Plant Archives Vol. 19 No. 1, 2019 pp. 587-592 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

BLACK WALNUT (*JUGLANS NIGRA* L.) ROOTS EXTRACT EFFECTS ON SEEDS GERMINATION AND GROWTH OF TOMATO (*SOLANUM LYCOPERSICUM* L.) AND RADISH (*RAPHANUS RAPHANISTRUM* L.)

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Abstract

Allelopathy is an important mechanism of plant intervention mediated by releasing of allelochemicals into the soil rhizosphere. Black walnut roots produce allelo chemicals, mainly juglone that negatively or positively affects plants growth. The allelopathic effects of black walnut roots extract on seeds germination and growth of tomato and radish plants were investigated. Our results showed that both seeds germination and growth of tomato were significantly inhibited by walnut roots extract. Only 10% of tomato seeds was germinated; the shoot fresh weight and length of grown plants were significantly reduced, $P< 0.05$. However, seeds germination and growth of radish plant were unaffected. From allelopathic point of viewsensitive species to jugloneshouldnot be planted in areas adjacent to walnut trees. With the increasing importance of organic agriculture, extract from walnut roots can be commercially used to synthesize juglone-based bio-herbicides.

Key words : Allelopathy, Allelochemicals, Germination, Growth, Juglone.

Introduction

Allelopathy refers to the release of chemical substances into the environment by one plant species that inhibit or enhance growth of other species (Walsh, 2013; Nowicka, Żądło *et al*., 2017). The chemical substances can be found in several parts of plant, and the active compounds constitute about 5% of dry weight (Willis, 2007). Allopathic plants have several mechanisms such asexudation, volatilization, and leaching to release their allelochemicals into the environment. Allelochemicals range from acids and basis to simple organic substances that serve to reduce the effects of competition between plant species for various limiting factors such as nutrients, light, and space (Willis, 2007). Allelopathy can impact many features of plant that include plant growth, diversity, dominance, structure of plant communities, and plant succession (Ferguson and Rathinasabapathi, 2003). Allopathic suppression in plants is more complex and may include different interaction of chemicals such as alkaloids, amino acids, terpenoids and phenolic compounds with mixture of different substances that have a stronger effect of allelopathy than individual substance alone (Ferguson and Rathinasabapathi, 2003). Allelopathy has an important role in diseases resistance of plants whereby many plants produce various compounds, either before or after infection by specific pathogens, making the plants more resistant to diseases caused by the pathogens (Anuar and Ahmad, 2015).

Juglone, 5-hydroxy-1, 4-naphtoquinone, is an aromatic organic compound that is found in nature. Juglone, gallic acid, caffeic acid, myricetin and quercetin terpenoids and flavonoids are phenolic compounds produced by black walnut tree (*Juglans nigra*) (Sharma *et al*., 2009). Juglone is an allelopathic chemical found in all parts of black walnut tree (Nowicka, Żądło *et al*., 2017). Juglone's precursor or juglone is producedmainly in the black walnut tree roots and buds; however, it is also found in fresh walnut leaves (Solar *et al*., 2006), stems bark (Mouhajir *et al*., 2001), husks (Stampar *et al*., 2006) and inner roots bark (Hedin *et al*., 1979). Black walnuts, long-lived trees, are valuable hardwood timber trees, which are grown

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for their edible nuts and as shade trees (McCoy Ye *et al*., 2017). A mature black walnut tree has a toxic zone varies from 50-60 feet radius from its trunk, while the toxic area of a young black walnut tree is twice of the tree high. Juglone has a low solubility in water and does not diverge into the soil. Black walnut toxicity can remain in the soil several years after a tree is removed because decomposition of walnut roots remains releasing juglone into the soil. Most of the researches on walnut toxicity is focused on juglone and walnut leaf extract. In this study, two common plants; tomato (*Solanum lycopersicum*) and radish (*Raphanus raphanistrum*) were chosen as target species. Seedling, shoot fresh weight and length of grown plantswere used as indices to examine black walnut roots extract effects on tomato and radish in term of enhancing or suppressing seeds germination and plants growth.

Materials and Methods

Black walnut roots extract preparation

The hydro-extract was prepared by drying black walnut roots at 70^0C for 48 h. Then, 10gm of the dried roots was homogenized with 100 ml of distilled water by a blender. The solution was filtered and centrifuged at 3000 rpm for 3 min. The supernatant was used to make the dilution 10% (v/v) with distilled water. This dilution was used in both seeds germination and watering grown plants. The roots were taken from old trees because the younger than 7 years black walnut trees do not have sufficient juglone to cause toxicity.

Germination experiment

Tomato and radish seeds, the germination papers, and the commercial potting mix; PRO-MIX® Organic Vegetable & Herb Mix with MYCOACTIVE®1cu ft Loose Fill were purchased from the American Seed Company (Pennsylvania, USA), Hoffman manufacturing, Inc. (Oregon, USA) and the PRO MIX® (Pennsylvania, USA), respectively. The seeds were surface sterilized with 1% sodium hypochloride. Four sets of Petri dishes as germination environments were set in triplicates; 1) untreated control, 2) untreated control, 3) treated with black walnut roots extract and 4) treated with black walnut roots extract. The germination sets were set into two groups. Each group contained two sets; the untreated control and the treated with black walnut root extractPetri dishes, in triplicates. A germination paper and soil substrates were placed in each Petri dish of the control triplicates and soaked with tap water. The treated Petridishes contained germination papers and soil substrates that were soaked with black walnut roots extract 10% (v/v). In the first germination group, 10 tomato seeds were spread on each filter paper of the control and the treated Petri dishes. Likewise, 10 radish seeds were spread on the filter papers of the control and the treated Petri dishes of the second germination group. The dishes were labeled and placed ona cupboard in the Troy University's greenhouse (Alabama, USA) at 25^0C. The dishes were checked every day and after 7days,the germinated seeds were counted in the control and the treated dishes for both tomato and radish experimental sets.

Growth experiment

Four sets of planting jars; 1) untreated control, 2) untreated control 3) treated with black walnut roots extract, 4) treated with black walnut roots extract were filled with the commercial potting mix. The planting sets were organized into two groups, each group contained the untreated control and the treated with black walnut root extract, in triplicates. In the first group, 20 tomato seeds were planted in each of the control and the treatment planting jars. Similarly, 20 radish seeds were planted in each of the control and the treatment planting jars of the second group. The planting jars were labeled and placed in the green house. They were watered with tap water every day for 7 days. After 7 days, the grown plants were counted; 10 plants were left only and labeled in each planting jar. Then, the treated jars were watered every day with 10% (v/v) black walnut roots extract for 7 weeks. After that, the plants were cleaned from the soil residues and the shoot fresh weight and length were measured by a sensitive balance and a ruler, respectively for both tomato and radish plants.

Data analysis

The experiment was conducted using a randomized design with triplicates for both the untreated control and the treated with black walnut root extract. ANOVA *f*-test was used to determine the difference between the control and the treatment variances in plant fresh weight and length for both tomato and radish plants.

Results and Discussion

Walnut roots extract effects on seeds germination

There was 100% germination for both tomato and radish seeds in the control Petri dishes. Only 10% of tomato seeds was germinated in the treated Petri dishes; 2, 0 and 1 were the number of the germinated tomato seeds in each Petri dish of the treated triplicates. Allopathic sensitive plants show decreased germination rate while allopathic resistant plants show increased germination rate. Radish seeds germination was 100%

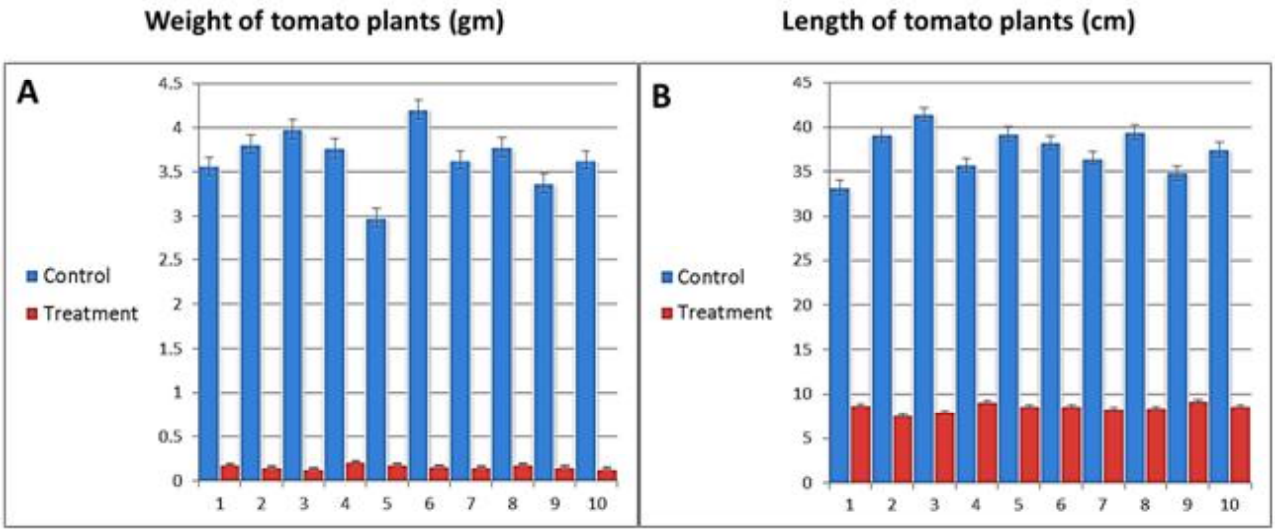

Fig. 1 : Black walnut roots extract effects on tomato plant after 7 weeks of watering the plants with 10% (v/v) walnut extract. (A) The extract effects on the weight of tomato plants. (B) The extract effects on the length of tomato plants.

Table 1 : Shoot fresh weight (gm) after 7 weeks of growth of tomato plants in the untreated control and treated with black walnut roots extract. The symbols C1, C2, C3 and T1, T2, T3 represent the individual triplicates of the control and the treatment, respectively.

C1	C2	C3	Mean	T1	T2	T3	Mean
3.652	3.836	3.215	3.567	0.178	0.172	0.196	0.182
3.256	3.972	4.230	3.819	0.150	0.181	0.140	0.157
4.291	3.722	3.942	3.985	0.140	0.128	0.135	0.134
3.585	3.692	4.041	3.772	0.275	0.188	0.176	0.213
3.300	2.450	3.193	2.981	0.206	0.185	0.171	0.187
4.317	3.983	4.338	4.212	0.183	0.154	0.167	0.168
3.686	3.215	4.015	3.638	0.185	0.132	0.150	0.155
3.780	3.576	4.000	3.785	0.210	0.183	0.168	0.187
3.850	2.875	3.403	3.376	0.161	0.173	0.140	0.158
3.717	3.224	3.964	3.635	0.150	0.123	0.145	0.139

in the treated with black walnut extract Petridishes. Similar to our finding, Azizi and Fuji (2005) found radish seeds germination was not affected by different concentrations of hydro-alcoholic extract of black zira (*Bunium pesicum*). Kocacë Aliskan and Terzi (2001) reported that the allelopathic effects of black walnut leaf extract and juglone inhibited seeds germination of tomato; however, seeds germination of radish was not affected by either walnut leaf extract or juglone addition.

Black walnut roots extract effects on tomato plants growth

Walnut roots extract had significant inhibitory effects on growth of tomato plants. In comparison with the untreated control, the fresh weight and length were significantly reduced after 7 weeks of watering tomato

Table 2 : *F*-test for the mean of weight triplicates of tomato plants in the untreated control and the treated with black walnut roots extract.

***F-Test* Two-Sample for Variances**

	Variable 1	Variable 2
Mean	3.677	0.168
Variance	0.112483	0.000592
Observations	10	10
df	9	9
F	189.9332	
P(F<=f) one-tail	3.55E-09	
F Critical one-tail	3.178893	

plants with 10% (v/v) walnut roots extract (tables 1 and 3; fig. 1). The means of shoot fresh weight and length of treated plants were reduced (tables 2 and 4). The correlation between variances of weight and length of the untreated tomato plants and the treated with walnut roots extract was significant, $P<0.05$ (tables 2 and 4). The correlation denotes to the phytotoxic activity of walnut roots extract on tomato plants. Wang *et al*. (2014) identified 28 compounds in black walnut leaf litter and several of these compounds were phytotoxic; of these phytotoxic compounds is juglone, which is produced mainly in the walnut tree roots. Juglon eaffects plant's growth by increasing oxidative stress (Segura-Aguila *et al*., 1992), disrupting photosynthesis (Jose and Gillespie, 1998; Huang *et al*., 2013), altering the hormonal balance in plant tissues (Bogatek and Gniazdowska, 2007) and inhibiting root and leaf respiration rates, stomatal conductance and transpiration (Jose and Gillespie, 1998). Wang *et al*. (2014) reported that the addition of black walnut leaf litter to lettuce plant reduced chlorophyll a, b and carotenoid

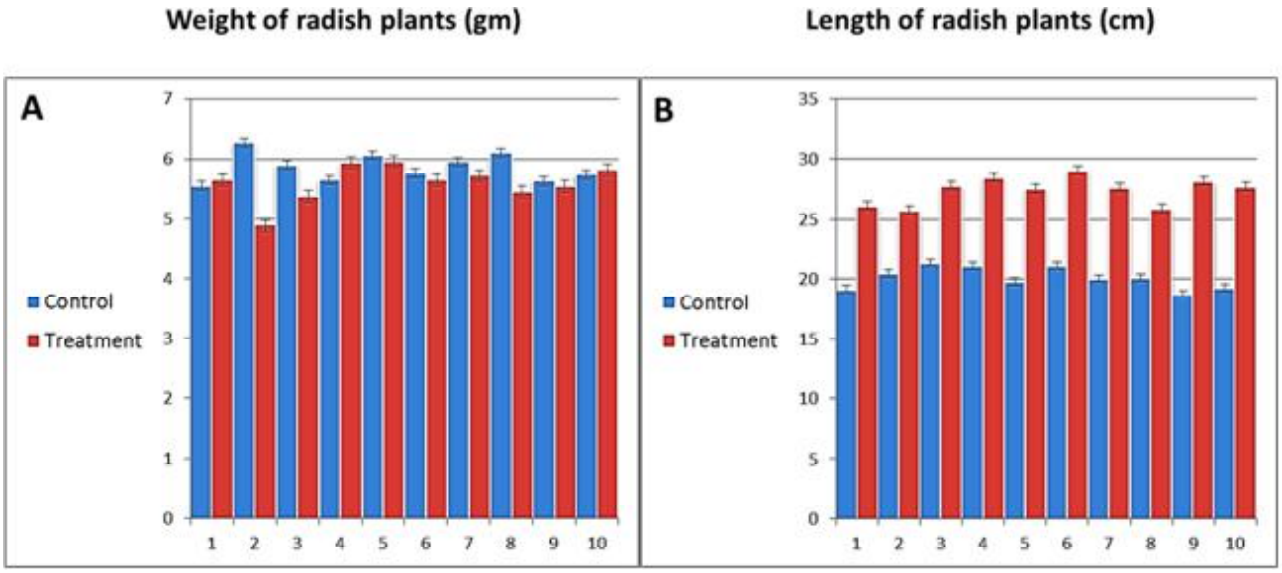

Fig. 2 : Black walnut roots extract effects on radish plant after 7 weeks of watering the plants with 10% (v/v) walnut extract. (A) The extract effects on the weight of radish plants. (B) The extract effects on the length of radish plants.

Table 3 : Shoot length (cm) after 7 weeks of growth of tomato plants in the untreated control and the treated with black walnut roots extract. The symbols C1, C2, C3 and T1, T2, T3 represent the individual triplicates of the control and the treatment, respectively.

C1	C2	C3	Mean	T1	T2	T3	Mean
32.05	32.37	35.35	33.25	8.22	8.24	9.80	8.75
35.75	42.18	39.62	39.18	8.30	7.76	6.83	7.63
37.95	46.05	40.37	41.45	8.15	7.38	8.43	7.98
38.00	32.38	36.85	35.74	8.53	9.98	8.85	9.12
36.25	44.10	37.54	39.29	8.74	9.33	7.96	8.67
35.85	37.74	41.25	38.28	8.63	8.84	8.70	8.72
37.52	35.35	36.75	36.54	8.70	9.16	7.19	8.35
34.80	39.85	43.74	39.46	8.53	9.20	7.78	8.50
35.75	32.50	36.28	34.84	8.68	9.89	9.20	9.25
38.38	40.00	34.30	37.56	8.83	8.47	8.74	8.68

contents. Kocacë Aliskan and Terzi (2001) demonstrated that tomato and garden cress are the most sensitive plants to juglone phytotoxicity. Tomato belongs to nightshade family (*Solanaceae*). Nightshade family species cannot survive near roots of black walnut trees because they produce and release juglone into the soil that causes mortality and alter plant species composition. The observed decreases in mean shoot weight and length of tomato plant might have resulted from juglone effects on photosynthesis, leaves and roots respiration and hormonal balance in plant tissues.

Black walnut roots extract effects on radish growth

After 7 weeks of watering the grown radish plants with walnut roots extract, the plants were slightly

Table 4 : *F*-test for the mean of length triplicates of tomato plants in the untreated control and the treated with black walnut roots extract.

***F-Test* Two-Sample for Variances**

	Variable 1	Variable 2
Mean	37.559	8.565
Variance	6.133899	0.236294
Observations	10	10
df	9	9
F	25.95871	

P(F<=f) one-tail 2.17E-05
F Critical one-tail 3.178893

Table 5 : Shoot weight (gm) after 7 weeks of growth of radish plants in the untreated control and the treated with black walnut roots extract. The symbols C1, C2, C3 and T1, T2, T3 represent the individual triplicates of the control and the treatment, respectively.

C1	C2	C3	Mean	T1	T2	T3	Mean
5.20	5.42	6.05	5.55	5.00	5.76	6.21	5.65
5.36	7.00	6.46	6.27	5.05	5.13	4.54	4.90
5.61	6.78	5.33	5.90	5.50	5.70	4.95	5.38
5.54	6.05	5.38	5.65	5.61	5.80	6.42	5.94
5.75	6.50	5.94	6.06	5.50	5.93	6.42	5.95
5.52	5.95	5.82	5.76	5.71	6.30	4.96	5.65
5.43	6.78	5.65	5.95	4.85	6.27	6.05	5.72
5.53	6.93	5.84	6.10	5.47	6.15	4.75	5.45
5.76	5.45	5.70	5.63	5.20	6.33	5.10	5.54
5.76	6.05	5.43	5.74	5.77	6.53	5.18	5.82

Table 6 : *F*-test for the mean of weight triplicates of radish plants in the untreated control and the treated with black walnut roots extract.

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	5.861	5.6
Variance	0.054766	0.096711
Observations	10	10
df	9	9
F	0.56628	

P(F<=f) one-tail 0.204871
F Critical one-tail 0.314575

Table 7 : Shoot length (cm) after 7 weeks of growth of radish plants in the untreated control and the treated with black walnut roots extract. The symbols C1, C2, C3 and T1, T2, T3 represent the individual triplicates of the control and the treatment, respectively.

C1	C2	C3	Mean	T1	T2	T3	Mean
20.05	21.15	16.28	19.16	24.78	26.25	27.44	26.15
19.85	20.90	20.62	20.45	25.72	27.10	24.35	25.73
19.73	20.85	23.38	21.32	25.85	28.50	29.05	27.80
21.15	19.86	22.15	21.05	26.70	30.15	28.53	28.46
19.76	18.45	21.15	19.78	25.95	29.38	27.27	27.53
23.20	20.35	19.76	21.10	30.76	31.15	25.26	29.05
18.70	20.45	21.05	20.06	28.05	26.73	28.17	27.65
20.00	18.56	21.80	20.12	26.28	27.50	23.85	25.87
20.05	17.88	18.25	18.72	25.85	30.25	28.38	28.16
17.75	20.80	19.20	19.25	28.34	25.62	29.15	27.70

Table 8 : *F*-test for the mean of length triplicates of radish plants in the untreated control and the treated with black walnut roots extract.

***F-Test* Two-Sample for Variances**

	Variable 1	Variable 2
Mean	20.101	27.41
Variance	0.792032	1.2716
Observations	10	10
df	9	9
F	0.622863	

P(F<=f) one-tail 0.245841
F Critical one-tail 0.314575

affected. The shoot fresh weight of treated plants was lower than the control (table 5, fig. 2A). The length of the treated radish plants was higher than the control (table 7, fig. 2B). The correlation between variances of shoot fresh weight and length of untreated radish plants and treated with walnut extract was insignificant, $P>0.05$ (tables 6 and 8). The correlation indicates that walnut roots extract has no phytotoxic effects on radish plants. Kocacë Aliskanand Terzi (2001) found that seedling growth of radish was slightly affected by either juglone or walnut leaf extract; however, seedling growth of muskmelon was increased. Phytotoxicity is a function of both plant species and the toxic compound (Dornbos and Spencer, 1990). The study showed that walnut extract has no toxic effects on radish and the extract addition to radish may enhance the growth of radish plants.

Conclusion

Black walnut roots extract was not phytotoxic to radish; seeds germination was unaffected by the extract and plant growth might have enhanced. However, the extract induced allelopathic stress in tomato, both seed germination and plant growth were inhibited. Juglone, which is mainly produced in black walnut roots, may be responsible for these inhibitory effects. The inhibition phenomenon of juglone should be tested on weed species as a natural weed control that has no environmental impacts. Black walnut roots extract can be commercially manufactured and used as an effective bio-herbicides for organic farming or in areas, where chemical herbicides are not allowed.

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