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Allelopathic potential of radish (*Raphanus sativus* L.) on germination and growth of some crop and weed plants

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Key words: Allelopathy, Allelochemicals, Weed, Herbicide.

<http://dx.doi.org/10.12692/ijb/9.1.394-403>

Article published on July 31, 2016

Abstract

This study was conducted in the laboratories of Agricultural college, Salahaddin University to investigate the allelopathic potential of *Raphanus sativus* L. roots aqueous extracts on germination and growth of wheat, and barley crops with three endemic weed plants (*Avina fatua*, *Phalaris minorand*, *Brassica napus*), in order to indicate the potentiality of utilizing this extract as a herbicide. Results showed the significant effect of different concentrations 5%, 10%, 15% and 20% of aqueous extracts compared to control 0% treatments on germination percentage, germination inhibition percentage, germination speed, radicle and plumule length, radicle and plumule elongation velocity, radicle and plumule dry weight, seedlings total dry weight, root-shoot ratio and seedling vigor index. Among five studied plant species wheat then barley were stronger than the three studied weed plants. In this study the effect was concentration dependent on all studied parameters, meanwhile the effect of combination between plant species and concentration caused significant differences with distinguished characteristics for both crop plants comparing to the three weed plants. So the radish aqueous extracts imposed herbicidal effect on studied weed plants.

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Introduction

Allelopathy was defined as any beneficial or harmful effect of one plant on another including microorganisms by producing allelochemicals from donor plants that affects recipient plants germination, growth and development (Rice, 1984). Allelopathy separated from plant competition in that when plants are competing for environmental resources such as water, minerals, light and space, meanwhile with allelopathic plants relationship there are chemical substances that added to the environment by donor plants to affect plants in vicinity (Ali, 2001). There was significant increase in allelopathic studies on crops and weeds using different plant part extracts, exudates or residues in order to indicate the allelopathic interference between crops and weeds (Ali and Sakri, 2010; Foy and Inderjit, 2001). There are different agricultural methods that utilize allelopathic plant relationship such as: allelopathic cover crops, allelopathic rotational crops, toxic extracts from allelopathic plants, mulch or soil incorporation with plant residue, natural herbicides, and breeding allelopathic crop cultivars with weed suppressing ability (Wu *et al.*, 1999; Chee-Ma *et al.*, 2005). There is a new strategy working on indicating bio-herbicides from plants as an alternative tool for crop production due to its ecofriendly characteristics when compared to chemical herbicides (Jones and Medd, 2005).

The allelopathic studies in the weed control field focused on utilization of crop plants to suppress weed growth in crop fields, It has been reported that corn gluten meal or corn gluten hydrolysate (Liu and Christians, 1996) which is a water-soluble material derived from the action of bacterial proteinase which was demonstrated to be more active than corn gluten meal to three grass species which were perennial ryegrass *Lolium perenne*, smooth crabgrass *Digitaria ischaemum*, and creeping bent-grass *Agrostis palustris* (Liu *et al.*, 1994).

Radish (*Raphanus sativus* L.) belongs to Brassicaceae family shows allelopathic effect on different studied plants (Lawley *et al.*, 2012) due to its allelochemicals compounds such as p-hydroxy benzoic acid, and isothiocyanates (isothiocyanate benzyl, Isothiocyanateallyl) (Peterson *et al.*, 2001; Uremis *et al.*, 2009).

It was recorded that some crops and weed plants were sensitive toward extracts of radish plant (Koseli, 1991; Norsworthy, 2003; Kadioglu and Yanar, 2004). The objective of this study was to indicate the allelopathic activity of garden radish on germination and some seedling growth characteristics of two crop plants and three weed plants as a first step toward finding ecofriendly bio-herbicides.

Materials and methods

Seed Sources

Crop and weed seeds of Wheat *Triticum aestivum*, Barley *Hordeum vulgare*, wild oat *Avena fatua*, Canary grass *Phalaris minor* and Black mustard *Brassica napus* were received from Erbil Research center. Roots of Radish *Raphanus sativus* were collected from grdarasha fields.

Extract Preparation

Radish roots were washed with tap water after that it was brought to the laboratory, cut in to 4-5cm pieces to be put in blender to get homogenized extract without adding any water except intercellular moisture next it was passed through cheesecloth and Whitman filter paper #1, finally the prepared aqueous extract kept in dark plastic jars and stored at -20°C in deep-freeze until use.

Bioassay

The stored extract was considered as (crude solution), from which other concentrations were prepared as 5, 10, 15, and 20% by adding distilled water to reach desired concentration, distilled water was considered as control treatment (Dogan and Uygur, 2005) and (Kivi *et al.*, 2010). Twenty five seeds of each species were placed between two sheets of filter paper #1 in 9 cm petridishes then each petridish treated with 8ml of the studied concentrations of Radish aqueous extracts, each petridishes were sealed and placed in growth chamber under 20°- 25°C temperature and continuous darkness. On 3rd, 5th, 7th and 10th day germination percentages of each petridish was determined, finally plants were harvested, Plumule and radicle lengths were measured, fresh and dry weights were determined.

Recorded Data

The list of recording data were germination Percentage; Inhibition Percentage; speed of germination (seed/day) (Norsworthy, 2003; Ali and Aziz, 2002); radicle length (cm); radicle elongation velocity (cm/day); plumule length (cm); plumule elongation velocity (cm/day) (Jiang and Lafitte, 2007); seedlings dry weight (mg); seedling vigor index (Abdul Baki and Anderson, 1970), and shoot/root ratio according to equations shown below (De-Oliveira *et al.*, 2013).

$$\text{Germination \%} = \frac{GS}{TTS} \times 100 \dots \dots \dots (1)$$

$$\text{IOG \%} = \frac{GPC - GPT}{GPC} \times 100 \dots \dots \dots (2)$$

$$\text{SG} \left(\frac{\text{seed}}{\text{day}} \right) = \left(\frac{N1}{1} + \frac{N2}{2} + \frac{N3}{3} + \frac{N}{n} \right) \times 100 \dots \dots \dots (3)$$

$$\text{REV or PEV} \left(\frac{\text{cm}}{\text{day}} \right) = \frac{\text{RL or PL}}{\text{TD}} \dots \dots \dots (4)$$

$$\text{RGI or PGI \%} = \left(1 - \frac{VUS}{VNS} \right) \times 100 \dots \dots \dots (5)$$

$$\text{RSR} = \frac{\text{RDW}}{\text{SDW}} \times 100 \dots \dots \dots (6)$$

$$\text{SVI} = \frac{\text{SL} \times \text{GP}}{100} \dots \dots \dots (7)$$

Where GS = germinated seeds, TTS = Total tested seeds, IOG= Inhibition of germination, GPC= germination percentage of control, GPT= germination percentage of treatment, SG= speed of germination, N1, N2, N3...N = proportion of seeds which germinate on days 1, 2, 3 ...n , REV = radicle elongation velocity, PEV= plumule elongation velocity, RL= radicle length, PL= plumule length, TD= total days, RGI= radicle growth inhibition, PGI= plumule growth inhibition, VUS= dry weight under stress, VNS= dry weight under non stress conditions, RSR= root shoot ratio, RDW= root dry weight and SDW= shoot dry weight, SVI= Seedlings Vigor Index, SL= Seedling length (cm) and GP = Germination Percentage.

Radicle or plumule dry weight were recorded by placing samples in an electrical oven 40°C for 72 hours or until weight consistency to record plant parts dry weights. Radicle or plumule growth inhibition was calculated according to equation (5) by depending on dry weight of radicle or plumule.

Statistical Analysis

This study was designed as completely randomized factorial experiment (Factorial C.R.D) consists of five plant species and five levels of radish aqueous extracts with three replications. The data were subjected to standard analysis of variance and means were compared at significant 1% level by Duncan test using SPSS computer analysis according to (Weinberg and Abramowitz, 2008) and (Field, 2005).

Results and discussion

Results of this study could be summarized to three categories as the studied factors and the interaction between both of them

Effect of plant species on studied plant characteristics

Table (1) indicated significant effect of radish aqueous extracts on recorded data, whereas the highest germination percentage where 49.33% recorded for wheat seeds and the lowest where 21.33% with *Phalaris minor* weed seeds, while for germination speed wheat seeds was the fastest (6.22 seeds/ day) and the slowest data was (2.48 seeds/day) documented with wild oat weed seeds, according to inhibition of germination data it was obvious that highest data for inhibition of germination was 77.64% with *Phalaris minor* weed plants and the lowest was 49.39% with wheat crop seeds, seedlings radicle length data recorded highest level (6.71 cm) in wheat seedlings and lowest (2.09 cm) with wild oat weed plants, on the other hand highest data for plumule length was (6.58 cm) with Barley crop seedlings and the shortest (3.11 cm) with black mustard weed seedlings. Dry weight data for radicle, plumule and total seedlings of studied species indicates the significant effect of aqueous extracts of radish on radicle, plumule and total dry weight and the highest data was (4.38, 5.23 and 8.63 mg) for wheat radicle, Barley plumule and wheat's total dry weights respectively, while lowest data were (0.31 mg) with black mustard radicle, (0.43 and 0.81 mg) canary grass weeds plumule and total dry weight respectively.

Radicle and plumule elongation velocity were significantly affected by radish aqueous extracts where the highest record were with elongation velocity with radicle of wheat and plumule of barley meanwhile lowest record were with elongation velocity with radicle of wild oat and plumule of black mustard weeds (Fig.1), radish aqueous extracts caused significant differences on studied plants root shoot ratio where the highest ratio were with wheat seedlings and the lowest with black mustard weed seedlings (Fig.2). Seedlings vigor index was used in this study to evaluate Seedlings vigor ability to emerge through soil and grow vigorously under stressed and non-stressed environmental conditions (Tekron Yand Egli, 1991) where there was significant differences between studied plant species and the most vigorous seedling was wheat plant seedlings and

the lowest data was with wild oat seedlings under the abiotic stress of allelopathic compounds of radish aqueous extracts (Fig. 3). It was obvious that wheat Radicle growth inhibition data scored the lowest inhibition ratio, while seedlings of canary grass imposed highest sensitivity among other studied plant species, meanwhile plumule growth inhibition data shows that black mustard seedlings was the most sensitive one comparing to other studied species (Fig. 4). Results indicates the potential strength of radish aqueous extracts toward different plants species on the other hand it is well known that plant species differ in responding to allelochemicals due to genetic variation properties, beside crop and weed plants included in this study showed different levels of sensitivity toward allelochemics of radish plants (Wu *et al.*, 2002; Uremis *et al.*, 2009), (table 2).

Table 1. The effect of plant species on germination and inhibition percentage with some growth parameters.

| Plant Species | Germination Percentage % | Germination Speed (Seed/day) | Inhibition Percentage % | Radicle Length (cm) | Plumule Length (cm) | Radicle Dry Weight (mg) | Plumule Dry Weight (mg) | Total Dry Weight (mg) |
|---------------|--------------------------|------------------------------|-------------------------|---------------------|---------------------|-------------------------|-------------------------|-----------------------|
| Wheat | 49.33 a | 6.22 a | 49.39 d | 6.71 a | 6.43 a | 4.25 a | 4.38 b | 8.63 a |
| Barley | 36.00 b | 6.13 ab | 63.05 c | 5.07 b | 6.58 a | 3.21 b | 5.23 a | 8.44 a |
| Wild Oat | 23.20 d | 2.48 d | 72.25 b | 2.09 e | 4.69 b | 1.66 c | 3.39 c | 5.05 b |
| Canary Grass | 21.33 d | 3.10 c | 77.64 a | 3.47 c | 4.46 c | 0.38 d | 0.43 d | 0.81 c |
| Black Mustard | 27.73 c | 5.55 b | 69.61 b | 3.08 d | 3.11 d | 0.31 d | 0.52 d | 0.83 c |

Common letter means that there was a non-significant difference at 1% probability level by Duncan's test.

Table 2. The effect of concentrations of aqueous extracts of radish on germination and inhibition percentage with some growth parameters.

| Concentration | Germination Percentage % | Germination Speed (Seed/day) | Inhibition Percentage % | Radicle Length (cm) | Plumule Length (cm) | Radicle Dry Weight (mg) | Plumule Dry Weight (mg) | Total Dry Weight (mg) |
|---------------|--------------------------|------------------------------|-------------------------|---------------------|---------------------|-------------------------|-------------------------|-----------------------|
| control | 84.53 a | 12.83 a | 15.47 e | 12.13 a | 12.99 a | 4.97 a | 6.00 a | 10.97 a |
| 5 % | 51.73 b | 7.92 b | 40.25 d | 5.98 b | 7.66 b | 2.91 b | 4.15 b | 7.06 b |
| 10 % | 15.20 c | 1.87 c | 82.61 c | 1.87 c | 3.26 c | 1.53 c | 2.73 c | 4.27 c |
| 15 % | 6.13 d | 0.85 d | 93.61 b | 0.44 d | 1.36 d | 0.40 d | 1.07 d | 1.47 d |
| 20 % | 0.00 e | 0.00 e | 100.00 a | 0.00 e | 0.00 e | 0.00 e | 0.00 e | 0.00 e |

Common letter means that there was a non-significant difference at 1% probability level by Duncan's test.

Effect of different concentrations of Radish aqueous extracts on some studied plant characteristics

Table 3. elucidate the significant effect of different concentrations of aqueous extracts of radish on germination percentage, germination speed, inhibition of germination percentage, radicle and plumule length, radicle, plumule and seedlings total dry weight where the highest data was with control

treatments meanwhile lowest values were with 20% concentration for all above mentioned data, except germination inhibition percentage which the highest value was with 20% concentration and lowest value was with control treatment. (Fig. 5) shows the significant influence of radish aqueous extract concentrations on tested plants radicle and plumule elongation velocity where the highest values

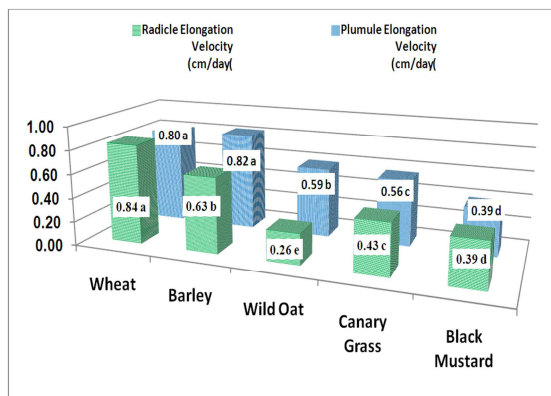


Fig. 1. The effect on radicle and plumule elongation velocity.

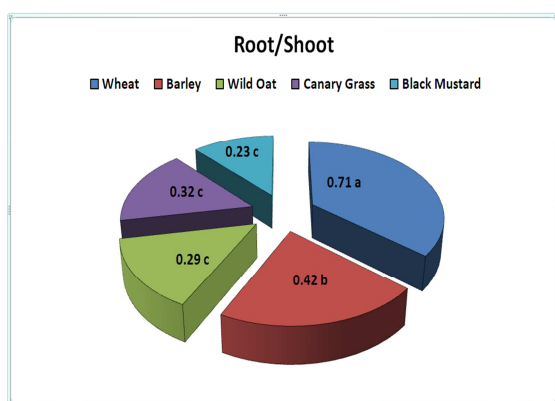


Fig. 2. The effect on Seedlings root-shoot ratio.

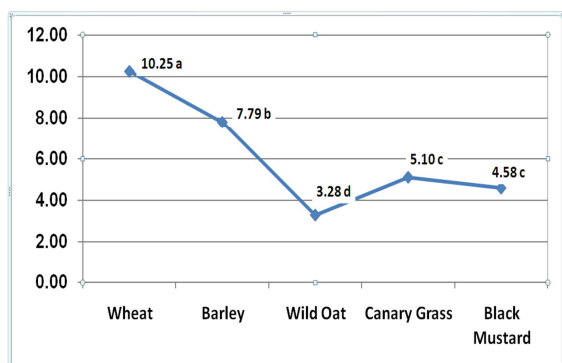


Fig. 3. The effect on seedlings vigor index.

documented with control treatments for both radicle and plumule elongation velocity while lowest data were documented with highest concentration level for both radicle and plumule elongation velocity, the same criteria was obvious for both root, shoot ratio and seedlings vigor index where the highest data were with control treatment and lowest data were with 20% concentration of radish aqueous extracts (Fig. 6 and 7).

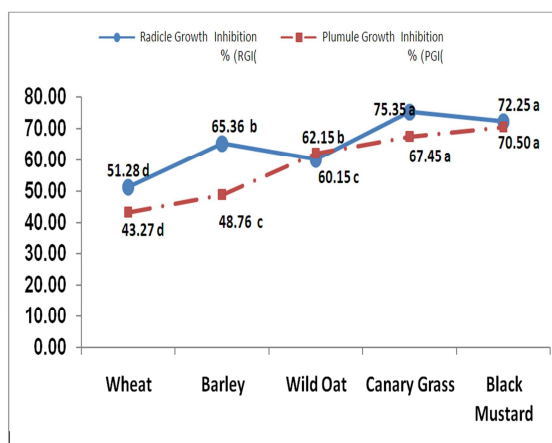


Fig. 4. The effect of plant species on radicle and plumule growth inhibition.

Radicle and plumule growth inhibition data was obvious with the highest concentration level for both seedling parts, but the lowest data was recorded with control treatments which means increasing concentration levels caused increasing growth inhibition ratio for radicle and plumule seedlings part (Fig. 8). These results elucidate the negative effect of increasing concentration of the aqueous extracts which suggest different plant response to concentration in such way it may be the perfect evidence of its potentiality to be used as a herbicide for these tested weed plants due to its concentration dependent behave (Rafiqul Hoque *et al.*, 2003) and (Ussalam *et al.*, 2011).

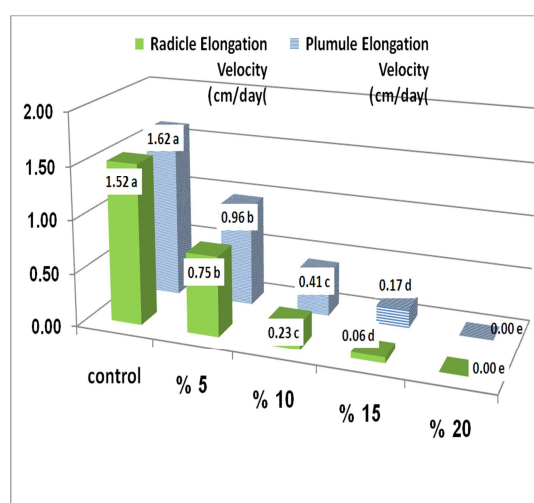


Fig. 5. The effect of concentrations of aqueous extracts on radicle and plumule elongation velocity.

Table 3. The effect of combination of plant species and concentrations of aqueous extracts of radish on germination and inhibition percentage with some growth parameters.

| Combination | Germination Percentage % | Germination Speed (Seed/day) | Inhibition Percentage % | Radicle Length (cm) | Plumule Length (cm) | Radicle Dry Weight (mg) | Plumule Dry Weight (mg) | Total Dry Weight (mg) | |
|--------------|--------------------------|------------------------------|-------------------------|---------------------|---------------------|-------------------------|-------------------------|-----------------------|---------|
| Wheat | control | 96.00 a | 12.73 b | 4.00 g | 16.92 a | 14.00 b | 8.73 a | 7.73 c | 16.47 b |
| | 0.05 | 78.67 b | 9.05 c | 17.90 f | 10.67 e | 8.68 e | 6.90 b | 6.33 d | 13.23 c |
| | 0.1 | 46.67 e | 5.58 e | 51.39 d | 4.94 j | 6.40 h | 4.33 c | 5.33 e | 9.67 e |
| | 0.15 | 25.33 g | 3.71 f | 73.64 c | 0.99 p | 3.09 m | 1.30 f | 2.50 f | 3.80 g |
| | 0.2 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |
| Barley | control | 94.67a | 16.03 a | 5.33 g | 13.71 b | 14.36 b | 9.27 a | 10.20 a | 19.47 a |
| | 0.05 | 68.00 c | 12.28 b | 28.20 e | 7.26 f | 9.00 e | 3.83 c | 7.37 c | 11.20 d |
| | 0.1 | 12.00 h | 1.80 j | 87.32 b | 3.16 m | 5.85 j | 2.27 e | 5.70 e | 7.97 f |
| | 0.15 | 5.33 j | 0.55 j | 94.38 b | 1.22 p | 3.70 k | 0.69 g | 2.87 f | 3.55 gh |
| | 0.2 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |
| Wild Oat | control | 68.00 c | 7.51 d | 32.00 e | 6.38 g | 11.65 c | 4.17 c | 8.97 b | 13.13 c |
| | 0.05 | 30.67 f | 2.91 g | 54.92 d | 2.84 n | 7.76 f | 3.07 d | 5.37 e | 8.43 f |
| | 0.1 | 17.33 h | 1.99 h | 74.33 c | 1.26 p | 4.06 k | 1.07 g | 2.63 f | 3.70 g |
| | 0.15 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |
| | 0.2 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |
| Canary Grass | control | 82.67 b | 12.15 b | 17.33 f | 12.13 c | 15.15 a | 1.57 f | 1.33 h | 2.90 h |
| | 0.05 | 24.00 g | 3.35 f | 70.87 c | 5.23 h | 7.13 g | 0.33 hk | 0.83 h | 1.17 j |
| | 0.1 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |
| | 0.15 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |
| | 0.2 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |
| Wild Rape | control | 81.33 b | 15.73 a | 18.67 f | 11.52 d | 9.82 d | 1.13 fg | 1.77 g | 2.90 h |
| | 0.05 | 57.33 d | 12.01 b | 29.38 e | 3.90 k | 5.74 j | 0.43 hk | 0.83 h | 1.27 j |
| | 0.1 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |
| | 0.15 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |
| | 0.2 | 0.00 j | 0.00 k | 100.00 a | 0.00 s | 0.00 n | 0.00 k | 0.00 j | 0.00 k |

Common letter means that there was a non-significant difference at 1% probability level by Duncan's test.

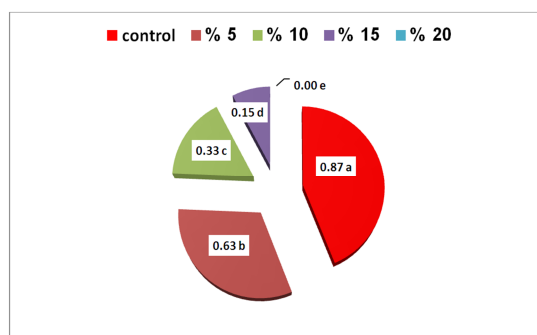


Fig. 6. The effect of concentrations of aqueous extracts on shoot, root ratio.

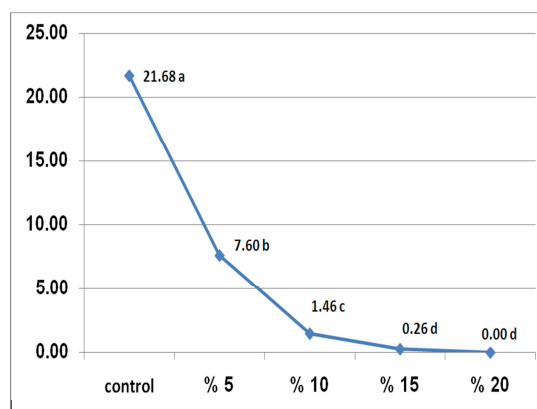


Fig. 7. The effect of concentrations of aqueous extracts on seedlings vigor index.

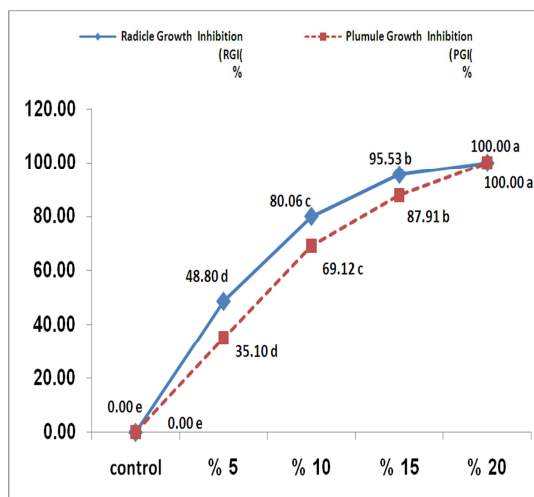


Fig. 8. The effect of concentrations of aqueous extracts on radicle and plumule growth inhibition.)

Effect of the combination between plant species and different concentrations of Radish aqueous extracts on some studied plant characteristics

Table (4) indicated significant effect of the combination of plant species and concentrations of radish aqueous extracts, whereas for germination percentage the highest germination percentage was recorded with wheat seeds under control treatment and the lowest with wheat and Barley under the 20% concentrations, wild oat under concentrations 15 and 20%, Canary grass and black mustard weeds under concentrations 10,15 and 20%, while for germination speed the highest value was with wheat crop seedling under control treatments and the lowest were with wheat and Barley under the 20% concentrations, wild oat under concentrations 15 and 20%, Canary grass and black mustard weeds under concentrations 10,15 and 20%. Data of inhibition of germination percentage recorded highest level for wheat and Barley under the 20% concentrations, wild oat under concentrations 15 and 20%, Canary grass and black mustard weeds under concentrations 10,15 and 20% meanwhile the lowest was with control treatment of wheat crops. Radicle length and elongation velocity elucidate highest data for wheat radicle under control treatments, on the other hand lowest data were with wheat and Barley under the 20% concentrations, wild oat under concentrations 15 and 20%, Canary grass and black mustard weeds under concentrations 10,15 and 20%.

Plumule length, plumule elongation velocity and data for radicle, plumule and total dry weight indicate significant differences between recorded values and the highest value is with control treatment of barley crop and the lowest were with wheat and Barley under the 20% concentrations, wild oat under concentrations 15 and 20%, Canary grass and black mustard weeds under concentrations 10, 15 and 20%, the highest record of root shoot ratio was with wheat crops and lowest data were with wheat and Barley under the 20% concentrations, wild oat under concentrations 15 and 20%, Canary grass and black mustard weeds under concentrations 10, 15 and 20%.

The used index in this study was seedling vigor index where the most vigor seedling was wheat seedling under control treatment and the lowest were with wheat and Barley under the 20% concentrations, wild oat under concentrations 15 and 20%, Canary grass and black mustard weeds under concentrations 10, 15 and 20%, the data for radicle and plumule growth inhibition indicates highest data for wheat and Barley under the 20% concentrations, wild oat under concentrations 15 and 20%, Canary grass and black mustard weeds under concentrations 10,15 and 20% meanwhile the lowest was with control treatment of all tested plant species. It is important to evaluate the sensitivity of tested plant species toward extracts of radish plant in order to name the most sensitive plant that could be the key for new studies toward the selectivity of allelochemicals by plant species (Belz, 2007) and (Macias *et al.*, 1999).

Thus in above combination of plant parts and species it is obvious that the three tested weed plants wild oat, canary grass and black mustard weeds were the most sensitive and the three plant species are regarded as important weed plants in cereal fields (Ali, 2009).

Isolation and identification of some poly-phenols from Radish Root parts

This part of study was conducted in Agricultural college, Salahaddin University laboratories using high performance liquid chromatography (HPLC) Merck Hitachi Lachrom UV Detector L-7400 and Pump L-7110, Germany made instrument and monitoring wave length 225 nm to identify poly-phenols of radish root parts.

Isocratic elution's were performed with a mobile phase of acetonitrile 30% and deionized water 70%, the injection volume was 10 μ l of solution (Takatori *et al.*, 2011; Ali, 2009; Sakakibara *et al.*, 2003). Standards of Caffeic acid (3,4-Dihydroxy-cinnamic acid),

Vanillic acid (4-hydroxy-3-methoxybenzoic acid), Chlorogenic acid (1,3,4,5- Tetra hydrox-ycyclohexane carboxylic acid), Ferulic acid (4-hydroxy-3-methoxycinnamic acid), Protocat-echuic acid (3,4-dihydroxy benzoic acid) and Syringic acid (4-hydroxy-3,5-Dimethoxy-benzoic acid) were used as slandered library for polyphenolic compounds. Results indicated only three peaks from studied standard curves that have been mentioned previously (Fig. 9). Root part compounds were vanillic acid, Ferulic acid and an unknown compound.

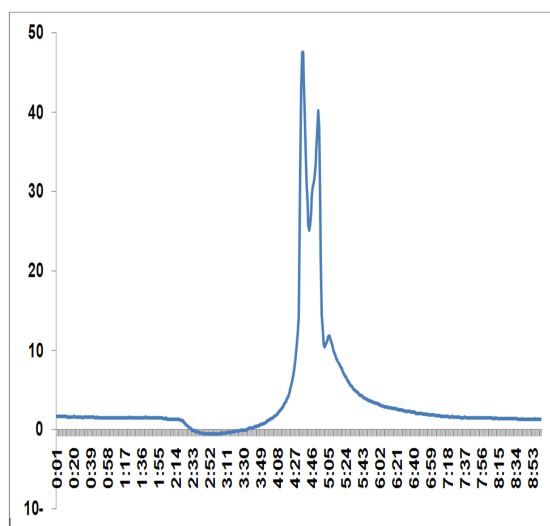


Fig. 9. The HPLC graph of Radich aqueous extract.

1) Vanillic acid (4-hydroxy-3-methoxybenzoic acid): found in the in roots aqueous extracts. It was reported as a cause for shoot and root inhibitor in wheat, annual bluegrass and rice, as well as inhibition of germination and growth parameters in lettuce plants (Einhelling, 2004 and Zhou and Yu, 2006).

2) Ferulic acid (4-hydroxy-3-methoxycinnamic acid): found in radish root aqueous extracts. It was reported as inhibitor of soybean germination and growth due to its inhibitory effect on protein synthesis. The same act was reported on cucumber and lettuce plants (Blum, 1998; Li *et al.*, 2010)

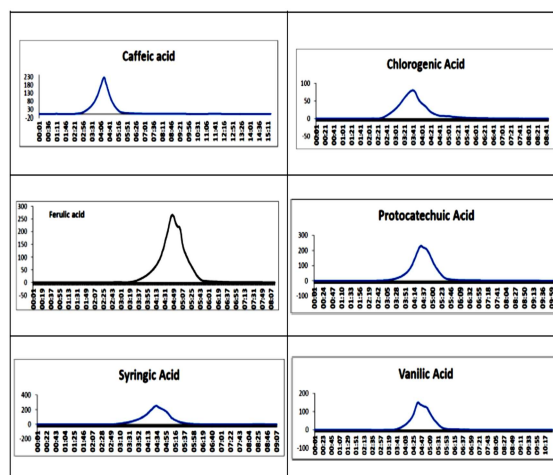


Fig. 10. Chromatograms phenolic acids expected in Radish extracts.

Conclusion

Results of this study reveals that radish root parts contain an effective biochemics which might be extracted, purified to control or eradicate the prevalence of noxious weeds in crop fields without any pollution or environment damages compared to synthetic herbicides due to its ecofriendly characteristics.

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