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Allelopathic Effects of Juglone and Walnut Leaf and Fruit Hull Extracts on Seed Germination and Seedling Growth in Muskmelon and Cucumber

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In this study, effects of the extracts of walnut fruit hulls and leaves on muskmelon (*Cucumis melo* cv. Galia) and cucumber (*Cucumis sativus* cv. Beith Alpha) seed germination percentage and post-germination seedling growth were investigated. Extracts were obtained by homogenization and centrifugation from walnut fruit hulls and leaves. Muskmelon and cucumber seeds were germinated in Petri dishes at 25 ºC. Seeds germination rates, seedling elongation and dry weights were determined at day 10. In conclusion, no significant effect of walnut extracts on muskmelon and cucumber seed germination percentage was determined. However, cucumber germination percentage was affected negatively by 1/4 walnut leaf extract and all juglone treatments. While the 1/4 diluted walnut fruit hull extract and 1/8 diluted walnut leaf extract increased root and stem growth of muskmelon seedlings, both walnut fruit hull and leaf extracts decreased root and stem growth of cucumber.

Key Words: Cucumber, Juglone, Muskmelon, Seed germination, Seedling growth, Walnut extracts.

INTRODUCTION

Allelochemicals are present in many types of plants and are relased into the rhizosphere by a variety of mechanism, including decomposition of residues, volatilazation and root exudation. Allelochemicals are known to affected germination, growth, devolopment, distribution and reproduction of a number of plant species[1].

The inhibitory effect of black walnut (*Juglans nigra*) on associated plant species is one of the oldest examples of allelopathy. The chemical responsible for walnut allelopathy is juglone (5-hydroxy-1,4 naphthoquinone)[2-7]. Juglone has been isolated from many plants in the walnut family (*Juglandaceae*) including *Juglans nigra*, *Juglans regia* and the others[8,9]. A colourless non-toxic reduced form called harmless hydrojuglone is abundant, especially in leaves, fruit hulls, stem and roots of walnut. When exposed to the air or to some oxidizing substance hydrojuglone is oxidized to its toxic form, juglone[10-13]. Rain washes juglone from the leaves and carries it into the soil. Thus, neighbour plants of the walnut are affected by absorbing juglone through their roots[3]. Walnut has been reported to be toxic to both herbaceous and

woody plants[3,14]. Affected plants turn brown, wilt and die. Most vegatable crops are vulnerable to juglone toxicity[15].

Juglon's allelopathic effects on plants are generally toxic, but beneficial on some plants[16-19]. In a previous study, it has been found that seedling growth of tomato, cucumber, garden cress and alfalfa were inhibited strongly by juglone and walnut leaf extracts, but seedling growth of muskmelon was increased by the juglone treatments[20]. Plants sensitive to the presence of walnut in the landscape and garden include tomato, potato, pea, apple, cucumber, watermelon, bean, garden cress, corn and many ornamental ericaceous species such as rhododendron and azalea[15]. Common midwest crops are susceptible to the presence of black walnut include corn and soybeans[21,22], wheat and alfalfa[13]. There are a handful of vegetable crops that are reportedly tolerant to juglone, including onions (*Allium cepa* L.)[23], Jerusalem artichokes (*Helianthus tuberasum* L.)[24], sugar beet (*Beta vulgaris* L.)[25] and certain species of bean (*Phaseolus* spp.)[23].

The physiological action of juglone is not well understood. Few studies have been done about juglon's inhibitory effect during seed germination and seedling growth[26]. Juglone inhibits plant growth by reducing photosynthesis and respiration[20,21,27-29], increasing oxidative stress[12], reducing chlorophyll content and some anatomical structures such as stomata, xylem vessel[21,26,29].

However, no study has been encountered about allelopathic effects of walnut fruit hull extracts. Therefore, based on our previous studies[20], indicated positive or negative results depending on plant species used, it was aimed here to determine the effects of juglone and fruit hull and leaf extracts of walnut (*Juglans regia* L. cv. Yalova-4) on seed germination and seedling growth of muskmelon and cucumber.

EXPERIMENTAL

Seeds of cucumber (*Cucumis sativus* cv. Beith Alpha) and muskmelon (*Cucumis melo* cv. Galia) were obtained from AGROMAR Company. The seeds were surface sterilized with 1 % sodium hypochlorite. At least 20 seeds were placed in a Petri dish layered with sheets of filter paper moistened with distilled water (control) or with the other treatments. Then the dishes were left in an incubator at 25 ºC in continuous dark[30]. After 10 d, germination percentages were recorded and post-germinative growths of the seeds were determined by measuring lengths and dry weights of the seedlings.

The treatments were as follow: (1) (a) Juglone (10^{-3} M), (b) Juglone (10^{-4} M), (c) Juglone (10^{-5} M), (2) walnut leaf and fruit hull extract (1/4 diluted), (3) walnut leaf and fruit hull extract (1/8 diluted) and (4) distilled water (control).

Walnut extracts were prepared from leaves and fruit hulls of *Juglans regia* 'Yalova-4' in the first week of August. The leaves were dried at 70 ºC in oven for 48 h. Later 10 g of walnut leaf and fruit hull was homogenized in 100 mL of distilled water by Waring Blender and filtered. The filtrate was centrifuged at 3000 rpm. The supernatant was used in experiments diluting 1/4, 1/8 ratios with distilled water.

Leaves of 12 year old walnut trees were used in obtaining the extracts because, walnut trees younger than 7 years old do not contain sufficient juglone to cause toxicity[9,25]. The leaves and fruit hulls picked in first week of August, since the juglone contents of walnut (*J. regia*) was found to be highest in the last week of July and the first week of August[31].

Juglone solution was prepared by dissolving in distilled water by stirring at 40 ºC for 24 h[20]. Afterwards, 10^{-3} M juglone were diluted with distilled water into 10^{-4} M and 10^{-5} M. These concentrations of juglone were used, since it occurs in soil of walnut plantation at this concentrations depending on walnut species and season[3,21,32,33]. Juglone (5-hydroxy-1,4-naphthoquinone) was purchased from SIGMA. Each treatment was replicated 3 times. Twenty seedlings were used in each replicate. To determine statistical difference between the treatments, variance analysis and least significant difference (LSD) tests were performed. In the tables, the standard deviations and errors as well as the LSD values (0.05) were indicated.

Percentage growth inhibition was calculated using the following equation:

$$\text{Percentage inhibition (\%)} = \frac{\text{Control value} - \text{Treatment value}}{\text{Control value}} \times 100$$

RESULTS AND DISCUSSION

No effect of walnut leaf and fruit hull extract as well as juglone on the percentage of muskmelon seed germination was observed (Table-1). That is, germination of muskmelon seed was % 100 in all the treatments. In previous studies, juglone and walnut leaf extract did not influence muskmelon seed germination[20,34]. The fact that muskmelon seed germination is not influenced by toxic effects of juglone indicates that there may be mechanism(s) for tolerance to juglone in muskmelon seed coat.

TABLE-1
EFFECTS OF JUGLON AND WALNUT LEAF AND FRUIT HULL EXTRACTS ON MUSKMELON AND CUCUMBER SEED GERMINATION (IN PERCENTAGES)

Treatment	Muskmelon	Cucumber
Control (H_2O)	100	100
Juglone (10^{-3} M)	100	50
Juglone (10^{-4} M)	100	60
Juglone (10^{-5} M)	100	80
1/4 Diluted walnut fruit hull extract	100	90
1/8 Diluted walnut fruit hull extract	100	100
1/4 Diluted walnut leaf extract	100	80
1/8 Diluted walnut leaf extract	100	100

LSD (0.05): 13.48

Cucumber seed germination was inhibited significantly by both juglone and one-fourth diluted walnut leaf extract (Table-1). Similarly, in previous studies, the seed germination of cucumber was affected negatively in a concentration dependent

manner by juglone and walnut leaf extract[20,26]. But in the present study, walnut fruit hull extract did not affected the cucumber seed germination.

While the root and stem elongations of muskmelon seedlings were increased by both 1/4 diluted walnut fruit hulls and 1/8 diluted walnut leaf extract, juglone and other dilutions (1/8 walnut fruit hulls and 1/4 diluted walnut leaf extract) decreased the same growth parameters (Table-2). It was found in the previous studies that juglone and walnut leaf extracts increased the growth of muskmelon seedlings[20,34]. The juglone decrease the muskmelon seedling elongation may be due to different variety of muskmelon used in this study. It is because the sensitivity to juglone varies from plant to plant as well as between subspecies[35,36]. Orcutt and Nilsen[37] suggested that plants can tolerate to allelochemicals due to (i) an ability to reduce uptake of allelochemicals from the root surface (ii) compartmentalization of allelochemicals away from molecular target sites or (iii) detoxification of allelochemicals.

TABLE-2
EFFECTS OF JUGLON AND WALNUT LEAF AND FRUIT HULL EXTRACTS ON MUSKMELON AND CUCUMBER SEEDLING ELONGATION (cm)

Treatment	Muskmelon		Cucumber	
	Root	Stem	Root	Stem
Control (H_2O)	7.7±0.40	5.9±0.35	9.9±0.36	4.5±0.25
Juglone (10^{-3} M)	6.3(-18)±0.37	4.5(-23)±0.41	1.9(-81)±0.29	1.8(-60)±0.25
Juglone (10^{-4} M)	6.5(-16)±0.34	4.9(-13)±0.38	6.4(-35)±0.37	2.9(-36)±0.41
Juglone (10^{-5} M)	7.2(-6)±0.40	5.2(-12)±0.41	6.6(-33)±0.42	4.1(-8)±0.38
1/4 Diluted walnut fruit hull extract	8.7(+13)±0.36	6.9(+13)±0.21	6.8(-31)±0.62	2.9(-36)±0.36
1/8 Diluted walnut fruit hull extract	4.7(-39)±0.60	3.9(-33)±0.15	7.2(-27)±0.30	3.1(-31)±0.40
1/4 Diluted walnut leaf extract	4.7(-39)±0.65	4.1(-31)±0.25	7.1(-28)±0.35	3.2(-29)±0.35
1/8 Diluted walnut leaf extract	8.4(+9)±0.36	6.2(+5)±0.35	7.3(-26)±0.25	3.3(-27)±0.45

LSD (0.05):1.49; (-) per cent inhibition; (+) per cent stimulation.

In the present study, walnut leaf and fruit hull extracts and juglone generally prevented cucumber root and stem elongation (Table-2). Similar results were indicated with juglone in previous studies in cucumber[20,26,31], tomato and bean[38], alfalfa[39], wheat and corn[21]. It is possible that juglone may decrease cucumber growth either by stimulating the synthesis of abscisic acid (ABA), a growth inhibitory hormone, or by preventing the synthesis of a growth-promoting hormone.

An interesting observation in this study is the blackening of root tips in the cucumber seeds during germination in juglone and walnut extracts. The reason of this is unknown but, it has been implicated with root sensitivity to juglone[25]. Apart from allelochemical stress this has also been reported in salt stress of bean and corn seeds related to polyphenol oxidase activity and the oxidation of phenols to quinones[40]. Affected plants turn brown, wilt and finally die. Most vegatable crops are vulnerable to juglone toxicity[15]. Blackening of root tips observed in this study may thus be due to quinones (normally brown) by means of polyphenol oxidase activity. On the

other hand, roots of cucumber were observed to grow upward as an antigravitropistic movement during seed germination (Fig. 1), that behaviour might be as a result of root escape from juglone. Similar root movement was observed in walnut extracts as well. As seen in Fig. 1, root deformation was also observed in juglone treated cucumber seeds.

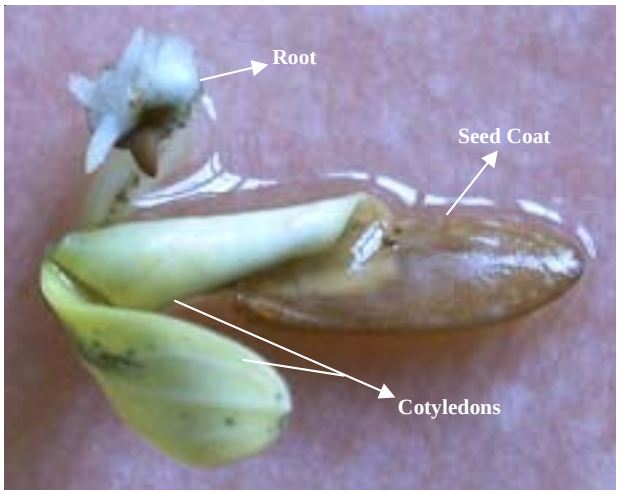

Fig. 1. Germination of a cucumber seed in juglone solution

While the root and stem dry weight of muskmelon seedlings were increased by both 1/4 walnut fruit hulls and 1/8 diluted walnut leaf extract, juglone and other dilutions (1/8 walnut fruit hulls and 1/4 diluted walnut leaf extract) decreased the same parameters (Tables 2 and 3). In previous studies, it was found that juglone and walnut leaf extracts increased the dry weights of muskmelon seedlings[20,34]. Some plants appear to have a protective capacity against oxidative stress from juglone by emitting enzymes that metabolize the coumpound to less toxic hydrojuglones[12]. Dry weights of root and stem of cucumber seedlings was influenced negatively by walnut leaves and fruit hull extracts and juglone, depending on the concentration. There is similarity between root and stem elongations. In pervious studies, it was determined that juglone and walnut leaf extracts decreased cucumber dry weights[20,26]. The stress caused by juglone is called allelochemical stress because juglone is an allelochemical. This condition can cause stressful situation in several plants[4,16]. In present study, the stress especially as a result of juglone treatment resulted a decrease in the seedling dry weight.

Rietveld[3] found that elongation and dry weight accumulation of root was less affected than the shoot by juglone in 16 plant species. Whereas in present study, inhibitory effects of treatments on elongation and dry weights of root and shoot were almost the same in the species studied. This discrepancy may be partly due to the differential response of various plant species to the juglone treatments.

TABLE-3
EFFECTS OF JUGLON AND WALNUT LEAF AND FRUIT HULL EXTRACTS ON MUSKMELON AND CUCUMBER SEEDLING DRY WEIGHT (mg)

Treatment	Muskmelon		Cucumber	
	Root	Stem	Root	Stem
Control (H_2O)	0.41±0.014	0.57±0.018	0.40±0.017	0.38±0.013
Juglone (10^{-3} M)	0.37(-9)±0.017	0.44(-23)±0.021	0.08(-80)±0.011	0.03(-85)±0.004
Juglone (10^{-4} M)	0.39(-5)±0.023	0.38(-33)±0.033	0.24(-40)±0.023	0.30(-31)±0.031
Juglone (10^{-5} M)	0.43(+5)±0.028	0.44(-23)± 0.036	0.30(-25)±0.023	0.32(-28)±0.031
1/4 Diluted walnut fruit hull extract	0.46(+12)±0.015	0.67(+17)±0.024	0.37(-8)±0.016	0.33(-16)±0.014
1/8 Diluted walnut fruit hull extract	0.37(-10)±0.016	0.41(-28)±0.014	0.38(-9)±0.016	0.32(-16)±0.014
1/4 Diluted walnut leaf extract	0.29(-29)±0.018	0.39(-32)±0.015	0.38(-9)±0.021	0.34(-16)±0.011
1/8 Diluted walnut leaf extract	0.44(+7)±0.027	0.59(+4)±0.031	0.37(-7)±0.021	0.36(-16)±0.019

LSD (0.05):1.49; (-) per cent inhibition; (+) per cent stimulation.

The physiological roles of allelochemicals have not been completely determined in plants[4,16]. While juglone was found to decrease the growth of several plants, it exerts growth stimulatory effect on some other plants[3,20]. The present study is in agreement with previous studies in this respect. The conclusion that derived from the present study is that root and stem elongation, dry weight of cucumber seedlings are negatively affected. In the case of muskmelon seedlings, the elongation of root and stem as well as the dry weight were increased positively by both 1/4 diluted walnut fruit hull and 1/8 diluted walnut leaf extract. Nonetheless, juglone, 1/8 diluted walnut leaf extract decreased the root and stem elongation as well as the dry weight of muskmelon seedlings (Tables 2 and 3).

As conclusion, the results presented have indicated that there is a significant amount of juglone in walnut hulls, based on the inhibitory effects of walnut hull extract on cucumber seedling growth without any effects on muskmelon growth, except 1/4 diluted leaf extract and 1/8 diluted fruit hull extract. Although allelopathic effects of walnut leaf extracts were examined previously, no studies related to the effects of walnut fruit hull extracts were reported to date. Further experiments are required to test the effects of the juglone from walnut leaf and fruit hull extracts on a wide varieties of plant species.

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