Full Length Research Paper

Allelopathic effects of Juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination and seedling growth

I. Terzi

Department of Biology, Faculty of Education, Dumlupınar University, Kütahya, Turkey. E-mail: iterzi@dumlupinar.edu.tr. Tel: + 90 0274 2652031. Fax: (Office): +90 0274 2672057.

Accepted 4 April, 2008

In this study, effects of juglone and decomposed walnut leaf juice on muskmelon (*Cucumis melo* cv. Galia) and cucumber (*Cucumis sativus* cv. Beith Alpha) seed germination percentage and postgermination seedling growth were investigated. Decomposition was carried out by keeping the leaves in distilled water. Muskmelon and cucumber seeds were germinated in Petri dishes at 25 °C. Seed germination, seedling elongation and weights were determined at day 10. No significant effect of juglone and decomposed walnut leaf juice on muskmelon seed germination percentage was observed. However, germination of cucumber seed was inhibited significantly. It was found that while both the dry weight root and stems elongation of muskmelon and cucumber seedlings were affected negatively by juglone and undiluted decomposed walnut leaf juices. The negative effect decreased as dilution ratios of decomposed leaf juice increased.

Key words: Cucumber, germination, juglone, muskmelon, walnut leaf.

INTRODUCTION

Allelochemicals are present in many types of plants and are released into the rhizosphere by a variety of mechanism, including decomposition of residues, volatilazation, and root exudation. These chemicals are known to affect germination, growth, devolopment, distribution and reproduction of a number of plant species (Inderjit and Malik, 2002)

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) (Davis, 1928; Rietveld, 1983; Rice, 1984; Ponder and Tadros, 1985; Willis, 2000; Jose, 2002). Juglone has been isolated from many plants in the walnut family (*Juglandaceae*) including *Juglans nigra*, *Juglans regia* and the others (Daglish 1950; Prataviera et al., 1983). A colourless nontoxic reduced form called harmless hydrojuglone is abundant, especially in leaves, fruit hulls, stem and roots of walnut. When exposed to air or oxidizing substances, hydrojuglone is oxidized to its toxic form, juglone (Lee and Campbell, 1969; Dana and Lerner, 1990; Segura-Aguilar et al., 1992; Bertin et al., 2003). Rain

washes juglone from the leaves and carries it into the soil. Thus, neighbouring plants of the walnut are affected by absorbing juglone through their roots (Rietveld, 1983). Walnut has been reported to be toxic to both herbaceous and woody plants (Funk et al., 1979; Rietveld, 1983). Affected plants turn brown, wilt, and die. Most vegatable crops are vulnerable to juglone toxicity (Crist and Sherf, 1973).

Juglon's allelopathic effects on plants are generally toxic but beneficial on some cases (Hale and Orcutt, 1987; Rice, 1979; Whittaker and Feeny, 1971; Rizvi and Rizvi, 1992). 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 treatments (Kocaçalışkan and Terzi, 2001). Plants sensitive to the presence of walnut in the land-scape and garden include tomato, potato, pea, apple, cucumber, watermelon, bean, garden cress, corn and many ornamental ericaceous species such as rhododendron and azalea (Crist and Sherf, 1973). Common Midwest (USA) crops are susceptible to the presence of

black walnut, and these include corn and soybeans (Jose and Gillespie, 1998; Hejl and Koster, 2004), wheat and alfalfa (Bertin et al., 2003). There are a handful of vegetable crops that are reportedly tolerant to juglone, including onions (*Allium cepa* L.) (Macdaniels and Pinnow, 1976), Jerusalem artichokes (*Helianthus tuberasum* L.) (Ross 1996), sugar beet (*Beta vulgaris* L.) (Piedrahita 1984), and certain species of bean (*Phaseolus* spp.) (Macdaniels and Pinnow 1976).

The physiological action of juglone is not well understood. Very few studies have been done about juglon's inhibitory effect during seed germination and seedling growth (Terzi et al., 2003b). Juglone inhibits plant growth by reducing photosynthesis and respiration (Einhelling, 1986; Balke et al., 1987; Hejl et al., 1993; Jose and Gillespie, 1998; Kocaçalışkan and Terzi, 2001), increasing oxidative stress (Segura-Aguilar et al., 1992), reducing chlorophyll content and some anatomical structures such as stomata, xylem vessel (Hejl et al., 1993; Jose and Gillespie, 1998; Terzi et al., 2003b)

In previous studies, effects of juglone and walnut leaf extracts on various plant species were investigated (Einhellig, 1986; Hejl et al., 1993; Kocaçalışkan and Terzi, 2001). However, allelopathic effects of decomposed walnut leaves were not reported. This study aims here to determine the effects of juice of decomposed walnut leaves on seed germination and seedling growth of muskmelon and cucumber.

MATERIALS AND METHODS

Seeds of cucumber (*Cucumis sativus* cv. Beith Alpha) and musk-melon (*Cucumis melo* cv. Galia) were obtained from AGROMAR Company. The seeds were surface sterilized with 1% sodium hypochloride. At least 20 seeds were placed in a Petri dish furnished 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 °C in continuous darkness (Şeniz, 1993). After 10 days, 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; juglone (10⁻³ M), juglone (10⁻⁴ M), juglone (10⁻⁵ M), decomposed walnut leaf juice (undiluted), decomposed walnut leaf juice (1/2 diluted), decomposed walnut leaf juice (1/4 diluted), decomposed walnut leaf juice (1/8 diluted), and distilled water (Control).

Decomposed walnut leaf juice was prepared from leaves of *Juglans regia* 'Kr-2 Yavuz-1'. The leaves were dried at 70 °C in oven, for 48 h. Later, 10 g walnut leaves were left in the 1 L distilled water for 24 months. During that time decomposed leaf parts were precipitated and the upper juice phase was taken to use. The juice was used in experiments diluting 1/2, 1/4, 1/8 ratios with distilled water or without diluting. Leaves of twelve-year-old walnut trees were used in obtaining the juice because, walnut trees younger than seven-years-old do not contain sufficient juglone to cause toxicity (Prataviera et al., 1983; Piedrahita, 1984). The leaves 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 (Tekintaş et al., 1988).

Juglone (5-Hydroxy-1,4 naphthoquinone) 10^3 M solution was prepared by dissolving in distilled water by constantly stirring at 40^0 C for 24 h. Afterwards, 10^3 M juglone were diluted with distilled

water into 10⁻⁴ and 10⁻⁵ M. These concentrations of juglone were used, since it occurs in soil of walnut plantation at these concentrations depending on walnut species and season (Rietveld, 1983; Jose and Gillespie, 1998; Thevathasan et al., 1998; Thevathasan and Gordon, 2004). The juglone was purchased from SIGMA. Each treatment was replicated three times. 20 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: Percentage inhibition (%) = [(Control value treatment value)/Control value] x 100.

RESULTS AND DISCUSSION

No effect of decomposed walnut leaf juice 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 (Kocaçalışkan and Terzi 2001; Terzi et al., 2003a). 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. Some plants appear to have a protective capacity against oxidative stress from juglone by emitting enzymes that metabolize the coumpound to less toxic hydrojuglones (Segura-Aguilar et al., 1992; Matvienko et al., 2001).

Cucumber seed germination was inhibited significantly by both juglone and decomposed walnut leaf juice with exception of 1/4 and 1/8 dilutions of the walnut juices (Table 1). Juglone is known to inhibit germination of various plant species (Fisher, 1978; Reynolds, 1987; Rietveld, 1983). Similarly, in several previous studies, the seed germination of cucumber is affected negatively in a concentration dependent manner by juglone and walnut leaf extract (Tekintaş et al., 1988; Orcutt and Nilsen, 2000; Kocaçalışkan and Terzi, 2001). On the other hand, in the present study juglone and decomposed walnut leaf juices decreased the cucumber seed germination in concentration-dependent manner.

While the root elongation of muskmelon seedlings in control and 1/4 diluted decomposed walnut leaf juice was the same, both the root and the stem elongation of muskmelon seedlings in all juglone and other decomposed walnut leaf dilutions decreased slightly (Table 2). It was found in the previous studies that juglone and walnut leaf extracts increased the growth of muskmelon seedlings (Kocacalıskan and Terzi, 2001; Terzi et al., 2003a). That juglone decreased muskmelon seedling elongation in the study may be due to different cultivars of muskmelon used. This is because the sensitivity to juglone varies from plant to plant and between subspecies (Bettina et al., 1990; Yu and Matsui, 1997). Orcutt and Nilsen, (2000) suggested that plants can tolerate to allelochemicals due to (i) an ability to reduce uptake of allelochemicals from the root surface, (ii) compartmen-

Table 1. Effects of juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination.

Treatment	Muskmelon (%)	Cucumber (%)
Control (dH₂O)	100	100
Juglone (10 ⁻³ M)	100	50
Juglone (10 ⁻⁴ M)	100	60
Juglone (10 ⁻⁵ M)	100	80
Undiluted decomposed walnut juice	100	70
1/2 diluted decomposed walnut juice	100	80
1/4 diluted decomposed walnut juice	100	90
1/8 diluted decomposed walnut juice	100	100

LSD (0.05): 19.1.

Table 2. Effects of juglone and decomposed walnut leaf juice on muskmelon and cucumber seedling elongation (cm).

	Muskmelon		Cucumber	
Treatment	Root	Stem	Root	Stem
Control (dH ₂ O)	7.5 ± 0.42	4.7± 0.35	10.3± 0.54	4.7± 0.37
Juglone (10 ⁻³ M)	6.3 ± 0.37 (16)	4.2 ± 0.30 (11)	2.1 ± 0.29 (80)	1.8 ± 0.25 (62)
Juglone (10 ⁻⁴ M)	6.5 ± 0.34 (13)	3.9 ± 0.38 (17)	6.2 ± 0.37 (40)	3.7 ± 0.41 (21)
Juglone (10 ⁻⁵ M)	7.2 ± 0.40 (4)	4.4 ± 0.41 (6)	6.8 ± 0.42 (34)	4.1 ± 0.38 (9)
Undiluted decomposed walnut juice	6.8 ± 0.43 (9)	3.3 ± 0.29 (30)	7.1 ± 0.49 (31)	4.0 ± 0.35 (15)
1/2 diluted decomposed walnut juice	6.6 ± 0.38 (12)	3.5 ± 0.33 (26)	7.4 ± 0.55 (28)	4.2 ± 0.32 (11)
1/4 diluted decomposed walnut juice	7.5 ± 0.42 (0)	3.8 ± 0.35 (19)	8.3 ± 0.61 (19)	4.4 ± 0.36 (7)
1/8 diluted decomposed walnut juice	7.1 ± 0.37 (15)	4.2 ± 0.31 (11)	9.2 ± 0.58 (11)	4.5 ± 0.42 (4)

[±] SD, LSD (0.05): 1.53.

Values in parantheses are percent inhibition.

talization of allelochemicals away from molecular target sites or (iii) detoxification of allelochemicals. Some plants appear to have a protective capacity against oxidative stress from juglone by emitting enzymes that metabolize the coumpound to less toxic hydrojuglone (Segura-Aguilar et al., 1992; Matvienko et al., 2001).

In the present study, juglone and decomposed walnut leaf juice significantly prevented cucumber root elongation. While cucumber stem elongation was significantly affected in the presence of 10⁻³ M juglone concentration. other concentrations of juglone or decomposed walnut leaf slightly interfered with cucumber stem elongation. Similar results were indicated with juglone in earlier studies in cucumber (Tekintaş et al., 1988), tomato and bean (Neave and Dawson, 1989), alfalfa (Dornbos and Spencer, 1990), wheat and corn (Jose and Gillespie, 1998). Likewise, previous studies indicated that cucumber seed elongation was decreased by juglone and walnut leaves. Also, it was stated that cucumber seedling elongation was decreased by juglone and walnut leaf extract (Kocacalıskan and Terzi, 2001; Terzi et al., 2003 b). It is possible that juglone decreased cucumber growth either by stimulating the synthesis of 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. Although the reason of this is unknown, it has been implicated with root sensitivity to juglone (Orcutt and Nilsen, 2000). 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 (Hejl et al., 1993). Affected plants turn brown, wilt, and die. Most vegatable crops are vulnerable to juglone toxicity (Crist and Sherf, 1973).

While the root dry weight of muskmelon seedlings in control and 1/4 diluted decomposed walnut leaf was the same, both the root and the stem dry weight of muskmelon seedlings in all juglone and other decomposed walnut leaf dilutions decreased slightly (Table 3). In previous studies, it was found out that juglone and walnut leaf extracts increased the dry weights of muskmelon seedlings (Kocaçalışkan and Terzi, 2001; Terzi et al., 2003a). Dry weights of root and stem of cucumber seedlings was influenced negatively by decomposed walnut leaves and juglone, depending on the concentration. There is similarity between root and stem elongations. In several pervious studies, it was determined that juglone and walnut leaf extracts decreased cucumber

	Muskmelon		Cucumber	
Treatment	Root	Stem	Root	Stem
Control (dH ₂ O)	0.44± 0.025	0.46± 0.031	0.59± 0.043	0.58± 0.045
Juglone (10 ⁻³ M)	0.38±0.019(14)	0.40±0.028(13)	0.11±0.016(81)	0.07± 0.021(99)
Juglone (10 ⁻⁴ M)	0.39± 0.023(4)	0.38±0.033(17)	0.24± .023(60)	0.40± 0.031(31)
Juglone (10 ⁻⁵ M)	0.43± 0.028(5)	0.44± 0.036(5)	0.30±0.023(50)	0.40± 0.031(28)
Undiluted decomposed walnut juice	0.40± 0.025(9)	0.30± 0.027(35)	0.32±0.029(46)	0.44± 0.052(24)
1/2 diluted decomposed walnut juice	0.39± 0.029(4)	0.32± 0.024(31)	0.34± 0.037(42)	0.48± 0.043(17)
1/4 diluted decomposed walnut juice	0.44± 0.027(0)	0.35± 0.028(24)	0.38± 0.041(36)	0.51± 0.047(12)
1/8 diluted decomposed walnut juice	$0.42 \pm 0.023(6)$	0.40± 0.034(13)	0.43± 0.039(24)	0.53± 0.044(10)

Table 3. Effects of juglone and decomposed walnut leaf Juice on muskmelon and cucumber seedling dry weight (mg).

Values in parantheses are percent inhibition.

± SE, LSD (0.05): 0.08.

dry weights (Kocaçalışkan and Terzi, 2001; Terzi et al., 2003b). The stress caused by juglone is called allelochemical stress. This condition can cause stressful situation in several plants (Rice, 1984; Rizvi and Rizvi, 1992). In the present study, the stress as a result of juglone treatments resulted in a decrease in the plant dry weight.

Rietveld (1983) found that elongation and dry weight accumulation of root was less affected than the shoot by juglone in 16 plant species. Whereas in the present study, inhibitory effects of the 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.

The physiological roles of allelochemicals have not been completely determined in plants. An allelochemical can be beneficial in one plant, or harmful in another plant. This depends on allelochemicals type, density, and on the time of treatment. (Whittaker and Feeny, 1971; Rice, 1979; Hale and Orcutt, 1987; Rizvi and Rizvi, 1992). The conclusion that derived from in the present study is that root and stem elongation, dry weight of cucumber seedlings are negatively affected. It was found that in the case of muskmelon seedlings, as well as the dry weight, the elongation of root and stem decreased with juglone and diluted decomposed walnut leaf juices in a concentration-dependent manner (Table 2 and 3).

Although allelopathic effects of walnut leaf extracts have been examined previously, no studies related to the effects of decomposed walnut leaf have been reported to date. Further experiments are required to test the effects of the juglone obtained from decomposed walnut leaf on a wide varieties of plant species.

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