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Effects of Aqueous Extracts Obtained from Two Radish Cultivars (Raphanus sativus L. and Raphanus sativus L. var. radikula) Using Liquid Nitrogen Is Germination of Barnyard Grass (Echinochloa crus-galli) and Allelopathic Effect on Seedling Growth

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Abstract
This study was conducted to evaluate the allelopathic effects of extracts obtained from different parts (root, stem and mixed) of radish varieties (*Raphanus sativus* L. and *Raphanus sativus* L. var *radicula*) on germination and seedling growth of barnyard grass as well as the identification of the phytotoxic chemicals responsible for allelopathic activity. During the extraction process, the plant parts of the radish varieties were frozen with the help of liquid nitrogen and then, crushed and powdered. Aqueous solutions prepared with certain doses of radish powders (0%, 1%, 2%, 4%, 8%, 16%) were applied to weed seeds. The seeds were kept in an incubator at 25 °C for 15 days. At the end of the period, the germination percentage of the weed seeds was determined by measuring the length of the root and stem. The results indicated that the allelopathic potential of radish on barnyard grass varied depending on the varieties, plant parts and extract concentrations tested. Among the test parameters, the highest allelopathic effects were obtained from the root elongation of barnyard grass. Stem extracts of little radish were the most inhibitory for all test parameters. As a result of GC-MS analysis of little radish stem extract, the presence of many bioactive compounds that may be the source of the allelopathic effect has been revealed. Overall, these results suggest that little radish could be used in bioherbicide development.
Keywords: liquid nitrogen, allelopathy, turnip, barnyard grass, germination, elongation,
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1. Introduction

Allelopathy, which plays an important role in plant-plant interaction, is a phenomenon mediated by secondary metabolites called allelochemicals. Allelochemicals released from different organs of a plant can inhibit the germination or growth of plants of the same or different species (Rice 1984; Putnam 1985; Macias *et al*. 2003; Inderjit *et al*. 2008).

The increased use of herbicides in weed control leads to the development of herbicide resistance and many problems in areas such as food safety, human and animal health, and pollution. Various strategies are being developed to reduce the use of herbicides in agricultural systems (Xuan *et al*. 2004). Allelochemicals, which have a mode of action similar to herbicides, are considered as an environmentally friendly alternative to synthetic herbicides (Soltys *et al*. 2013).

The species belonging to the Brassicaceae family mainly contain glucosinolates and their hydrolysis products (nitriles, isothiocyanates, thiocyanates, oxazoliolines, and epithionitriles), phenolic acids and many allochemicals (Carvalho *et al*. 2008; Jafariehyazdi & Javidfar 2011; Al-Sherif *et al*. 2013). Garden radish (*Raphanus sativus* L.) and little radish (*R. sativus*. *radicula* L.), belong to the Brassicaceae family, has been reported to have an allelopathic effect on the germination growth of many weeds and crop plants (Arslan *et al*. 2005; Uremis *et al*. 2009; De Moraes Gomes *et al*. 2017). It is known that some biologically active compounds in the structure of these plants have antioxidant, antimutagenic, antimicrobial and anticarcinogenic effects (Tierens *et al*. 2001; Barbieri *et al*. 2008; Jahangir *et al*. 2009) However, there are many studies on the herbicidal effects of some glucosinolates, which are commonly found in radish species and their degradation products such as isothiocyanates (Brown & Morra, 1997; Al-Khatib *et al*. 1997; Petersen *et al*. 2001; . El-Wakeel *et al*. 2019).

The main objectives of the current study were to determine the allelopathic effects of the extracts obtained with liquid nitrogen from different parts (stem, root, root and stem mixture) of two radish varieties (*R. sativus* and *R. sativus*. *radicula*) on germination and seedling growth of barnyard grass (*Echinochola-cruss galli*) and the detection of allelochemicals that may be the cause of this effect by GC-MS analysis.

2. Material and Method

2.1 Plant Material

Two radish varieties (*R*. *sativus* and *R*. *sativus*.*radicula*) were cultivated to be used as donor plant in the Plant

Protection Research Fields of Ege University located in 38˚27' 11"N and 27˚13' 35" E, İzmir, Turkey in March 2017. The maturing radishes were harvested at the end of the growing season (March-June). Barnyard grass 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

2.2 Preparation of Plant Extracts and Bioassays

After the radishes were taken to the laboratory, they were washed with tap water and separated into their roots and stems. The separated plant parts were cut into smaller pieces for the preparation of root, shoot and mix (root 50%+stem 50%) extracts. Fresh plant pieces from each part were crushed in a mortar with the aid of liquid nitrogen and powdered. To prepare fresh aqueous extracts at five concentrations, 1, 2, 4, 8 and 16 g of each powder sample (root, shoot and mixture) were taken and soaked in 100 ml each for 12 hours and distilled water was used as control. The prepared solutions were stirred in a magnetic stirrer for five minutes at 1200 rpm at 24^{0}C. The extracts were homogenized by filtering through a double layer of cheesecloth to remove plant residues. Homogenized aqueous extracts were distributed as 2 ml in petri dishes containing 15 barnyard grass seeds on Whatman No: 1 filter paper in two layers. The experiments were conducted in a completely randomized design with four replications for each treatment. All petri dishes were wrapped with parafilm to prevent contamination. The petri dishes were kept in the incubator at 25˚C in darkness for 15 days to evaluate germination and seedling development of barnyardgrass. After a 15-day incubation period, the germinated seeds were counted, the root and shoot lengths of the seeds were measured and any seed with a root length of more than 0.5 cm was considered germinated. The percentage of inhibition/ stimulation was calculated using the following equation:

Inhibition / stimulation percentage = [(Control - Aqueous extract)/Control × 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.

2.4 GC/MS Analysis

The chemical analysis of two radish species, made from different parts, were carried out with the GC / MS device. 10 g of the radish sample powdered with liquid nitrogen was taken out and transferred to a 50 ml centrifuge tube and 10 ml of acetonitrile was added to it and shaken for 1 minute. 4 g of magnesium sulfate ($MgSO_4$), 1 g of sodium chloride NaCl, 1 g of trisodium citrate ($Na_3C_6H_5O_7$), 0.5 g of disodium citrate sesquihydrate ($C_{12}H_{18}Na_4O_{17}$) were added to the mixture and the tube was mixed again for 1 minute and then it was centrifuged at 3000 rpm for 5 minutes. It was taken 1 ml from the formed phase at the top of the tube, mixed with 150 mg MgSO4 and 50 mg PSA (primary secondary amine) for 1 min and centrifuged again for 5 minutes at 3000 rpm. After centrifugation, 0.5 ml was taken from the upper phase into a vial and transferred to the GC-MS device.

GC-MS (Agilent GC6890 N, 5973 MSD) device with HP-SMS column (length: 30 m, diameter: 0.25 mm and film thickness: 0.25 µm fixed phase: 5% phenyl-methyl polysiloxane) was used to determine the content of the prepared plant extracts. Helium gas with flow rate of 2 mL / min was used as the carrier gas. The injection volume of 0.5 µI was employed (split ratio of 10:1) and injection temperature is set at 250 ^{0}C, MS transfer line is set at 280 ^{0}C. The oven temperature of the device was initially set to 50 ^{0}C and kept at this level for 0.75 minutes, then it was gradually increased to 280 ^{0}C. After a waiting time of 15 minutes at 280^{0}C, the final temperature was raised to 290 ^{0}C. Total running time of the GC is 20 minutes. The NIST02 (National Institute of Standards and Technology), having more than 62,000 patterns, the database has been utilized in the identification of the components.

3. Results and Discussion

3.1 Germination Percentage

The effect of radish extracts obtained from radish varieties on the germination of barnyard grass seeds varies depending on the part of the plant from which the extract is obtained and the concentration. All doses of garden radish extracts excluding 1 g 100 ml^{-1} concentration of mixed extract negatively affected germination of barnyard grass. The highest negative effect (27,44%) observed on the germination of the barnyard grass seeds was achieved by the 16 g 100 ml^{-1} concentration of the mixed extract, while the lowest negative effect was taken from the seeds in the root extract at the concentration of 1 g 100 ml^{-1} (Figure 1.).

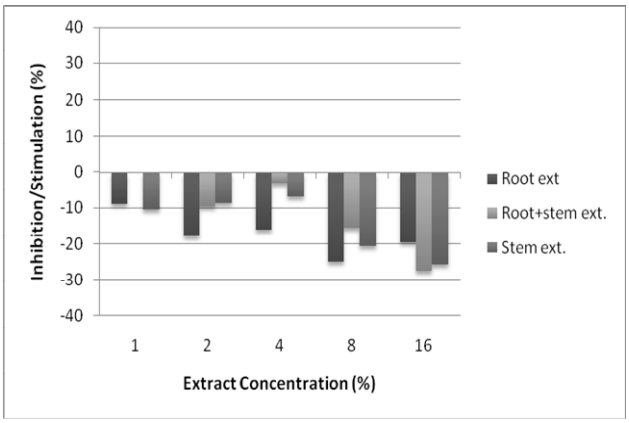

Figure 1. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of garden radish on the germination of barnyard grass seeds.

When examining the effects of little radish on the seed germination of barnyard grass, it was found that all extracts negatively affected germination at high concentrations (8 g 100 ml-1 and 16 g 100 ml-1). The degree of inhibition differs depending on the concentrations of the extracts. It was observed that germination was stimulated at low concentrations. The increased rate of germination was found to be 4.88% by 1 g 100 ml-1 concentration of stem extract, while it was 2.08% by root extract at 4 g 100 ml-1 concentration. Compared to the control, the highest negative effects on germination were achieved at almost the same rate (65%) as mixed and stem extracts at a concentration of 16 g 100 ml-1 (Figure 2.).

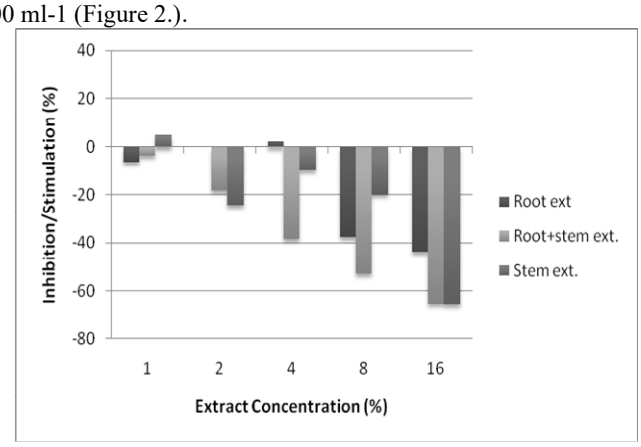

Figure 2. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of little radish on the germination of barnyard grass seeds.

In experiments established with both radish varieties, It was found that the effect of root extract was less than that of other extract applications. It was also found that the little radish has a higher negative effect on germination than the garden radish. Both garden radish and little radish applications showed statistically significant differences between the concentrations ($p < 0.05$) (Table 1).

Table 1. Allelopathic effect of extracts of Antep radish and little radish on germination of barnyard grass seeds.

Ext. Conc. (%)	Germination Rate (%) ± SE*					
	Graden Radish			Little Radish		
	Root ext.	Stem ext.	Root+Stem ext.	Root ext.	Stem ext.	Root+Stem ext.
0 (H_2O)	$93,33^{b}$±4,22	$96,67^{d}$±2,10	85^{b} ±5	80^{b} ±5,16	$68,33^{b}$ ±6,54	$91,67^{c}$ ±4,01
1	85^{ab}±6,19	$86,67^{bcd}$ ±4,94	85^{b} ±4,28	75^{b}±5,63	$71,67^{b}$ ±7,92	$88,33^{c}$ ±4,77
2	$76,67^{ab}$±5,58	$81,67^{abc}$±3,07	$76,67^{ab}$±9,55	80^{b}±7,30	$51,67^{b}$±9,80	$75,0^{c}$±4,28
4	$78,33^{ab}$±8,33	90^{cd}±4,47	$81,67^{ab}$±5,43	$81,67^{b}$±3,07	$61,67^{b}$±4,77	$56,67^{b}$ ±8,82
8	70^{a}±7,30	$76,67^{ab}$±4,22	$71,67^{ab}$ ±8,33	50^{a}±10,65	55^{b}±6,19	$43,33^{ab}$±7,15
16	75^{ab}±6,70	$71,67^{a}$±4,77	$61,67^{a}$±7,92	45^{a}±8,06	$23,33^{a}$ ±4,21	$31,67^{a}$ ±7,03

*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).

It is observed that the root development of the barnyard grass was reduced by extracts from garden radish in

all concentrations. The significant decrease in root length occurred at concentrations of 8 g 100 ml^{-1} and 16 g 100 ml^{-1} of the extracts. Among the garden radish applications, the highest negative effect compared to the control was observed with an average rate of 62% with germinated seeds in root and mixed extract at a concentration of 16 g 100 ml^{-1}. However, the lowest negative effect was found with 8.26% in seeds, which were found in 1 g 100 ml^{-1} of a concentration of stem extract (Figure 3.).

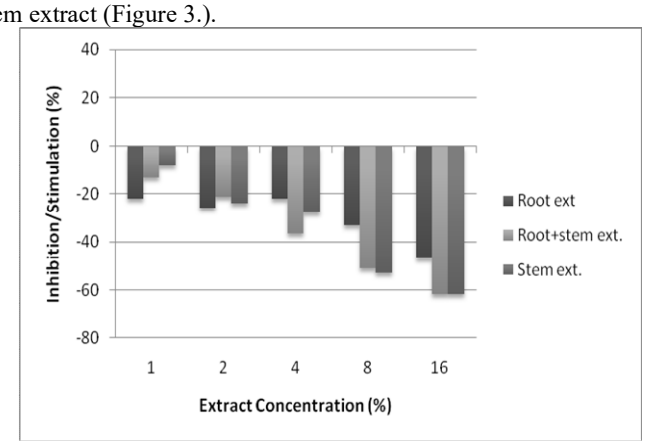

Figure 3. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of garden radish on root elongation of barnyard grass seeds.

Little radish has been found to have strong negative effects on root elongation of banyard grass. The rate of reduction in root length has increased drastically, especially from 4 g 100 ml^{-1} to 16 g 100 ml^{-1} concentration. It has been found that the most effective extract in root elongation is stem extract from little radish and inhibits root elongation by 96% at a concentration of 16 g 100 ml^{-1} . These results support studies that showing that the degree of inhibition correlates with concentration (Turk & Tawaha, 2003; Xuan *et al*. 2004; Geimadil *et al*. 2015; Rigon *et al*.2018).

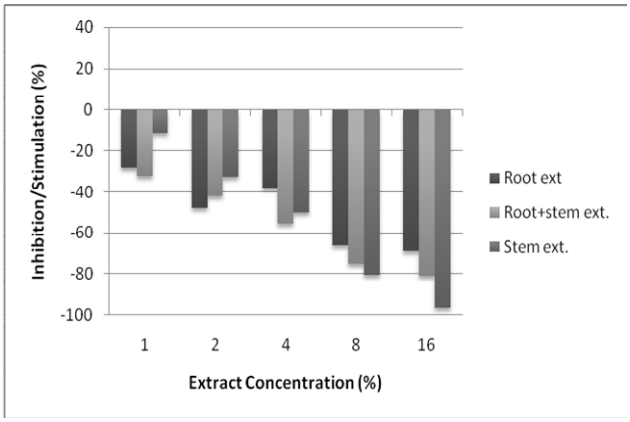

Figure 4. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of little radish on root elongation of barnyard grass seeds.

All extracts from varieties belonging to radish varieties have been found to have a significant effect on root elongation of barnyard grass, particularly at high concentrations (8 g 100 ml^{-1} and 16 g 100 ml^{-1}) and different statistical groups are formed among the concentrations. It has been found that little radish extracts have a greater effect on root elongation. In addition, the effect of the root extract was less effective in both radish applications than in the other extracts.

Table 2. Allelopathic effect of extracts of Antep radish and little radish on root elongation of barnyard grass seeds.

Root elongation (cm)±SE*						
Ext. Conc. (%)	Graden Radish			Little Radish		
	Root ext.	Stem ext.	Root+Stem ext.	Root ext.	Stem ext.	Root+Stem ext.
0 (H_2O)	$3,77^{c}$±0,22	$3,51^{c}$ ±0,32	$3,69^{c}$±0,51	$4,14^{c}$±0,37	$2,50^{d}$±0,38	$4,07^{d}$±0,32
1	$2,95^{b}$±0,19	$3,22^{bc}$ ±0,26	$3,20^{bc}$±0,20	$2,98^{b}$±0,30	$2,22^{cd}$±0,35	$2,76^{c}$±0,20
2	$2,80^{b}$±0,20	$2,68^{b}$ ±0,25	$2,91^{bc}$±0,32	$2,61^{b}$±0,19	$1,68^{bc}$±0,23	$2,36^{bc}$±0,12
4	$2,94^{b}$±0,25	$2,54^{b}$ ±0,16	$2,34^{ab}$ ±0,24	$2,56^{b}$±0,19	$1,26^{b}$±0,13	$1,83^{b}$±0,40
8	$2,53^{ab}$ ±0,32	$1,66^{a}$ ±0,23	$1,81^{a}$±0,26	$1,41^{a}$±0,37	$0,49^{a}$±0,12	$1,0^{a}$±0,20
16	2^{a}±0,21	$1,34^{a}$ ±0,22	$1,41^{a}$±0,27	$1,30^{a}$±0,29	$0,1^{a}$±0,05	$0,78^{a}$±0,22

*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).

The effects of extracts obtained from different parts of the garden radish on the seedling growth of the barnyard grass differ depending on the type and concentrations of the extract. The shoot elongation was stimulated by the root and mixed extracts of the garden radish at concentrations less than 8 g 100 ml-1. The results show parallels to previous studies in which allelochemicals at low concentrations promoted plant growth (Narwal 1994; Santos 2002; De Moraes Gomes *et al*. 2017; Sisek *et al*. 2019). However, it was found that stem extract had a negative effect on shoot elongation in all concentrations. The effect of the stem extract gradually increased in parallel with the increasing concentration. At the highest concentration, the stem extract which was found to be most effective decreased the shoot elongation of the banyard grass by 32.6%.

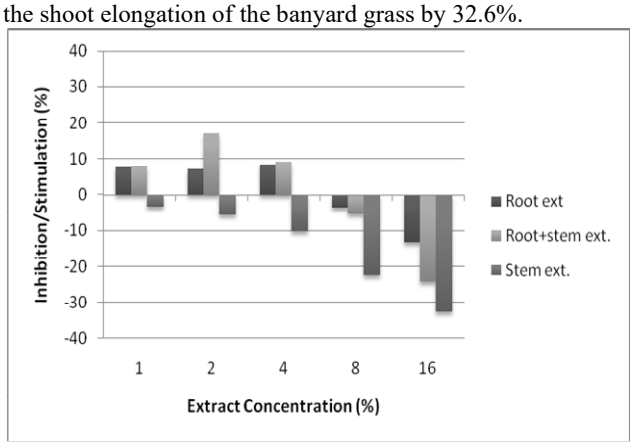

Figure 5. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of garden radish on shoot elongation of barnyard grass seeds.

The extracts from little radish have been found to have a strong inhibitory effect on the shoot devolopment of the barnyard grass seeds. Although the stem and mixed extracts stimulated shoot elongation up to a concentration of 4 g 100 ml^{-1}, all extracts had a negative effect at the higher concentrations. It was observed that the stem extract of little radish, which has the most significant effect, decreased the shoot elongation by 76% at 16 g 100 ml^{-1} concentration. Root and mixed extracts in the same concentration reduced shoot elongation by 58% and 70%, respectively, compared to the control.

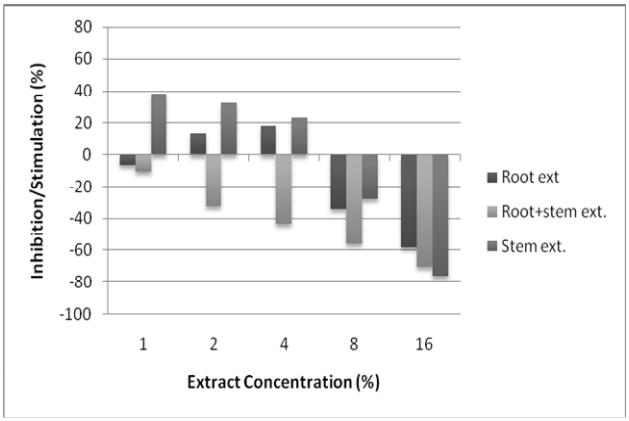

Figure 6. Effects (%) of extracts from different parts (root, stem and root + stem extracts) of little radish on shoot elongation of barnyard grass seeds.

it was observed that the stem and mixed extract were more effective than the root extract among all extract applications. It was found that the shoot elongation of barnyard grass was affected by little radish extracts more than garden radish extracts. While all the extract applications of little radish at the highest concentration significantly decreased the shoot elongation compared to the control, the effect of garden radish root and mixed extract was found statistically insignificant.

Table 3. Allelopathic effect of extracts of Antep radish and little radish on shoot elongation of barnyard grass seeds.

Shoot elongation (cm)±SE*						
Ext. Conc. (%)	Graden Radish			Little Radish		
	Root ext.	Stem ext.	Root+Stem ext.	Root ext.	Stem ext.	Root+Stem ext.
0 (H_2O)	6,90^{a}±1,11	10,92 ^{c} ±0,48	5,52^{a}±0,69	7,12 ^{c}±0,46	3,94b^{c}±0,42	8,90^{d}±0,74
1	7,43^{a} ±1,00	10,57 ^{bc} ±,087	5,95^{a}±0,59	6,66 ^{bc}±0,60	5,44^{c}±0,49	7,94^{cd}±0,87
2	7,39^{a} ±1,14	10,30 ^{bc} ±0,15	4,57^{a}±0,92	8,08 ^{c}±1,05	5,22^{c}±1	6^{bc}±0,72
4	7,48^{a} ±1,15	9,81 ^{bc} ±0,72	6,03^{a}±0,68	8,39 ^{c}±0,11	4,86^{c}±0,56	5,06^{b}±0,90
8	6,66^{a} ±0,98	8,46 ^{ab} ±0,58	5,22^{a}±0,56	4,69 ^{ab}±1,03	2,84^{b}±0,44	3,90^{ab}±0,86
16	5,99^{a} ±0,82	7,36 ^{a} ±1,07	4,18^{a}±0,60	2,98 ^{a}±0,71	0,94^{a}±0,24	2,67^{a}±0,54

*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).

Many studies have illustrated that aqueous extracts from different tissues of a plant show different allelopathic effects on the test plants (Turk & Tawaha 2002; Sodaeizadeh *et al*. 2009; Sisek *et al*. 2019). Similarly, the current study has shown that the allelopathic response of the barnyard grass to radish plants varies according to the plant tissue from which the extract is prepared.

In all the parameters tested, it was determined that the barnyard grass was less affected by root extract applications. In previous studies, leaf extracts were found to have a higher inhibitory effect compared to root extracts (Turk & Tawaha 2002; Sodaeizadeh *et al*. 2009). In the current study, leaf extract was not used alone. However, since the leaves are included in the root extract, results similar to previous studies have been obtained.

The allelopathic content of the plant changes depending on the environmental conditions, the plant part, the development stage as well as the variety (Kobayashi 2004). Our findings were emphatically proved that, different varieties of the Brassicaceae family have varying effects on the tested plant. Similarly, previous studies have found that different Brassicaceae plants inhibit the germination and growth of many weeds at different rates. Uremis *et al*. (2005) reported that among 6 Brassicaceae species, garden radish is the most effective species on the root length of the ground cherry. Uremis (2009) also found that black radish is the most effective on johnsongrass sprouting under laboratory. In the current study, both germination and seedling growth of the barnyard grass was found to be most affected by little radish extracts. The reduction in the root and shoot length of the seedlings can be attributed to the decreased rate of cell division and elongation due to the presence of allelochemicals in the aqueous extracts (Bukolova 1971).

3.2 GC/MS Analysis

GC-MS analysis was carried out to determine the phytochemical composition of little radish stem extract. List of the identified phytochemicals of little radish the stem extract and their retention time (RT), molecular weight (MW), molecular Formula and peak area (%PA), are shown in Table 4.

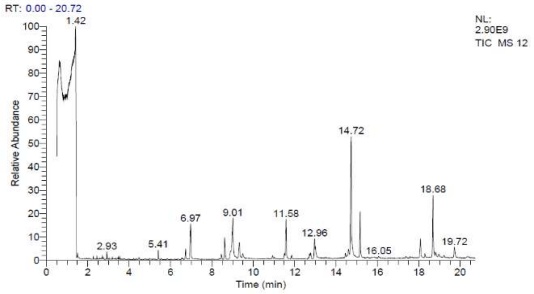

Figure 7. GC-MS chromatograph for stem extract of little radish

Table 4. Major phytocomponents identified in little radish stem extract by GC-MS.

N	RT	Bileşik Adı	Mol. Form.	MA	%PA

1	2,93	2-(1,2-Epoxy-2-methylcyclohexyl)-4-phenyl-3 -butyn-2-ol	$C_{17}H_{20}O_2$,	256	0,552
2	5,41	Pluchidiol	$C_{13}H_{20}O_2$	208	1,198
3	6,97	Hexadecanoic acid (CAS) (Palmitic acid)	$C_{16}H_{32}O_2$	256	5,662
4	9,01	Ethyl lınoleolate	$C_{20}H_{36}O_2$	308	11,279
5	11,58	9-Octadecenamide (CAS)	$C_{18}H_{35}NO$	281	6,903
6	12,96	Octadecanoic acid, 2-hydroxy-1,3-propanediylester (CAS)	$C_{39}H_{76}O_5$,	624	4,802
7	14,72	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (CAS)	$C_{21}H_{42}O_4$	358	20,283
8	16,05	Oleic acid, eicosyl ester (CAS)	$C_{38}H_{74}O_2$	562	0,309
9	18,68	Stıgmast-5-en-3-ol, (3á,24S)-	$C_{29}H_{50}O$	414	11,202
10	19,72	4,5α-Epoxy-3-methoxy-17-methyl-7à-(4-phenyl-1,3-butadienyl)-6á,7á-(oxymethylene)morphinan	$C_{29}H_{31}NO_3$	441	2,553

Analysis of the peaks by mass spectroscopy revealed that the ten major compounds in the stem of little radish extract (Figure 7 and Table 4). The high concentration of compounds consists of fatty acids (Palmitic acid (5,66%), 9-Octadecenamide (6,90%)) their esters (Ethyl lınoleolate (11,27%), Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (20,28%), Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (4,80%) Oleic acid, eicosyl ester (0,31%)) and steroids (Stıgmast-5-en-3-ol (11,20%)) (Table 4). Comparative concentrations of the components (PA%) were calculated by the GC-MS analyzer based on peak heights (Table 4).

Table 5. Other phytocomponents identified in little radish stem extract by GC-MS.

N	RT	Bileşik Adı	Mol. Form.	MA	%PA
1	2,19	2,7-Diphenyl-1,6-dioxopyridazino[4,5-2',3']pyrrolo[4',5'-D]pyridazine	$C_{20}H_{13}N_5O_2$	355	0,011
2	2,27	Phenol, 3-ethyl- (CAS)	$C_8H_{10}O$	122	0,207
3	2,44	2,3-Dıhydro-benzofuran	C_8H_8O	120	0,233
4	2,58	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	0,089
5	2,70	Phenol, 2-methyl-5-(1-methylethyl)- (CAS)	$C_{10}H_{14}O$	150	0,413
6	2,76	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0,176
7	3,19	Desulphoglucoerucin	$C_{12}H_{23}NO_6S_2$	341	0,119
8	3,54	2-tert-Butyl-4-isopropyl-5-methylphenol	$C_{14}H_{22}O$	206	0,231
9	3,95	2-Amino-4-chlorophenol	$C_{16}H_{24}O_4$	280	0,090
10	4,13	2-(6-[1-Ethyl-4-[4-(1h-pyrrole-2-carb onyl)-2,3,3a,4,5,7a-hexahydro-1H-ınınden-5-yl]-buta-1,3-dıenyl]-5-methyl-tetrahydro-pyran-2-yl)-propıonıc acıd DEN-5-YL]-BUTA-1,3-DIENYL]-5-METHY L-TETRAHYDRO-PYRAN-2-YL)-PROPIO NIC ACID	$C_{29}H_{39}NO_4$	465	0,002
11	4,34	3-oxo-à-ionol	$C_{13}H_{20}O_2$	208	0,114
12	4,48	6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol	$C_{13}H_{22}O_3$	226	0,298
13	4,58	Spiro[7H-cyclohepta[b]furan-7,2'(5'H)-furan]-2,5'(3H)-dione, octahydro-8-hydroxy-6,8-dimethyl-3-methylene-, [3aS-(3aà,6á,7à,8á,8aà)]- (CAS)	$C_{15}H_{20}O_5$	280	0,029
14	4,89	Oxiranepentanoic acid, 3-undecyl-, methyl ester, trans- (CAS)	$C_{19}H_{36}O_3$	312	0,073
15	4,98	Pseudojervine	$C_{33}H_{49}NO_8$	587	0,229
16	5,10	Dotriacontane (CAS)	$C_{32}H_{66}$	450,	0,026
17	5,15	Tetradecanoic acid (CAS) (Myristic acid)	$C_{14}H_{28}O_2$	228	0,167
18	5,35	1,25-Dıhydroxy vıtamın D2	$C_{28}H_{44}O_3$	428	0,002
19	5,55	Indole 4-carboxaldehyde	C_9H_7NO,	145,	0,137
20	5,74	1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo[f]quinazoline	$C_{26}H_{20}Cl_2N_2$	430	0,062
21	5,82	Ethanol, 2-(9-octadecenyloxy)-, (Z)- (CAS)	$C_{20}H_{40}O_2$	312	0,083
22	6,08	01297107001 Tetraneurın - a - dıol	$C_{15}H_{20}O_5$	280	0,024
23	6,42	Quercetın 7,3',4'-trımethoxy	$C_{18}H_{16}O_7$	344	0,048
24	6,54	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	0,230
25	6,61	Pentadecanoic acid, 14-methyl-, methyl ester (CAS)	$C_{17}H_{34}O_2$	270	0,143
26	7,11	Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS)	$C_{19}H_{38}O_4$	330	0,002

N	RT	Bileşik Adı	Mol. Form.	MA	%PA
27	7,59	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methylester (CAS)	$C_{25}H_{42}O_2$	374	0,034
28	7,86	Dı-2-benzothıazole dısulfane	$C_{14}H_8N_2S_4$,	332	0,027
29	8,31	Isochıapın B	$C_{19}H_{26}O_6$	350	0,002
30	8,46	9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)- (CAS)	$C_{19}H_{32}O_2$	292	0,888
31	8,62	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]- (CAS)	$C_{20}H_{40}O$,	296,	3,379
32	9.32	Octadecanoic acid (CAS)	$C_{18}H_{36}O_2$	284	3,254
33	9,49	Hexadecanamide (CAS)	$C_{16}H_{33}NO$	255	0,901
34	10,93	Hexadecanoic acid, 2-hydroxy-1,3-propanediylester (CAS)	$C_{35}H_{68}O_5$	568	0,610
35	11,43	6,9,12,15-Docosatetraenoic acid, methyl ester (CAS)	$C_{23}H_{38}O_2$	346	0,028
36	12,14	N,N-Bis(trimethylsilyl)-2-(2-thienyl)quinolin-4-amine	$C_{19}H_{26}N_2SSi_2$	370	0,085
37	12,37	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-(CAS)	$C_{12}H_{10}FN_5$	243	0,125
38	12,72	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- (CAS)	$C_{21}H_{36}O_4$	352	1,023
39	12,77	2-Monolınolenın	$C_{21}H_{36}O_4$	352	0,824
40	13,12	Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester (CAS)	$C_{35}H_{68}O_5$	568	0,005
41	14,45	Heptanoic acid, docosyl ester (CAS)	$C_{29}H_{58}O_2$	438	0,510
42	15,16	9-Octadecenamide, (Z)- (CAS)	$C_{18}H_{35}NO$	281	6,847
43	15,95	24,25-Dihydroxycholecalciferol	$C_{27}H_{44}O_3$	416	0,023
44	16,12	3-Hydroxy-N-(p-methoxyphenyl)-4-[(S)-2,2-dimethyl-1,3-dioxolan-4-yl]-3-[3,4-bis(methoxycarbonyl)phenyl]azetidin-2-one	$C_{25}H_{27}NO_9$	485,	0,147
45	16,25	1,3-Dimethyl-2,4-dioxo-6-(1-naphthoyl)-8-(pchlorophenyl)-1,2,3,4-tetrahydro[1.2.4]triazolo[3,4-f]purine	$C_{25}H_{17}ClN_6O_3$	484,	0,066
46	16,37	trans-N-Diphenylphosphinoyl-2-(á-trimethylsilyl)vinyl-3-(p-trifluoromethylphenyl)aziridine	$C_{26}H_{27}F_3NOPSi$	485	0,044
47	16,43	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0,005
48	16,83	4H-1-Benzopyran-4-one,2-(3,4-dimethoxyphenyl)-5,7-dihydroxy-(CAS)	$C_{17}H_{14}O_6$	314	0,032
49	17,03	2,7-Di-tert-Butyl-3,6-diphenylbiphenylene	$C_{32}H_{32}$	416	0,137
50	17,14	Dımethoxyglycerol docosyl ETHER	$C_{27}H_{56}O_5$,	460,	0,004
51	17,38	Cholest-5-en-3-ol (3á)- (CAS)	$C_{27}H_{46}O$,	386,	0,333
52	17.57	5,10-Dihexyl-5,10-diihydroindolo[3,2-b]indole-2,7-dicarbaldehyde	$C_{28}H_{34}N_2O_2$	430	0,230
53	17,65	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)- (CAS)	$C_{29}H_{50}O_2$	430	0,146
54	17,71	Lucenin 2	$C_{27}H_{30}O_{16}$	610	0,090
55	17,96	Rhodopin	$C_{40}H_{58}O$	554	0,026
56	18.07	(E)-5,10-secocholest-1(10)-en-3,5-dione	$C_{27}H_{44}O_2$	400,	3,443
57	18,29	Stigmasta-5,22-dien-3-ol, (3á,22E)- (CAS)	$C_{29}H_{48}O$	412,	0,713
58	18,82	Stigmasta-5,24(28)-dien-3-ol, (3á)- (CAS)	$C_{29}H_{48}O$	412	0,604
59	18,97	Cholest-5-en-3-one (CAS)	$C_{27}H_{44}O$	384	0,653
60	19,98	03027205002 Flavone 4'-oh,5-oh,7-dı-o-glucosıde	$C_{27}H_{30}O_{15}$	594,	0,049

Apart from major compounds, 60 compounds with low concentrations have been identified in the little radish stem extract by GC-MS (Table 5).

Table 6. Bioactive components and their chamical classes identified in little radish stem extract by GC-MS

Name of substance	Mol. Form.	Chemical classification
Desulphosinigrin	$C_{10}H_{17}NO_6S$	Glucosinolate

Name of substance	Mol. Form.	Chemical classification
Desulphoglucoerucin	$C_{12}H_{23}NO_6S_2$	Glucosinolate
Phenol, 3-ethyl- (CAS)	$C_8H_{10}O$	Phenol
Phenol, 2-methyl-5-(1-methylethyl)- (CAS) (Carvacrol)	$C_{10}H_{14}O$	Phenol
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	Phenol
2-tert-Butyl-4-isopropyl-5-methylphenol	$C_{14}H_{22}O$	Phenol
2-Amino-4-chlorophenol	$C_{16}H_{24}O_4$	Phenol
Quercetın 7,3',4'-trımethoxy	$C_{18}H_{16}O_7$	Flavonoid
4H-1-Benzopyran-4-one,2-(3,4-dimethoxyphenyl)-5,7-dihydroxy-(CAS) (Kaempferol)	$C_{17}H_{14}O_6$	Flavonoid
Lucenin 2	$C_{27}H_{30}O_{16}$	Flavonoid
03027205002 Flavone 4'-oh,5-oh,7-dı-o-glucosıde	$C_{27}H_{30}O_{15}$	Flavonoid
3-oxo-à-ionol	$C_{13}H_{20}O_2$	Ketone
7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	Ketone
Pseudojervine	$C_{33}H_{49}NO_8$	Alkoloid
4,5α-Epoxy-3-methoxy-17-methyl-7à-(4-phenyl-1,3-butadienyl)-6á,7á-(oxymethylene)morphinan	$C_{29}H_{31}NO_3$	Alkoloid
1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo[f]quinazoline	$C_{26}H_{20}Cl_2N2$	Alkoloid
1,25-Dıhydroxy vıtamın D2	$C_{28}H_{44}O_3$	Vitamin
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]- (Phytol)	$C_{20}H_{40}O$,	Terpene
Rhodopin	$C_{40}H_{58}O$	Terpene
Octadecanoic acid (CAS) (Steraic acid)	$C_{18}H_{36}O_2$	Fatty acid
Hexadecanamide (CAS)	$C_{16}H_{33}NO$	Fatty acid
Hexadecanoic acid (CAS) (Palmitic acid)	$C_{16}H_{32}O_2$	Fatty acid
9-Octadecenamide, (Z)- (CAS)	$C_{18}H_{35}NO$	Fatty acid
Tetradecanoic acid (CAS) (Myristic acid)	$C_{14}H_{28}O_2$	Fatty acid
Hexadecanoic acid, 2-hydroxy-1,3-propanediylester (CAS)	$C_{35}H_{68}O_5$	Fatty acid ester
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-(CAS)	$C_{21}H_{36}O_4$	Fatty acid ester
Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester (CAS)	$C_{35}H_{68}O_5$	Fatty acid ester
Heptanoic acid, docosyl ester (CAS)	$C_{29}H_{58}O_2$	Fatty acid ester
Octadecanoic acid, 2-hydroxy-1,3-propanediylester (CAS)	$C_{39}H_{76}O_5$,	Fatty acid ester
Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (CAS)	$C_{21}H_{42}O_4$	Fatty acid ester
Oleic acid, eicosyl ester (CAS)	$C_{38}H_{74}O_2$	Fatty acid ester
Ethyl lınoleolate	$C_{20}H_{36}O_2$	Fatty acid ester
Pentadecanoic acid, 14-methyl-, methyl ester (CAS)	$C_{17}H_{34}O_2$	Fatty acid ester
Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS)	$C_{19}H_{38}O_4$	Fatty acid ester
9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)- (CAS)	$C_{19}H_{32}O_2$	Fatty acid ester
24,25-Dihydroxycholecalciferol	$C_{27}H_{44}O_3$	Vitamin
Stigmasta-5,22-dien-3-ol, (3á,22E)- (CAS) (Stigmasterol)	$C_{29}H_{48}O$	Phytosterol
Stigmasta-5,24(28)-dien-3-ol, (3á)- (CAS)	$C_{29}H_{48}O$	Phytosterol
Stıgmast-5-en-3-ol, (3á,24S)- (Beta sitosterol)	$C_{29}H_{50}O$	Phytosterol

As a result of the GC-MS analysis, bioactive compounds belonging to various chemical groups were detected (Table 6) .

The allelopathic potential of the Brassicaceae species is mainly stem from glucosinolates and their biologically active hydrolysis products (nitriles, isothiocyanates, thiocyanates, oxazoliolines, and epithionitriles), as well as phenolic acids (Brown and Morra, 1997; Al-Khatib *et al*. 1997; Petersen *et al*. 2001; Jafariehyazdi and Javidfar, 2011; Jabran, 2017) . As a result of GC-MS analysis, 2 glycosinolates (desulphosinigrin, desulphoglucoerucin), 4 phenolic acid (Carvacrol, phenol, 3-ethyl-, 2-methoxy-4-vinylphenol, 2-tert-Butyl-4-isopropyl-5-methylphenol, 2-amino-4-chlorophenol) and 4 flavonoids (quercetın 7,3',4'-trımethoxy, 4H-1-Benzopyran-4-one,2-(3,4-dimethoxyphenyl)-5,7-dihydroxy, lucenin 2 , 03027205002 flavone 4'-oh,5-oh,7-dı-o-glucosıde) compounds were detected in the little radish stem extract. Desulfoglukoerucin has been identified in the root extract of the *Aurinia leucadea* (Guss.) (Blažević *et al*. 2013), in the tissue extract of *Bunias erucago* L. (Blažević *et al*. 2019) and in the extracts of *Degenia velebitica* aerial parts (De Nicola *et al*. 2011). Glucoerucin, the precursor of the biologically highly active erucin isothiocyanate, is abundant in *Eruca sativa* (Bennett *et al*.

2007) . El-Wakeel *et al*. (2019) demostrated that fresh shoot aqueous extract of *E. sativa* which has 12 GSL including glucoerucin can be used as a natural selective bioherbicide. Desulfosinigrin compound with known antibacterial activity, which is other found glucosinolates in the present study, was detected in some plant extracts (*Brassica oleracea*, *Annona reticulata*, *Euphorbia lathyrus*, *Aspergillus terreus*).

In a previous study, high concentration carvacrol (12%) was found to significantly inhibit *Sorghum bicolor* seed germination and completely stop germination of *Lactuca sativa* L. (Pinheiro *et al*. 2015).

In this study, it has been proved that some of flavonoids like lucenin 2, quercetin, kaempferol found as a result of GC-MS analysis have allelopathic effect. Parvez *et al*. (2004) have report that while quercetin significantly reduced the growth of *A. thaliana* seedlings, has no significant effect on *Neurospora crassa*. Basile *et al*. (2003) showed that lucenin 2 significantly reduced *R. sativus* seed germination and root length compared to control and inhibited *Tortula muralis* spore germination. It has also been stated that the kaempferol-3-O-β-D-glucoside compound isolated from the *Solidago canadensis* plant inhibited the seedling growth of the *Echinochloa colonum* (Omezzine *et al*. 2014).

The allelopathic effect of many terpene compounds has been previously reported (Zhao *et al*. 2009, Shao *et al*. 2019). In the present study, 3-oxo-inol, a sesquiterpene compound, has been identified. In a previous study, it was stated that the (6R,9S)-3-oxo-ionol compound isolated from *Brachiaria brizantha* extract reduced root and stem growth of garden cress depending on increased concentration (Kato-Noguchi *et al*. 2014).

The compounds in the fatty acids, fatty acid esters and sterols group determined in the current study are at a higher concentration than other metabolites (Table 4). Some of these compounds have been reported to be phytotoxic on germination and seedling growth of the test plants. For example, sodium salts of some fatty acids (palmitic acid, myristic acid, steraic acid, oleic acid) found to be allelopathic effects on seed germination and radicula growth of *Cynodon dactylon* (Alsaadawi *et al*. 1983). In addition, stigmasterol and beta sitosterol which are phytosterols and phytol which is a diterpen detected in our study, were purified from the aerial parts of the *Justica anselliana* and were found that negatively affected the germination, fresh weight and seedling development of the *Vigna ungiculata* (Kpoviessi *et al*. 2006).

All these allochemicals affect many physiological processes and functions, such as mitotic activity, photosynthesis, nutrient uptake, permeability of the cell membrane, cell division and respiration, as well as inhibition of enzyme activity and protein formation (Rice 1984; Wu *et al*. 2000; Xuan *et al*. 2004).

5. Conclusion

According to findings of current investigation, it was found that aqueous extracts of both cultivars of radish inhibit germination and seedling growth of *E. crus-galli* at high concentrations. Among all treatments, the most remerkable impacts was obtained from seeds grown in stem extract of little radish. As the result of GC-MS analysis of little radish stem extract, the presence of many bioactive compounds that may be the source of allelopathic effect has been revealed.

It is not known exactly which of the chemical compounds in the plant causes this effect. Further studies will be needed to isolate phytochemicals of little radish that can be used in bioherbicide development.

References

Al-Khatib, K., C. Libbey, & R. Boydston. (1997), "Weed suppression with Brassica green manure crops in green pea", *Weed Sci.* ***45****:439-445*

Alsaadawi, I. S., Rice, E. L., & Karns, T. K. (1983), "Allelopathic effects of Polygonum aviculare L", *Journal of Chemical Ecology*, ***9***(6), 761-774.

Al-Sherif, E., Hegazy, A., Gomaa, N., & Hassan, M. (2013), "Allelopathic effect of black mustard tissues and root exudates on some crops and weeds", *Planta Daninha*, **31**, 11–19.

Arslan, M., Uremis, I. and Uludag, A. (2005), "Determining Bio-Herbicidal Potential Rapeseed, Radish and Turnip Extracts on Germination Inhibition of Cutleaf Ground-Cherry (*Physalis angulata* L.) Seeds", *Agronomy Journal* , **4**, 134-137.

Barbieri, G., Pernice, R., Maggio, A., De Pascale, S., & Fogliano, V. (2008), "Glucosinolates profile of Brassica rapa L. subsp. Sylvestris L. Janch. var. esculenta Hort", *Food Chemistry*, **107**(4), 1687-1691.

Basile, A., Sorbo, S., Lopez-Saez, J. A., ve Cobianchi, R. C. (2003), "Effects of seven pure flavonoids from mosses on germination and growth of *Tortula muralis* HEDW.(Bryophyta) and *Raphanus sativus* L.(Magnoliophyta) ", *Phytochemistry*, **62**(7), 1145-1151.

Bennett, R. N., Carvalho, R., Mellon, F. A., Eagles, J., & Rosa, E. A. (2007), "Identification and quantification of glucosinolates in sprouts derived from seeds of wild *Eruca sativa* L.(salad rocket) and *Diplotaxis tenuifolia* L.(wild rocket) from diverse geographical locations", *Journal of agricultural and food chemistry*, **55**(1), 67-74.

Blažević, I., De Nicola, G. R., Montaut, S., ve Rollin, P. (2013), "Glucosinolates in two endemic plants of the Aurinia genus and their chemotaxonomic significance", *Natural Product Communications*, **8**(10).

Blažević, I., Dulović, A., Čikeš Čulić, V., Burčul, F., Ljubenkov, I., Ruščić, M., ve Generalić Mekinić, I. (2019) , *Bunias erucago* L.: "Glucosinolate Profile and In Vitro Biological Potential", *Molecules*, **24**(4), 741.

Brown, P. D., & Morra, M. J. (1997), *"*Control of soil-borne plant pests using glucosinolate-containing plants", *Advances in Agronomy,* **61**, 167–231.

Bukolova, T.P. (1971), "A study of the mechanism of action of water-soluble substances of weeds on cultivated plants", In: *Physiological biochemical basis of plant interactions in phytocenoses*. A.M. Grodzinsky (ed). **2**, 66-69.

Carvalho, C.A., Silva, M.B., Oliveira, T.G., Lima, J.M. and Rosa, M.B. (2008), "Spectrometric Study at Different Phenologic Stages of the Cabbage (Brassica oleraceae var. capitata) ", Revista Brasileira de Farmacognosia , **18**, 249-257.

De Moraes Gomes, M., Bertoncelli, D.J., Alves, G.A.C., Freiria, G.H., Furlan, F.F., Gomes, G.R., Favoretto, V.R., Neto, H.F.I., Omura, M.S. and De Souza, J.R.P. (2017), "Allelopathic Potential of the Aqueous Extract of *Raphanus sativus* L. on the Germination of Beans and Corn Seeds", Open Access Library Journal ,**4**, 1.

De Nicola, G. R., Blažević, I., Montaut, S., Rollin, P., Mastelić, J., Iori, R., ve Tatibouët, A. (2011), "Glucosinolate distribution in aerial parts of *Degenia velebitica*. *Chemistry ve Biodiversity"*, **8**(11), 2090-2096.

El-Wakeel, M. A., El-Desoki, E. R., & Abd El-Ghany, S. E. D. (2019), "Bioherbicidal activity of Eruca sativa fresh shoot aqueous extract for the management of two annual weeds associating *Pisum sativum* plants. *Bulletin of the National Research Centre"*, **43**(1), 87.

Geimadil, R., Shokati, B., & Shahgholi, H. (2015), "Allelopathic effects of medicinal plants of lemon balm, lemon verbena and bitter apple on seed germination and early seedling growth characteristics of wild mustard weed", *Journal of Medicinal Plants and By-products*, **2**, 249-254

Inderjit, Seastedt, T. R., Callaway, R. M., Pollock, J. L., & Kaur, J. (2008), "Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches", *Biological Invasions*, **10**(6), 875-890.

Jabran, K. (2017), "Brassicaceae allelopathy for weed control", In *Manipulation of Allelopathic Crops for Weed Control* (pp. 21-27). Springer, Cham.

Jafariehyazdi, E., & Javidfar, F. (2011), "Comparison of allelopathic effects of some brassica species in two growth stages on germination and growth of sunflower", *Plant*, *Soil and Environment*, **57**, 52–56.

Jahangir, M., Kim, H. K., Choi, Y. H., & Verpoorte, R. (2009), "Health-affecting compounds in Brassicaceae. Comprehensive Reviews in Food Science and Food Safety"*,* **8**(2), 31-43.

Kato-Noguchi, H., Kobayashi, A., Ohno, O., Kimura, F., Fujii, Y., ve Suenaga, K. (2014), "Phytotoxic substances with allelopathic activity may be central to the strong invasive potential of *Brachiaria brizantha*", *Journal Of Plant Physiology*, **171**(7), 525-530.

Kobayashi, K., Koyama, H., & Shim, I. S. (2004), "Relationship between behavior of dehydromatricaria ester in soil and the allelopathic activity of *Solidago altissima* L. in the laboratory", *Plant and soil*, **259**(1-2), 97-102.

Kpoviessi, D. S. S., Gbaguidi, F. A., Gbenou, J. D., Accrombessi, G. C., Haddad, M., Moudachirou, M., & Quetin-Leclercq, J. (2006), "Allelopathic effects on cowpea (Vigna unguiculata (L.) Walp) plant and cytotoxic activities of sterols and triterpenes isolated from Justicia anselliana (NEES) T. Anders", *Electronic Journal of Natural Sciences*, *1*, 12.

Macias, F. A., Marin, D., Oliveros-Bastidas, A., Varela, R. M., Simonet, A. M., Carrera, C., et al. (2003), "Allelopathy as a new strategy for sustainable ecosystems development", . *Biol. Sci*. Space **17**, 18–23.

Narwal SS. 1994. "Allelopathy in crop production", Scientific Publishers, Jodhpur, India, p 288.

Omezzine, F., Bouaziz, M., Simmonds, M. S., & Haouala, R. (2014), "Variation in chemical composition and allelopathic potential of mixoploid *Trigonella foenum-graecum* L. with developmental stages", *Food chemistry*, ***148***, 188-195.

Parvez, M.M., Yokotani, K. T., Fujii, Y., Konishi, T., ve Iwashina, T. (2004), "Effects of quercitin and its seven derivates on the growth of *Arabidopsis thaliana* and *Neurospora crassa", Biochemicals systematics and ecology*, **32**,631-635.

Petersen, J., R. Belz, F. Walker, and K. Hurle. (2001), "Weed suppression by release of isothiocyanates from turnip-rape mulch", *Agron. J.* **93**:37- 43

Pinheiro, P. F., Costa, A. V., Alves, T. D. A., Galter, I. N., Pinheiro, C. A., Pereira, A. F., ... & Fontes, M. M. P. (2015), "Phytotoxicity and cytotoxicity of essential oil from leaves of Plectranthus amboinicus, carvacrol, and thymol in plant bioassays",. *Journal of agricultural and food chemistry*, **63**(41), 8981-8990.

Putnam, A.R., 1985, Weed allelopathy (Chapter 5). In: Duke, S.O. (Ed.), Weed Physiology, Vol. I: Reproduction and Ecophysiology. CRC Press, Boca Raton, FL, pp. 131-155.

Rice, E.L. Allelopathy, 2nd ed.; Acadamic Press: Orlando, FL, USA, 1984; p. 422

Rigon, C. A. G., Salamoni, A. T., de Aguiar, A. C. M., & Cutti, L. (2018), "Allelopathic effect of aqueous extracts of different organs of three sunflower cultivars on germination of radish", *Bioscience Journal*, **34**(3).

Santos, J.C.F., Souza, I.D., Mendes, A.N.G., Morais, A.D., Conceicao, H.D. and Marinho, J.T.S. (2002), "Effect of Extracts of Coffee Husks and Rice on Emergence and Growth of Caruru-de-Mancha", *Pesquisa*

Agropecuaria Brasileira, **37**, 783-790.

Shao H, Wei C, Zhou S, Li W, Jiang C, Yang W, Han C, Zhang C (2019), "Chemical composition and allelopathic, phytotoxic and pesticidal activities of Atriplex cana Ledeb. (Amaranthaceae) essential oil", *Chem. Biodivers*.

Sisek, D., Turkseven, S., & Topal, S. (2019), "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", *Open Access Library Journal*, **6**(7), 1-9.

Sodaeizadeh, H., Rafieiolhossaini, M., Havlík, J., & Van Damme, P. (2009), "Allelopathic activity of different plant parts of *Peganum harmala* L. and identification of their growth inhibitors substances", *Plant Growth Regulation*, **59**(3), 227.

Soltys, D.; Krasuska, U.; Bogatek, R.; Gniazdowska, A. Allelochemicals as Bioherbicides Present and Perspectives. In Herbicides Current Research and Case Studies in Use; Price, A.J., Kelton, J.A., Eds.; IntechOpen: London, UK, 2013.

Tierens, K. F. J., Thomma, B. P., Brouwer, M., Schmidt, J., Kistner, K., Porzel, A., ... & Broekaert, W. F. (2001), "Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of Arabidopsis to microbial pathogens", *Plant physiology*, **125**(4), 1688-1699.

Turk, M.A. and Tawaha, A.M. (2003), "Allelopathic Effect of Black Mustard (*Brassica nigra* L.) on Germination and Growth of Wild Oat (*Avena fatua* L.) ", *Crop Protection*, **22**, 673-677.

Turk, M.A., Tawaha, A.M., (2002), "Inhibitory effects of aqueous extracts of black mustard on germination and growth of lentil.*Pak. J.Agronom*.**1** (1), 28–30.

Uremis, I., Ahmet, M., Uludag, A. and Sangun, M. (2009), "Allelopathic Potential of Residue of 6 Brassica Species on Johnsongrass [*Sorghum haplense* (L.) Pers.] ", *African Journal of Biotechnology*, **8**, 3497-3501.

Uremis, I., Arslan, M. ve Uludağ, A. (2005), "Allelopathic effects of some brassica species on germination and growth of cutleaf ground-cherry (*Physalis angulata* L.) seeds", *Journal of Biological Sciences*, **5**, 661- 665.

Xuan, T. D., Shinkichi, T., Hong, N. H., Khanh, T. D., & Min, C. I. (2004), "Assessment of phytotoxic action of *Ageratum conyzoides* L.(billy goat weed) on weeds", *Crop protection*, **23**(10), 915-922.

Xuan, T.D., Tsuzuki, E., Tawata, S. and Khanh, T.D. (2004), "Methods to Determine Allelopathic Potential of Crop Plants for Weed Control", *Allelopathy Journal* , **13**, 149-164.

Zhao, B., Lei, L., Vassylyev, D. G., Lin, X., Cane, D. E., Kelly, S. L., ... & Waterman, M. R. (2009), "Crystal structure of albaflavenone monooxygenase containing a moonlighting terpene synthase active site", *Journal of Biological Chemistry*, **284**(52), 36711-36719.