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The Extract Obtained from Two Radish Cultivars (*Raphanus sativus* L. and *Raphanus sativus* L. var. *radikula*) Using Liquid Nitrogen Is Germination of Sterile Oats (*Avena sterilis* Subsp. *sterilis*) and Allelopathic Effect on Seedling Growth

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Abstract

The purpose of this study is to determine the allelopathic effects of extracts obtained by using liquid nitrogen from the plant of Antep radish (*Raphanus sativus* L.) and little radish (*Raphanus sativus* L. var. *radikula*) on germination and seedling growth of sterile oats. The experiments were carried out in six groups with root, stem and root + stem mixed extracts of radish plants (Antep and little radish). A novel method that we developed based on the principle of liquid nitrogen crushing was used for extraction. According to this method, Antep radish and little radish plant parts were frozen with liquid nitrogen aid and then crushed to powder. These powders obtained were stored at −20˚C for nine months until the experiments were established. The aqueous solutions prepared at specific concentrations (0%, 1%, 2%, 4%, 8%, 16%) from the obtained radish powders were applied as 5 ml to per each petri dishes contain 10 seeds on Whatman No: 1 double layer filter paper. Experiments were carried out in six replicates for each concentration. The prepared petri dishes were kept in the incubator at 15˚C for 15 days. At the end of the experimental period, the number of germination of the seeds of sterile wild oat plant was evaluated by measuring the length of seedling root and stem. As a result, as the concentration of allelopathic solutions increased in all experimental groups, the number of germination and root and stem lengths of sterile wild oats decreased. Although allelopathic effects in all three experimental groups were observed in terms of germination number and seedling lengths,

the most significant effects were observed in the results of Antep radish root experiments. In this experiment group, the inhibition rate of root and stem length of weed seeds at a concentration of 16% were 86% and 82%, respectively, when compared to the control. In addition, the germination number was reduced by 74.36% in the seeds at the highest concentration compared to the control. As a result of the study, it was determined that samples of radish plant crushed with liquid nitrogen were stored for a long time and showed high allelopathic effect on sterile wild oats plant.

Subject Areas

Agricultural Science

Keywords

Allelopathy, *Avena sterilis*, Antep Radish, Liquid Nitrogen

1. Introduction

Allelopathic compounds called allelochemicals are released from plant tissues into the environment in various ways (root exudation, leaf leaching, volatilisation and residue decomposition) [1] [2]. The use of allelopathy in agriculture has become increasingly important to reduce the negative effects of herbicides on the environment, human and food health and to eliminate the problem of herbicide-resistance in plants [3].

Many members of Brassicaceae family were investigated for allelopathic effects on germination and seedling growth of plants [4] [5] [6] [7].

The species belonging to Brassicaceae commonly contain glucosinolates, the secondary metabolite group containing-sulfur. The level of glucosinolates produced varies according to plant species, tissue type, stage of development and environmental conditions [8] [9]. Glucosinolates are degraded with the myrosinase enzyme to the isothiocyanates (ITC's) responsible for the allelopathic effect. In addition to ITC's, various hydrolysis products of glucosinolates (nitriles, thiocyanates and oxazolidinethiones and organic cyanides) also occur [10] [11].

Raphanus sativus and *Raphanus sativus* L. var. *radikula* belong to Brassicaceae family are allelopathic cultivars that suppress the growth of weeds and a number of crops [12] [13] [14].

The purpose of this study is to determine the allelopathic effects of the extracts prepared by using liquid nitrogen from Antep radish (*Raphanus sativus* L.) and little radish (*Raphanus sativus* L. var. *radikula*) on germination and seedling growth of sterile oats (*Avena sterilis* subsp. *sterilis*).

2. Material & Methods

2.1. Plant Material

Antep and little radish were planted to be use as a donor plant in the Ege Un-

iversty Plant Protection Research Fields, İzmir, located in 38˚27' 15.5340 and 27˚13' 26.5008. Two radish cultivars (*Raphanus sativus* and *Raphanus sativus* L. var. *radikula*) that matured at the end of the growing season were harvested in May 2017. In addition, the sterile oat used as a test plant was collected from the Ege University campus. Identification of the collected plants was made by using "Flora of Turkey and the East Aegean Islands" [15].

2.2. Preparation of Aqueous Extracts and Experimental Design

The radish plants were washed with tap water and cut into separated parts for preparing root, shoot, mixture (root 50% + shoot 50%) extracts. Fresh tissues from each part were powdered aid of liquid nitrogen. The powder was diluted to give concentrations of 1, 2, 4, 8 and 16 $g \cdot kg^{-1}$ per 100 ml of distilled water. Prepared solutions were homogenized in a magnetic stirrer for five minutes at 1200 rpm and distributed as 2 ml to petri dishes containing 15 sterile oat seeds on two layers Whatman No: 1 filter paper. The seeds in control groups were moistened with distilled water. Experiments consisting of two groups, Antep radish and little radish, were conducted in a completely randomized design with four replications for each concentrate. The petri dishes kept in the incubator at 15˚C for 10 days to observe germination and seedling development of sterile oat seeds. At the end of the period, germinated seeds were counted, root and shoot lengths of germinated seeds were measured. A seed with 0.5 cm of root was considered germinated. The inhibition percentage was calculated using the following equation:

$$\text{Inhibition percentage} = \left[\left(\text{Control} - \text{Aqueous extract}\right)/\text{Control}\right] \times 100$$

2.3. Statistical Analysis

The data analysis was performed using one-way analysis of variance (ANOVA) in SPSS ver.16.0.Statistical differences ($p \leq 0.05$) between treatments were determined using Duncan's multiple range test.

3. Results and Discussion

3.1. Germination Percentage

All extracts obtained from different parts of Antep radish and little radish pronouncedly inhibited the seed germination of sterile oats at the highest concentration (16%). The degree of inhibition gradually increased with the concentration of Antep radish extracts. Among Antep Radish extracts, the most inhibitory effect was observed in root extracts, especially at 16% concentration with a reduction of 74.36% (**Figure 1**).

All extract applications of Antep radish were found to be more effective than little radish extracts on seed germination. Among the little radish extract applications at 16% concentration, it was determined that the most effective application was mixed extract (37.73%), while the least effective one was the stem

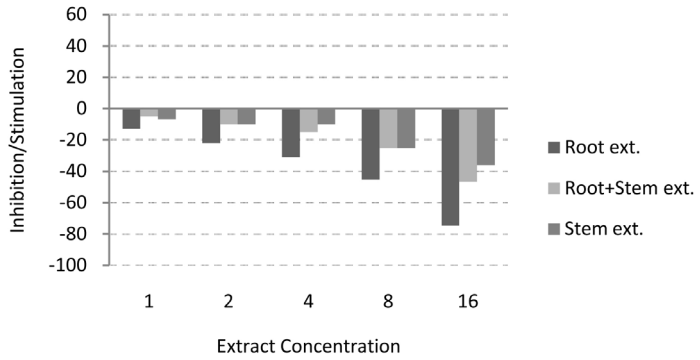

Figure 1. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of Antep radish on germination of sterile oat seeds.

extract application (26.32%). However, low concentrations of the little radish stem (1%) and mixture extracts (1% - 2%) stimulated the germination of sterile oats (**Figure 2**).

Both Antep radish and little radish applications showed statistically significant differences between concentrations ($p < 0.05$) (**Table 1**).

3.2. Shoot Elongation

The effects of extracts obtained from different parts of antep radish and little radish on shoot elongation of sterile oat are shown in **Figure 3** and **Figure 4**. All the extracts of Antep radish significantly reduced the shoot elongation at concentrations of 8% and 16% ($p < 0.05$) (**Table 2**).

Several studies have shown that extracts from different tissues of various allelopathic plants suppress the growth of test plants at different rates [16]. These findings were supported by the results of this study. It was observed that the root extract of Antep radish, which has the most significant effect, decreased the shoot elongation of the seeds at 16% concentration by 82.84%. However, the stem extract at the same concentration reduced the shoot elongation by 52.14%. It has been shown in previous studies that aqueous allelopathic solutions at low concentration may have a stimulatory effect. Similarly in this study, Antep radish stem extract at low concentrations (1% and 4%) stimulated the shoot elongation. The degree of inhibition gradually increased with the concentration of Antep radish extracts (**Figure 3**).

The effects of little radish extracts on shoot elongation varied at different concentrations. At 16% concentration, stem extract of little radish showed the most effective result (70.84%) on shoot elongation, while root extracts showed the lowest effect (30.26%). At 1% and 2% concentrations, mixture and stem extracts of little radish showed the stimulatory effect on shoot elongation (**Figure 4**).

The effect of the extracts of different parts of the Antep radish on the shoot elongation was in the following order: root > mixture > stem. However, the effect of little radish extracts is opposite; stem > mixture > root.

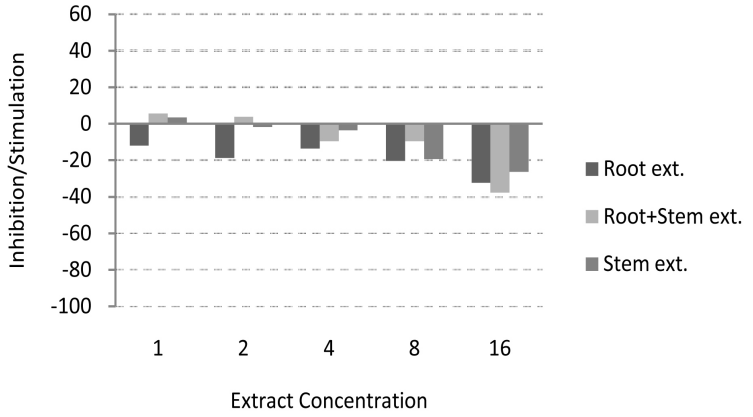

Figure 2. Effects of extracts (%) from different parts (root, stem and root + stem extracts) of little radish on germination of sterile oat seeds.

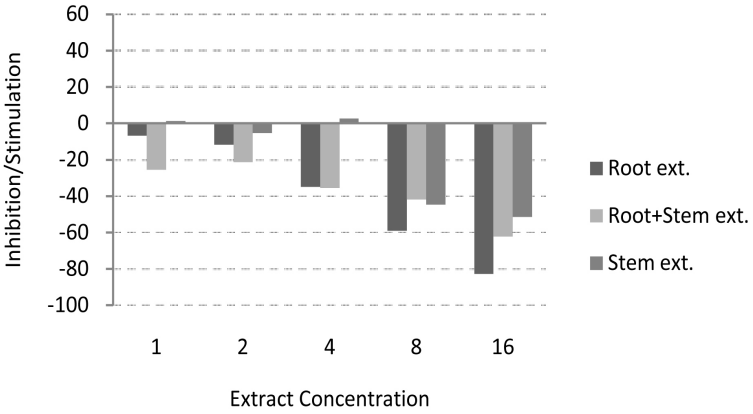

Figure 3. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of Antep radish on shoot elongation of sterile oat seeds.

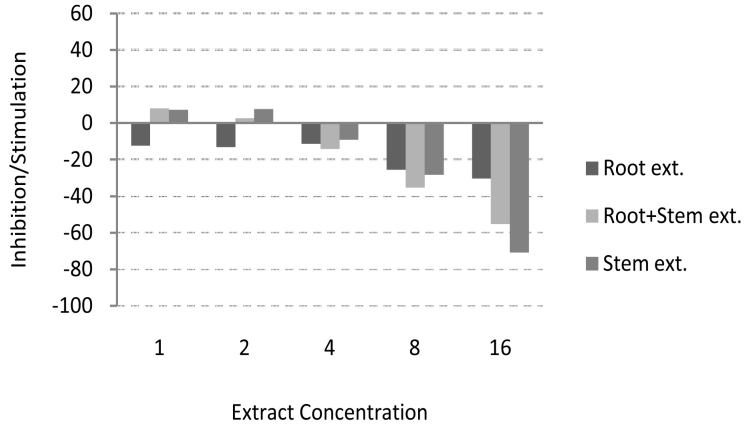

Figure 4. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of little radish on shoot elongation of sterile oat seeds.

3.3. Root Elongation

Both cultivars of radish significantly ($p < 0.05$) reduced the root elongation of sterile oat, especially at 16% concentration (**Table 3**).

Table 1. Allelopathic effect of extracts of Antep radish and little radish on germination of sterile oat seeds.

Germination Rate (%) ± SE*						
Ext. Conc. (%)	Antep radish			Little radish		
	Root ext.	Stem ext.	Root + Stem ext.	Root ext.	Stem ext.	Root + Stem ext.
0	$91^{d} \pm 3.07$	$100^{c} \pm 0$	$100^{c} \pm 0$	$98.33^{b} \pm 1.67$	$95.00^{b} \pm 3.41$	$88.33^{b} \pm 4.77$
1	$80^{cd} \pm 5.77$	$93.33^{c} \pm 3.33$	$95^{c} \pm 3.42$	$86.67^{ab} \pm 4.21$	$98.33^{b} \pm 1.67$	$93.33^{b} \pm 2.11$
2	$71.67^{bc} \pm 4.77$	$93.33^{c} \pm 3.33$	$90^{bc} \pm 6.32$	$80^{ab} \pm 5.77$	$93.33^{ab} \pm 3.33$	$91.67^{b} \pm 4.77$
4	$63.33^{bc} \pm 12.82$	$90^{bc} \pm 5.16$	$85^{bc} \pm 6.71$	$85^{ab} \pm 6.19$	$78.33^{ab} \pm 13.76$	$80^{b} \pm 6.83$
8	$50^{b} \pm 11.83$	$75^{ab} \pm 4.28$	$75^{b} \pm 5.63$	$78.33^{ab} \pm 5.43$	$76.67^{ab} \pm 9.88$	$80^{b} \pm 8.16$
16	$23.33^{a} \pm 10.22$	$63.33^{a} \pm 10.54$	$53.33^{a} \pm 9.55$	$66.67^{a} \pm 11.16$	$70.00^{a} \pm 6.83$	$55^{a} \pm 8.47$

*Means followed by different letters in the same column are significantly different from each other at 5% probability according to Duncan Test (SE: Standard Error).

Table 2. Allelopathic effect of extracts of Antep radish and little radish on shoot elongation of sterile oat.

Shoot elongation (cm) ± SE*						
Ext. Conc. (%)	Antep radish			Little radish		
	Root ext.	Stem ext.	Root + Stem ext.	Root ext.	Stem ext.	Root + Stem ext.
0	$10.72^{d} \pm 0.74$	$15.13^{b} \pm 1.46$	$15.81^{c} \pm 0.99$	$15.53^{b} \pm 0.84$	$13.79^{c} \pm 0.75$	$12.35^{c} \pm 0.76$
1	$10.01^{cd} \pm 0.70$	$15.32^{b} \pm 1.54$	$11.78^{b} \pm 1.18$	$13.62^{ab} \pm 0.81$	$14.79^{c} \pm 0.79$	$13.34^{c} \pm 0.70$
2	$9.46^{cd} \pm 1.11$	$14.32^{b} \pm 0.65$	$12.45^{b} \pm 1.33$	$13.49^{ab} \pm 1.09$	$14.84^{c} \pm 0.48$	$12.67^{c} \pm 0.70$
4	$6.98^{bc} \pm 1.38$	$15.54^{b} \pm 1.92$	$10.19^{b} \pm 1.05$	$13.78^{ab} \pm 0.86$	$12.53^{c} \pm 0.54$	$10.60^{bc} \pm 1.09$
8	$4.40^{ab} \pm 1.34$	$8.38^{a} \pm 0.91$	$9.19^{b} \pm 0.81$	$11.56^{a} \pm 1.14$	$9.88^{b} \pm 1.25$	$8.00^{ab} \pm 1.55$
16	$1.84^{a} \pm 0.99$	$7.24^{a} \pm 1.57$	$5.96^{a} \pm 1.14$	$10.83^{a} \pm 1.57$	$4.02^{a} \pm 0.80$	$5.52^{a} \pm 0.80$

*Means followed by different letters in the same column are significantly different from each other at 5% probability according to Duncan Test (SE: Standard Error).

Table 3. Allelopathic effect of extracts of Antep radish and little radish on root elongation of sterile oat.

Root elongation (cm)±SE*						
Ext. Conc. (%)	Antep radish			Little radish		
	Root ext.	Stem ext.	Root + Stem ext.	Root ext.	Stem ext.	Root + Stem ext.
0	$10.57^{d} \pm 0.39$	$13.80^{b} \pm 1.38$	$15.58^{d} \pm 0.92$	$12.11^{c} \pm 0.63$	$9.38^{c} \pm 0.48$	$10.16^{c} \pm 0.41$
1	$8.82^{d} \pm 0.88$	$13.86^{b} \pm 1.45$	$11.52^{c} \pm 1.00$	$8.09^{b} \pm 0.51$	$9.30^{c} \pm 0.23$	$8.64^{c} \pm 0.77$
2	$7.92^{cd} \pm 0.67$	$13.26^{b} \pm 0.63$	$11.17^{c} \pm 1.25$	$8.5^{b} \pm 0.61$	$8.75^{c} \pm 0.64$	$9.85^{c} \pm 0.81$
4	$5.82^{bc} \pm 1.28$	$12.22^{b} \pm 1.12$	$9.35^{bc} \pm 1.17$	$8.61^{b} \pm 1.43$	$9.96^{c} \pm 0.38$	$6.65^{b} \pm 0.48$
8	$3.87^{ab} \pm 1.02$	$7.77^{a} \pm 0.76$	$6.85^{ab} \pm 0.72$	$7.97^{b} \pm 0.81$	$6.14^{b} \pm 0.83$	$6.24^{b} \pm 1.02$
16	$1.44^{a} \pm 0.81$	$6.06^{a} \pm 1.18$	$4.51^{a} \pm 0.81$	$5.28^{a} \pm 0.76$	$2.98^{a} \pm 0.44$	$3.11^{a} \pm 0.36$

*Means followed by different letters in the same column are significantly different from each other at 5% probability according to Duncan Test (SE: Standard Error).

Root extract of Antep radish prominently reduced root elongation of sterile oat at all concentrations except 1% and 2%. High inhibitory effects were exhibited particularly in 8% and 16% concentration by 63.38% and 86.37% respectively (Figure 5).

Root elongation of sterile was affected approximately similar rates by all littler a dish extracts prepared at 8% concentration. At the highest concentration, the root extract was found to suppress the root elongation (56.40%) at a lesser rate than the stem (68.23%) and mixed extracts (69.39%) (Figure 6).

These results are supported by the finding of Uremıs *et al*. (2009), who reported that extracts of Antep radish and little radish had an inhibitory effect on the germination and seedling length of sterile oat [17]. However, when the findings are compared, it is clear that the extraction method used in our study gives more effective results than the other methods and the plant parts exhibit different allelopathic effects.

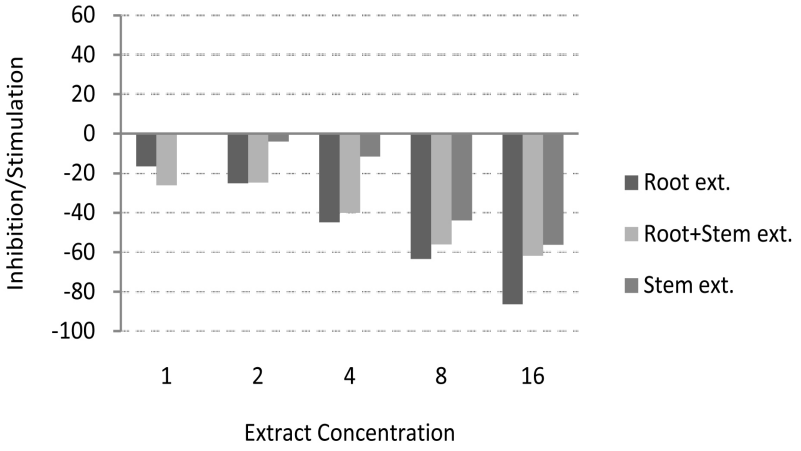

Figure 5. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of Antep radish on root elongation of sterile oat seeds.

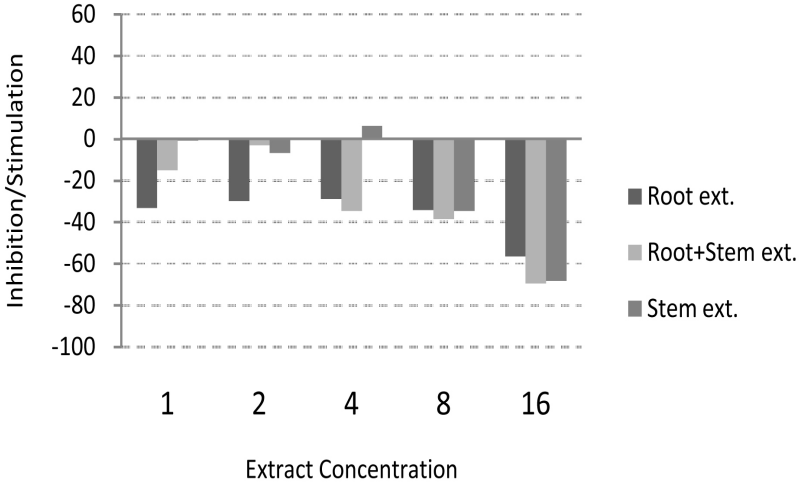

Figure 6. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of little radish on root elongation of sterile oat seeds.

4. Conclusion

In the study, it has shown that aqueous extracts prepared from radish cultivars have inhibitory effects on seed germination and seedling growth of wild oats. It has also emphasized that the use of liquid nitrogen as an extraction method can give effective results. We recommend that this study shed light on future studies for the production of natural herbicides from allelopathic plants and the results obtained from this study should be supported with field studies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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