0
Line 46	77.3	3.8	36.3	60.3
Line 47	75.7	5.6	58.0	36.5
Line 48	67.3	16.6	32.3	64.6
Line 50	73.3	8.1	45.0	50.7
Line 51	75.7	5.3	59.0	35.4
Line 52	72.3	9.1	72.7	20.3
Line 53	79.7	0.4	58.7	35.8
Line 55	78.0	2.6	69.0	24.6
Line 57	77.3	3.5	75.7	17.1
Line 58	82.0	-2.4	59.7	34.7
Line 59	71.3	11.0	78.3	14.2
Line 60	67.7	16.3	21.3	76.6
Line 62	45.7	43.4	46.0	49.6
Saragolla cv.	58.0	27.3	51.0	44.1
Svevo cv.	79.7	0.4	61.3	32.9
Control	80.3	0.0	91.3	0.0
Mean ± SE	58.2 ± 1.8	27.3 ± 2.3	51.6 ± 1.2	43.2 ± 1.3
F	15.9**	15.8**	8.5**	8.5**
LSD (p<0.05)	15.1	19.0	13.6	14.9

* P<0.05, ** P<0.01.

On the other hand, the maximum germination rates in einkorn experiment for redroot pigweed and perennial ryegrass were found in the control applications (76.7% and 88.7%) with cv. Svevo (79.7%) (Table 2). Aqueous extracts of einkorn lines of 1, 15, 49 and 56 inhibited the germination of redroot pigweed seeds completely. In addition, lines 21, 42, 43 and 86 inhibited germination of redroot pigweed over 90% (Table 2). The einkorn extracts were also successful for inhibiting germination of perennial ryegrass seeds. Einkorn lines of 15, 42 and 18 were the most remarkable applications with 95.8%, 92.4% and 91.2% inhibition rates, respectively. Einkorn lines 86, 21 and 5 also inhibited the germination of perennial ryegrass over 80% (Table 2). Especially, einkorn line 15 inhibited

almost completely germination of both weed species and lines 42, 21 56 and 86 were also determined as successful inhibitors for both weed species (Table 2). Variance analysis illustrated that statistically significant ($p<0.01$) differences were found among the einkorn lines for inhibiting germination of redroot pigweed and perennial ryegrass seeds (Table 2). Aqueous extracts of cultivars (cvs. Saragolla and Svevo) were not as successful as the einkorn and emmer lines for suppressing the germination of weed species (Table 1 and Table 2). Li et al. (1996) reported that the germination of *A. retroflexus* treated with wheat aqueous extract (500 mg kg^{-1}) was decreased by 86%. Our findings on inhibition rate of some einkorn and emmer wheat lines on pigweed seeds were quite similar to Li et al. (1996).

Table 2. Effects of einkorn lines on germination and inhibition rates of the weed species.

Çizelge 2. Siyez hatlarının yabancı ot türlerinin çimlenme ve inhibisyon oranları üzerine etkisi.

Einkorn Line/Cultivars	Redroot pigweed		Perennial ryegrass	
	Germination rate (%)	Inhibition rate (%)	Germination rate (%)	Inhibition rate (%)
Line 1	0.0	100.0	62.7	29.3
Line 2	49.3	36.0	75.0	15.2
Line 4	28.7	62.4	51.7	41.5
Line 6	9.0	88.1	34.7	60.7
Line 8	62.7	17.4	74.7	15.7
Line 9	18.3	76.5	53.0	40.4
Line 10	64.0	16.2	44.3	49.7
Line 12	75.3	1.3	60.3	32.0
Line 14	13.7	82.8	42.0	52.9
Line 15	0.0	100.0	3.7	95.8
Line 16	55.0	29.6	53.7	39.0
Line 18	29.3	62.3	7.7	91.2
Line 19	61.3	20.1	59.7	32.2
Line 20	52.0	32.7	64.7	27.3
Line 21	1.7	97.9	14.3	83.7
Line 22	44.7	42.6	55.0	37.1
Line 23	26.3	66.2	54.7	38.3
Line 24	54.3	28.3	61.7	30.4
Line 25	66.0	13.5	70.0	20.8
Line 26	52.3	32.6	44.0	49.9
Line 27	71.3	6.8	53.7	39.6
Line 28	61.0	20.5	62.7	29.1
Line 32	71.0	7.2	77.3	12.7
Line 33	26.3	65.4	41.0	53.6
Line 37	53.7	30.2	83.0	6.6
Line 38	62.0	19.4	74.7	16.1
Line 39	56.3	26.4	75.7	14.7
Line 40	14.0	82.0	27.7	68.9
Line 41	38.3	49.3	67.0	24.3
Line 42	4.3	94.4	6.7	92.4
Line 43	6.3	92.1	22.3	74.8
Line 49	0.0	100.0	20.3	76.9
Line 52	19.3	75.3	21.3	75.9
Line 55	47.3	38.4	71.0	19.8
Line 56	0.0	100.0	15.0	82.9
Line 86	7.0	90.9	12.7	85.7
Saragolla cv.	58.0	23.6	51.0	42.4
Svevo cv.	79.7	-4.5	51.3	42.0
Control	76.7	0.0	88.7	0.0
Mean ± SE	38.9 ± 2.5	49.3 ± 3.2	48.9 ± 2.2	44.6 ± 2.5
F	23.6**	22.6**	20.8**	21.2**
LSD ($p<0.05$)	15.2	20.3	14.5	16.2

* $P<0.05$, ** $P<0.01$.

Wu et al. (2000) claimed that wheat seedling allelopathy varied significantly with genetic backgrounds. They also reported that there was a considerable genetic variation of allelopathic activity in wheat (*Triticum aestivum* L.) germplasm on annual ryegrass (*Lolium rigidum*). Bertholdsson (2005) also studied to determine potential allelopathic activity of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) cultivars on perennial ryegrass (*Lolium perenne*) using an agar-based bioassay. He reported that allelopathic activity of barley and wheat cultivars on perennial ryegrass changed between 7-58% and 0-21%, respectively. We have witnessed a great variation between emmer and einkorn wheat lines as Wu et al. (2000) and Bertholdsson (2005) demonstrated in wheat and barley cultivars.

The most remarkable findings were actually determined in einkorn treatments in this study. Although there are variations in species, einkorn is a photoperiodic plant that requires long-day conditions for heading (Nakimichi, 2015). The species grows as prostrate or semi-prostrate from seedling growth to heading and it therefore has to compete with weeds for light, moisture, space and nutrients. In this process, allelopathic actions like the release of plant-produced secondary metabolites are vital sources for einkorn. Unlike this, even though emmer wheat may seem less allelopathic than einkorn according to our findings, it is actually very competitive against to weeds due to its early vigour and high plant height (Konvalina et al., 2014).

CONCLUSION

This preliminary study on allelopathic effects of different emmer and einkorn wheat lines showed that there were significant variations among these lines to inhibit germination of seeds of two important weeds. The inhibition effect of einkorn lines was higher than emmer lines on both weed species. Whereas some einkorn lines such as 15, 42, 21, 56 and 86 inhibited germination of both weed species, there were no any emmer wheat lines that inhibited both perennial ryegrass and pigweed seeds.

Bread or common wheat cultivars have been generally used as genetic material in such wheat allelopathy studies by now. To our knowledge, this is the first report to reveal allelopathic potential of einkorn and emmer wheats and detailed new studies to be conducted under both greenhouse and field conditions are needed by using these promising hulled wheat lines to get more concrete data.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DECLARATION OF AUTHOR CONTRIBUTION

TA and YEK designed the study. YEK and MT performed the experiments. YEK and MT analyzed the data. MT, TA and YEK wrote and edited the manuscript.

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