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Research Article

Evaluation of the Allelopathic Effect of Some Cruciferous Plants on Germination and Growth of Johnsongrass

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Abstract

Johnsongrass [*Sorghum halepense* (L.) Pers.] is a dangerous weed causes great losses in agricultural crops. It is difficult to control due to its reproduction by rhizomes, and its resistance to herbicides. This study aims to evaluate the allelopathic performance of five species of cruciferous plants on germination and some growth indicators of Johnsongrass seeds and rhizomes. Cruciferous species are white cabbage, red cabbage, broccoli, turnip, and garden rocket. The aqueous extracts of all these plants were prepared in 2%, 5%, 10%, and 20% concentrations. Germination inhibition rate, shoot, and radicle length of the seedlings of Johnsongrass were calculated over control environment. The powders of whole cruciferous plants were also analyzed using GC-MS. The results showed that garden rocket had the highest effect on the germination inhibition of both seeds and rhizomes. Garden rocket extracts at 10% and 20% concentrations reduced seeds germination by 100%. On the other hand, rhizomes germination inhibition for 10% and 20% concentrations were found 85.3% and 92.7% respectively. The effects of aqueous extracts of cruciferous plants on shoots and radicals were similar to their effects on the germination of Johnsongrass. The allelopathic effect also increased with increasing concentration of the aqueous extract. The most effective cruciferous plant on the biomarkers of Johnsongrass was garden rocket, while red cabbage was the least. The cruciferous plants, especially garden rocket, have a strong allelopathic effect that can be used as an alternative to herbicides in the control of Johnsongrass.

Introduction

Johnsongrass ranks 6th in the world in terms of the economic damage by weeds in 50 different cultivated plants (Holm et al., 1977; Peerzada et al., 2017). It was reported that it causes high yield losses in economically important crops such as soybean [*Glycine max* (L.) Meri.], corn (*Zea mays* L.), cotton (*Gossypium barbadense* L.), vegetables and fruits (Peerzada et al., 2017). High density of Johnsongrass caused yield losses of 70% in cotton, 88-100% in corn, 69% in sugarcane, and 59-88% in soybean (Barroso et al., 2016).

The allelopathic potential of Brassicaceae plants can be used for weed control in agricultural areas. It was found that *Brassica* sp. have a toxic effect on weeds and can be an alternative herbicide (Bangarwa and Norsworthy, 2014; Jabran et al., 2015). It was reported that fresh white cabbage with a concentration of 50% inhibited seeds germination of *Amaranthus retroflexus* L., *Chenopodium album* L. *Solanum nigrum* L., *Z. mays* and *Beta vulgaris* L. between 34 and 95% in laboratory conditions (Kural and Ozkan, 2020). The use of turnip plant residues was found to be effective in control of Johnsongrass in both field and laboratory conditions (Uremis et al., 2009). Broccoli and white cabbage reduced both the number of branches and the dry weight of *Orobanche crenata* Fors (Aksoy et al., 2016). Garden rocket defatted seedmeal at 0.5% and 1% concentrations completely inhibited the emergence of sicklepod (*Senna obtusifolia* L.) seeds (Vaughn et al., 2006). The allelopathic effect of the aqueous extract of fresh garden rocket plant was used to control *Phalaris minor* Retz. and *B. vulgaris*. The aqueous extract at a concentration of 80% achieved the maximum growth inhibiting effect of both plants (El-Wakeel et al., 2019). Isothiocyanates (ITCs) compounds are the most important allelochemicals synthesized by plants belonging to the Brassicaceae family. ITCs are produced by the enzymatic hydrolysis of glucosinolates (Jafariehyazdi and Javidfar, 2011). Brassicaceae plants

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contain different ITC compounds such as allyl-ITC, 2-phenylethyl-ITC, 3-butenyl-ITC, and benzyl-ITC, depending on the species and growing conditions. As the structure of the chemical chain changes, the type of glucosinolates and ITCs also changes (Bangarwa et al., 2011; Shah et al., 2016).

This study aimed to estimate the allelopathic effects of white cabbage, red cabbage, broccoli, turnip, and garden rocket on the germination and growth of Johnsongrass seeds and rhizomes in laboratory conditions, in addition to identify ITCs in these plants.

Materials and Methods

The experiment was carried out twice in 2021 in the laboratories of Agriculture Faculty, Kahramanmaras Sutcu Imam University, Turkey.

Source of plants materials

Johnsongrass seeds and rhizomes were collected in the fall 2020 from the fields of the Faculty of Agriculture at Kahramanmaras University. The rhizomes were washed with tap water, and placed in nylon bags in the fridge at a temperature of 2 °C. The seeds were soaked in 1% sodium hypochlorite solution for 15 minutes, then dried, placed in nylon bags, and kept at room conditions.

The varieties of cruciferous species used in this experiment are shown in Table 1. The seeds of cruciferous species varieties were obtained from Teta-Tohumculuk-seed company, Turkey. The cruciferous plants were planted in January 2020, then whole plants were taken in the head formation stage for the white and red cabbage, and in the flowering stage for broccoli, turnip, and garden rocket. The samples were cleaned and dried in room conditions for a month, then they were ground in a coffee grinder and placed in bags in the fridge at 4 °C.

Table 1. Varieties of cruciferous species used in the experiment

Species and Abbreviation	Scientific name	Varieties
White cabbage (WG)	*Brassica oleracea* var. *capitata* f. *alba*	Yellow sarmalik
Red cabbage (RG)	*B. oleracea* var. *capitata* f. *rubra*	Mohrenkopf
Broccoli (Br)	*B. oleracea* var. *italica*	Sakura F1
Turnip (Tp)	*B. rapa*	K.Maras salgam
Garden rocket (GR)	*Eruca vesicaria* ssp. *sativa*	Istanbul rokası

Preparation of cruciferous plant extracts

Aqueous extracts of cruciferous plants were prepared from powder of dried plants at 2, 5, 10, and 20% concentrations. To obtain a 20% concentration, 200 g of each cruciferous plant was placed in a separate erlenmeyer flask, and 1 liter of distilled water was added to it. The extracts were placed in a shaker at 200 rpm and 25 ±2 °C for 8 hours. Then the mixtures were filtered with filter papers twice. The other concentrations were prepared from extract of concentration of 20% by adding distilled water.

Experiment setup

Petri dishes with a 9 cm diameter were used with a double layer of filter papers. Petri dishes were sterilized with ethanol 90% and dried completely. To break Johnsongrass seeds dormancy mechanical method (sandpaper) was applied (AL Sakran et al., 2020). The rhizomes were cut 1 cm long so that each piece contained one bud. Then, 20 seeds or 10 rhizomes of Johnsongrass were added to Petri dishes. According to the design of the experiment, 5 ml of the extracts was added to each Petri dish and 5 ml of distilled water was added to the controls. Petri dishes were placed in the climate room at 25±2 °C and 12:12 light-dark hours. This experiment was designed in a completely randomized design. Each treatment had four replicates and the experiment was replicated twice.

Johnsongrass germination inhibition rate

The cruciferous plant's aqueous extracts inhibition rate of Johnsongrass seeds and rhizomes germination over control was determined by the formula (1).

Germination inhibition over control = $(G_n - G_0 / G_0) \times 100$ ...... (1)

Where G_n is the number of germinated seeds or rhizomes in treatment n, and G_0 is the number of germinated seeds or rhizomes in control.

Shoot and radicle length reduction rate of Johnsongrass

The shoot and radicle lengths of the germinated plants were measured by electronic ruler ten days after the beginning of the experiment. The percentage of reduction of shoot and radicle lengths by the extracts was calculated using the formula (2).

Reduction of lengths over control = $(L_n - L_0 / L_0) \times 100$ ...... (2)

Where L_n is the shoot or radicle length of seeds or rhizomes in treatment n, and L_0 is the shoot or radicle length of seeds or rhizomes in control.

Identification of major ITCs in cruciferous plants

Ten grams of powder from each cruciferous plant were defatted with hexane using soxhlet for 24 hours. Dried defatted powders were mixed with 30 ml potassium phosphate (0.05M) buffer. Dichloromethane (50 ml) was added to each mixture powder and then placed in the shaker at 25 °C and 200 rpm for 8 hours, 10 g of NaCl and Na2SO4 were added to the mixtures and mixed well. The mixture was filtered with filter paper with dichloromethane added (Vaughn and Berhow, 2005). Dichloromethane solutions were sent to the chemistry department of Ataturk University for a GC-MS analysis.

Data analysis

Data were statistically analyzed by IBM SPSS Statistics (version 24) and Excel programs (2013). The data were subjected to analysis of variance (ANOVA) at significant levels ($p<0.01$), and then means were compared by the Least Significant Difference (LSD) test. According to the t-test, there was no significant difference between the two replications of the experiment, so, the analysis of the average of data was performed.

Results and Discussion

Johnsongrass germination inhibition rate

The results of this experiment showed that garden rocket extracts inhibited the germination of Johnsongrass seeds and rhizomes at the highest rate, followed by turnip. The inhibition of seeds germination under the influence of garden rocket extracts at a concentration of 10% and 20% were 100%, while they were 85.3% and 92.7 % for rhizomes, under the effects of these concentrations respectively. Red cabbage and broccoli extracts were the least effective on germination inhibition of Johnsongrass seeds and rhizomes. A stimulating effect on seed germination was observed under the influence of low concentrations (2%) of extracts of all plants. The highest stimulation rate was 28.7% in the garden rocket (2%) extract treatment (Table 2).

Table 2. Effect of different concentrations of cruciferous plants on the germination inhibition rate of Johnsongrass seeds and rhizomes

Cruciferous plants	Germination inhibition rate of Johnsongrass over control (%)							
	Seed				Rhizome			
	2%	5%	10%	20%	2%	5%	10%	20%
WG	-19.3 b	16.3 c	65.9 c	82.9 c	6.3 c	20.9 c	55.6 bc	70.3 c
RG	-16.5 b	14.2 c	60.5 d	77.3 d	5.2 c	14.1 d	49.7 d	66.6 c
Br	-11.2 a	15.6 c	62.0 d	78.6 cd	6.1 c	16.9 cd	54.4 cd	70.1 c
Tp	-24.0 c	61.1 b	75.2 b	91.6 b	10.5 b	34.5 b	60.4 b	83.2 b
GR	-28.7 d	71.9 a	100.0 a	100.0 a	13.4 a	42.2 a	85.3 a	92.7 a
LSD	3.7	2.8	3.2	4.3	2.4	4.0	5.2	4.0

Similar letters in the same column express that no significant difference ($p<0.01$).

Earlier Shaker et al. (2010) reported garden rocket aqueous extract of 2% reduced the germination of Johnsongrass seeds by 62.5%. This differs from the results of our experiment, the reason may be due to the different varieties of garden rocket used and their content of glucosinolates. Li et al. (2021) found that there can be significant variation in the quantity and quality of glucosinolates between genotypes of plants, quantity can be more than 100-fold. White cabbage extract inhibited the germination of some plants by 34 - 95% (Kural and Ozkan, 2020). It was also reported incorporating broccoli into the soil inhibited weed density by 58.7% (Isci et al., 2010).

Shoot and radicle length reduction rate of Johnsongrass

The highest rate of shoot length reduction occurred in garden rocket and turnip treatments. The 20% concentration of the different extracts reduced the length of shoots germinated from the seeds and rhizomes by rates between 71.6 - 100% and 72.2 - 91.5%, respectively (Table 3).

Table 3. Effect of different concentrations of aqueous extracts of cruciferous plants on the shoot length of Johnsongrass

Cruciferous plants	Shoot length of Johnsongrass over control (%)							
	Seed				Rhizome			
	2%	5%	10%	20%	2%	5%	10%	20%
WG	-6.1 b	-4.1 c	41.9 b	86.0 b	4.2 c	22.5 c	53.0 c	80.1 bc
RG	-4.7 b	-0.7 c	12.8 c	71.6 c	3.4 c	17.8 cd	39.4 d	72.2 c
Br	-13.5 b	4.1 c	25.7 c	75.3 c	5.5b c	14.4 d	51.5 c	74.4 c
Tp	6.8 a	39.2 b	52.0 b	93.6 ab	10.4 b	44.5 b	74.2 b	88.6 ab
GR	9.7 a	85.1 a	100.0 a	100.0 a	16.9 a	56.4 a	83.9 a	91.5 a
LSD	10.3	17.6	13.2	8.4	5.8	7.4	8.1	8.6

Similar letters in the same column express that no significant difference ($p<0.01$).

The effects of cruciferous plant extracts at different concentrations on the length of the radicle were similar to their effects on the length of the shoot. A stimulating effect on shoot and radicle length growing from seeds was also recorded under the effect of a 2 and 5% concentration for white and red cabbage. The extracts at a concentration of 20% reduced the length of radicle growing from seeds and rhizomes by 78.6 - 100% and 79.5 - 92.0%, respectively (Table 4).

Table 4. Effect of different concentrations of aqueous extracts of cruciferous plants on the radicle length of Johnsongrass

Cruciferous plants	Radicle length of Johnsongrass over control (%)							
	Seed				Rhizome			
	2%	5%	10%	20%	2%	5%	10%	20%
WG	-7.3 b	-4.0 c	49.0 c	87.5 b	9.4 b	28.5 b	62.5 c	84.4 b
RG	-14.6 b	-5.3 c	38.5 c	78.6 c	5.2 b	18.4 b	47.9 d	79.5 b
Br	-16.1 b	5.8 c	40.1 c	79.2 c	5.9 b	25.3 b	60.1 c	81.0 b
Tp	4.2 a	40.6 b	67.2 b	92.9 ab	13.2 ab	52.4 a	74.7 b	86.1 ab
GR	4.7 a	86.0 a	100.0 a	100.0 a	18.1 a	59.7 a	88.5 a	92.0 a
LSD	10.8	11.6	10.1	8.0	7.7	12.8	9.1	7.5

Similar letters in the same column express that no significant difference ($p<0.01$).

Some research showed that extracts of aqueous cruciferous plants reduce the length of shoots and radicles of weeds in varying proportions (Ozdemir, 2007; Shaker et al., 2010; Aksoy et al., 2016). The results of this research are also consistent with many researches that low concentrations of allelopathic plants may stimulate the germination and growth of other plants, and this stimulus turns into an inhibitor with an increase in concentration in parallel (El-Kenany and El-Darier, 2013; Xiaobang, 2019; AL Sakran et al., 2021).

Identification of major ITCs in cruciferous plants

According to the results of GC-MS analysis, four ITCs were identified in white cabbage with a total percentage of 32.57% and the predominant were 3-(methylsulfinyl)propyl- (iberin) with a rate of 23.43%. Similarly, Wermter et al. (2020) found that one of the major compounds in white cabbage was 3-(methylsulfinyl)propil- (Table 5).

Both red cabbage and broccoli contained 6 ITCs but their total percentage of ITCs were the lowest (26.46% and 27.49%). Also, prevalent ITC was 4-(methylsulfonyl)butyl- (sulforaphane) in both plants with rates of 10.79% and 11.27% respectively. Some research determined that the predominant glucosinolate in red cabbage and broccoli was glucoraphanin, whose hydrolysis lead to produce 4-(methylsulfinyl)butyl- (Meyer and Adam, 2008; Wang et al., 2019).

There were 5 different ITCs in turnip with a total percentage of 47.64%. The most predominant ITC was 3-butenyl with 25.09% (Table 5). Klopsch et al. (2017) identified the dominant glucosinolate in both leaves and tubers of turnip was 3-butenyl-.

Only 4 ITCs were determined in garden rocket and the total percentage was 55.23%, while prevalent ITCs were 4-(methylthio)butyl- (erucin) with a rate of 27.12% (Table 5). Also, Bell et al. (2015) found that the main glucosinolates in garden rocket were 4-(methylthio)-butyl-, 4-(methylsulfonyl)-butyl- and 3-(methylthio)-propyl-.

Table 5. Percentage of types of ITCs in different cruciferous plants according to GC-MS analysis

Cruciferous plants	ITCs	Percentage(%)
White cabbage	3-(Methylsulfinyl)propyl	23.43
	2-Propenyl	4.42
	4-(Methylsulfonyl)butyl	2.79
	Indol-3-ylmethyl	1.93
	Totel	32.57
Red cabbage	4-(Methylsulfonyl)butyl	10.79
	(S)-2-Hydroxy-3-butenyl	4.62
	2-Propenyl	4.16
	3-Butenyl	2.76
	3-(Methylsulfinyl)propyl	2.50
	Indol-3-ylmethyl	1.63
	Totel	26.46

Cruciferous plants	ITCs	Percentage(%)
Broccoli	4-(Methylsulfonyl)butyl	11.27
	3-(Methylthio)propyl	7.14
	(S)-2-Hydroxy-3-butenyl	3.92
	3-Butenyl	2.95
	2-Propenyl	1.24
	Indol-3-ylmethyl	0.97
	Totel	27.49
Turnip	3-Butenyl	25.09
	2-hydroxy-3-butenyl-	9.24
	2-Phenylethyl	6.35
	4-Pentenyl	4.44
	Indol-3-ylmethyl	2.52
	Totel	47.64
Garden rocket	4-(Methylthio)butyl	27.12
	4-(Methylsulfonyl)butyl	14.32
	4-Mercaptobutyl	8.03
	3-(Methylthio)propyl	5.76
	Totel	55.23

The reason may be that garden rocket was more effective in reducing the growth indicators of Johnsongrass as it has the highest rate of ITCs. Therefore, the rate of prevailing ITC in it is high compared to other plants. This hypothesis is confirmed by the effect of turnip and other plants and their contents of ITCs.

Conclusion

The cruciferous plants used in this study have potential allelopathic effects on the germination and growth of Johnsongrass. The allelopathic effects were directly proportional to the concentration of the aqueous extract. The order of cruciferous plants according to the strength of the effects on the biomarkers of Johnsongrass was as follows, garden rocket, turnip, white cabbage, broccoli, and finally red cabbage. These plants, especially garden rocket, can be used as an alternative to herbicides to control of Johnsongrass. But it is necessary to study the effect of individual allelochemicals to determine which of them are responsible for the greatest effects.

Competing Interests

The authors have declared that no competing interests exist.

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