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BRIEF COMMUNICATION

Effects of juglone on growth of muskmelon seedlings with respect to physiological and anatomical parameters

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Abstract

Growth parameters (seedling elongation, fresh and dry masses) and protein content of *Cucumis melo* were enhanced by juglone (allelochemical occuring in the walnut family) treatment in pregerminative stage but decreased in postgerminative treatment. Catecholase and tyrosinase activities were also increased in both treatments. Xylem vessel radius of stem was enhanced significantly by the pregerminative treatment, whereas it decreased slightly by the postgerminative treatment. However, bundle radius of stem was enhanced by both treatments of juglone. Stomata length and number were not changed significantly. Xylem vessel radius of the stem was affected by juglone more than the other parameters.

Additional key words: anatomy, catecholase, *Cucumis melo*, elongation, fresh and dry mass, proteins, stem radius, stomata, tyrosinase, xylem vessel radius.

The inhibitory effect of black walnut (*Juglans nigra*) on associated plant species is one of oldest examples of allelopathy. Juglone (5-hydroxy-1,4-naphthoquinone) is an allelochemical responsible for walnut allelopathy (Davis 1928, Rice 1984). Juglone has been isolated from many plants of the walnut family (*Juglandaceae*) including *J. nigra* and *J. regia* (Daglish 1950, Prataviera *et al.* 1983). A colourless, nontoxic reduced form called hydrojuglone is abundant, especially in leaves, fruit hulls, and roots of walnut tree. When exposed to air or to some oxidizing substance, hydrojuglone is oxidized to its toxic form, juglone (Lee and Campbell 1969, Segura-Aguilar *et al.* 1992). 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 (Rietveld 1983). Walnut is toxic to both herbaceous and woody plants (Funk *et al.* 1979, Rietveld 1983). Juglone effects on plants are generally negative but it may be beneficial on seedling growth of muskmelon (Kocaçalışkan and Terzi 2001). Juglone inhibits plant growth by reducing rates of photosynthesis and respiration (Hejl *et al.* 1993, Jose and Gillespie 1998) and increasing oxidative stress (Segura-Aguilar *et al.* 1992). However, no information about the effect of juglone on anatomical parameters is available. Therefore, the aim of this study was to determine the effect mode of juglone on physiological and anatomical parameters of muskmelon.

Seeds of muskmelon (*Cucumis melo* cv. Kış kavunu) obtained from *Agromar Company*, Turkey were surface sterilized with 1 % sodium hypochloride. At least 20 seeds were placed in a Petri dish furnished with sheets of filter paper. 1 mM juglone (*Sigma*, USA) solution was prepared by dissolving in distilled water stirring at 40 °C for 24 h (Kocaçalışkan and Terzi 2001). 1 mM juglone was used since it occur at this concentration in soil of walnut plantations (Rietveld 1983). Juglone solution was applied either before germination into Petri dishes containing muskmelon seeds or into plastic pots with *Perlite* containing 7-d-old muskmelon seedlings. Distilled water was used as control for each group. Each treatment was replicated three times and at least 20 seeds were used in each replicate. After juglone treatments Petri dishes and plastic pots were left in a plant growth chamber for ten days (14-h photoperiod, irradiance of 54 W m^{-2}, day/night temperature of 25/18 °C and relative humidity 70/80 %).

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For measuring protein content and polyphenol oxidase activities, 0.5 g of leaves was homogenized in 5 cm^3 0.1 M phosphate buffer of pH 6.5 and centrifuged at 3 500 *g* for 10 min. The supernatant was used for determining protein content by spectrophotometric method (*Spectronic 20D*, Milton Roy, USA) of Bradford (1976). Catecholase and tyrosinase activities were determined spectrophotometrically by measuring absorbances of reaction products at 430 nm (Kabar and Kocaçalışkan 1990).

For anatomical examination, seedlings were stored in 70 % ethanol. Later, cross sections from leaf and stem, and superficial sections from subepidermal tissue of leaf were taken. The fixed preparations were obtained from the sections in glycerine-gelatine medium, and the sections were examined under light microscope. Leaf Xylem vessel and vascular bundle radius of stem and stomata length of leaf were measured using an ocular micrometer.

Pregerminative juglone application increased elongation of the muskmelon seedlings but post-germinative application decreased it significantly. Fresh and dry masses of the seedlings were also increased by the pregerminative treatment of juglone, and they were decreased by the postgerminative treatment (Table 1). Juglone increased significantly protein content and catecholase and tyrosinase activities of muskmelon in both pregerminative and postgerminative treatments (Table 1).

Vascular bundle radius of the stem was enhanced by juglone in both pregerminative and postgerminative treatments (Table 1). Stomata number and length of the leaves were not affected significantly, although stomata length was slightly less at the treatments (Table 1). Radius of xylem vessels of the stem was increased by pregerminative treatment of juglone but it decreased in the postgerminative treatment (Table 1). Three layers of palisade parenchyma in the leaves of juglone treated leaves were observed while two layers in control leaves. Furthermore, mesophyll tissue of the leaves was thicker in juglone treatments than in controls (values not shown). Hale and Orcutt (1987) indicated that while leaf surface was reduced, its thickness was increased under several stresses.

Juglone effects on muskmelon were different according to application stage. Seedling growth is much more sensitive to juglone than seed germination (Rietveld 1983, Tekintaş *et al.* 1988, Dornbos and Spencer 1990, Kocaçalışkan and Terzi 2001). On the other hand, seedling growth of muskmelon was enhanced significantly by juglone and walnut leaf extracts in the previous work (Kocaçalışkan and Terzi 2001). The growth stimulating effect was seen if juglone was applied on seeds in pregerminative stage. The reason of the negative effect of postgerminative application may be connected with translocation of juglone from roots directly to leaves by xylem vessels (Daglish 1950) and thus it may disturb leaf photosynthesis and respiration. The seed coat of muskmelon may be a barrier to absorption of juglone at the beginning of seed germination. On the other hand, some biochemical mechanism in muskmelon seeds may detoxify juglone as shown by Segura-Aguilar *et al.* (1992) for spruce seeds. In our study the growth stimulating effect of juglone was paralleled by increase in protein content, catecholase and tyrosinase activities, and in xylem vessel and bundle radius in the pregerminative juglone treatment. A similar correlation was not seen in the postgerminative treatment. This show that positive and negative mechanisms of juglone effect may be different.

Protein content was increased by juglone in both treatments and this effect agrees with findings of Ranade and David (1985) and Compton and Preece (1988). Catecholase oxidizes catechol substrate as an oxidative defence mechanism. Increase in the activities of catecholase and tyrosinase may be a reaction againts juglone.

The effect of juglone on muskmelon seems to be related to xylem vessel radius rather than to the other

Table 1. Effect of juglone on growth parameters, protein content, catachalose and tyrosinase activities, and some anatomical parameters of muskmelon seedlings. Means ± SD, $n = 3$, * - significant in each treatment with respect to control at $P < 0.05$.

		Pregerminative treatment			Postgerminative treatment		
		control	1 mM juglone	[%]	control	1 mM juglone	[%]
Elongation	[cm]	20.4 ± 0.4	24.4 ± 0.5*	119.53	20.2 ± 0.3	15.9 ± 0.4*	78.71
Fresh mass	[mg]	400.0 ± 11.0	430.0 ± 9.0	107.50	410.0 ± 3.3	200.0 ± 3.3*	48.78
Dry mass	[mg]	12.9 ± 0.5	13.6 ± 0.3	105.42	16.6 ± 0.2	13.1 ± 0.2	78.91
Protein	[mg g^{-1}(f.m.)]	5.8 ± 0.0	6.3 ± 0.0*	108.62	13.6 ± 0.0	14.2 ± 0.0*	104.41
Catecholase	[A_{430} g^{-1}(f.m.)]	7.8 ± 0.6	8.4 ± 0.1*	108.33	6.1 ± 0.1	11.7 ± 0.1*	191.80
Tyrosinase	[A_{430} g^{-1}(f.m.)]	2.5 ± 0.0	3.2 ± 0.0*	128.00	9.1 ± 0.0	17.6 ± 0.0*	193.62
Xylem vessel radius	[µm]	45.5 ± 5.2	52.7 ± 4.7*	115.82	28.4 ± 1.7	26.0 ± 2.8	91.54
Vascular bundle radius	[µm]	315.0 ± 11.5	372.0 ± 30.4*	117.95	344.0 ± 15.3	415.0 ± 19.7*	120.73
Stomata number	[mm^{-2}]	126.0	130.0	103.17	142.0	148.0	104.22
Stomata length	[µm]	33.0	32.8	99.39	23.2	22.0	94.82

parameters.

Similar changes were typical for xylem vessel radius and growth parameters. That is, xylem vessel radius was increased with increasing growth in pregerminative juglone treatment and decreased with decreasing growth in the postgerminative treatment. Narrowing of the xylem vessels in postgerminative treatment may be a defence mechanism of the seedlings to limit juglone translocation, but this may also cause limited translocation of water and minerals from roots to leaves. A 50 % decrease in fresh mass of the seedlings as compared with control proved this event.

References

Bradford, M.M.: A rapid and sensitive method for the quantitiation of microgram quantities of protein utilizing the principle of protein dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Compton, M.E., Preece, J.E.: Effects of phenolic compounds on tobacco callus and black berry shoot cultures. - J. amer. Soc. hort. Sci. **113**: 160-163, 1988.

Daglish, C.: The determination and occurrence of hydrojuglone glucoside in the walnut. - Biochem. J. **47**: 458-462, 1950.

Davis, E.F.: The toxic principle of *Juglans nigra* as identified with synthetic juglone and its toxic effects on tomato and alfalfa plants. - Amer. J. Bot. **15**: 620, 1928.

Dornbos, D.L., Spencer, G.F.: Natural products phytotoxity. - J. chem. Ecol. **16**: 339-352, 1990.

Funk, D.T., Case, P.J., Rietveld, W.J., Phlares, R.E.: Effects of juglone on the growth of coniferous seedlings. - Forest Sci. **25**: 452-454, 1979.

Hale, M.G., Orcutt, D.M.: The Physiology of Plants under Stress. - John Wiley and Sons, New York 1987.

Hejl, A.M., Einhellig, F.A., Rasmussen, J.A.: Effects of juglone on growth, photosynthesis and respiration. - J. chem. Ecol. **19**: 559-568, 1993.

Jose, S., Gillespie, A.R.: Allelopathy in black walnut *(Juglans nigra* L.) alley cropping: II. Effects of juglone on hydroponically grown corn *(Zea mays* L.) and soybean (*Glycine max* L. Merr.) growth and physiology. - Plant Soil **203**: 199-205, 1998.

Kabar, K., Kocaçalışkan, I.: Interactions among salinity, polyphenol oxidase and growth regulators in the germination of wheat seeds. - Turk. J. Bot. **14**: 235-251, 1990.

Kocaçalışkan, I., Terzi, I.: Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. - J. hort. Sci. Biotechnol. **76**: 436-440, 2001.

Lee, K.C., Campbell, R.W.: Nature and occurrence of juglone in *Juglans nigra* L. - HortScience **4**: 297-298, 1969.

Prataviera, A.G., Kuniyuki, A.H., Ryugo, K.: Growth inhibitors in xylem exudates of Persian walnuts (*Juglans regia* L.) and their possible role in graft failure. - J. amer. Soc. hort. Sci. **108**: 1043-1045, 1983.

Ranade, S., David, S.B.: Quinones as plant growth regulators. - Plant Growth Regul. **3**: 3-13, 1985.

Rice, E.L.: Allelopathy. - Academic Press, Orlando 1984.

Rietveld, W.J.: Allelopathic effects of juglone on germination and growth of several herbaceous and woody species. - J. chem. Ecol. **9**: 295-308, 1983.

Segura-Aguilar, J., Hakman, I., Rydstrom, J.: The effect of 5-OH-1,4-naphthoquinone on Norway spruce seeds during germination. - Plant Physiol. **100**: 1955-1961, 1992.

Tekintaş, E., Tanrısever, A., Mendilcioğlu, K.: [Effect of juglone on seed germination]. - Ege Univ. Zir. Fak. Dergisi **25**: 203-213, 1988. [In Turk.]