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

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Abstract

Effects of juglone on seedling growth of cucumber (*Cucumis sativus* cv. Beith Alpha) with respect to physiological and anatomical parameters were investigated. Growth parameters (seedling elongation, fresh and dry weights) were reduced by 1 mM juglone. Juglone also decreased chlorophyll a and b contents and reduced some anatomical tissues (xylem vessel and bundle radius of stem, stomata length and stomata number of the cotyledons). The anatomical changes in stem and cotyledon of the seedlings were related to growth inhibiting effect of juglone. On the other hand, increase in catecholase and tyrosinase activities by the effect of juglone were also recorded.

Introduction

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, Rice 1984). Juglone has been isolated from many plants in the walnut family (*Juglandaceae)* including *Juglans nigra, Juglans regia* and others (Daglish 1950, Prataviera *et al.* 1983). A colorless nontoxic reduced form called hydrojuglone is abundant, especially in leaves, fruit hulls and roots of walnut. 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 has been reported to be toxic to both herbaceous and woody plants (Funk *et al.* 1979, Rietveld 1983).

Juglone allelopathic effects on plants are generally toxic but beneficial to some. In the previous study, it was 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 (Kocaçalişkan and Terzi 2001).

The physiological action of juglone is not well understood. Very few studies have been done about juglone inhibiting effect during seed germination and seedling growth. Juglone inhibits plant growth by reducing 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 the anatomical parameters is available. Therefore, the aim of this work is to determine both physiological and anatomical changes created by juglone in cucumber seedlings.

Materials and Methods

Juglone application and seedling growth

Seeds of cucumber (*Cucumis sativus* cv. Beith Alpha) were obtained from BURSA TOHUMCULUK company. The seeds were surface sterilized with 1 % sodium hypochloride. At least 20 seeds were placed in a Petri dish laid with sheets of filter paper moistened with distilled water. After four days of germination in a growth chamber whose conditions are indicated below, seedlings were transferred into the plastic pots filled with perlit. Then 30 ml of 1 mM juglone solution or distilled water (control) were added into the pots. Juglone solution was prepared by dissolving in distilled water by stirring at 40 °C for 24 hours (Kocaçalişkan and Terzi 2001). The 1mM juglone solution was used, since it occurs in soil under field conditions at this concentration (Rietveld 1983). Juglone (5-hydroxy-1,4-naphthoquinone) was purchased from SIGMA. Each treatment was replicated three times. Twenty seedlings were used in each replicate. After the treatments, plastic pots containing cucumber seedlings were left in a plant growth chamber for seven days. Growth conditions of the chamber were "14 hours light (20 000 lux) / 25 °C / 70 % humidity and 10 hours dark / 18 °C / 80 % humidity " (Şeniz 1993).

Determinations

Chlorophyll and carotenoid contents of the cotyledons were determined by the method of Arnon (1949). For measuring protein content and the enzyme activities, tissue extracts of the cotyledons were prepared by homogenizing 0.5 g of the cotyledons in 5 ml phosphate buffer (pH: 6.5; 0.1 M) and centrifuging at 3500 x g for 10 min. The supernatant was used as protein source. Protein content was determined by the method of Bradford (1976).

Catecholase and tyrosinase activities were determined spectrophotometrically by measuring absorbances of reaction products at 430 nm (Kabar and Kocaçalişkan 1990). 10 mM of catechol and L-tyrosine were used as substrates for determining of catecholase and tyrosinase activities, respectively. The reaction mixture contained 1.5 ml of substrate and 1.5 ml of homogenizing buffer and 0.2 ml of the tissue extract. The mixtures were incubated at 37 °C for 2 min and 2 h for catecholase and tyrosinase, respectively. Then absorbance values were recorded in spectrophotometer. Omission of the extract in the mixture served as blank sample. Under the assay conditions, the absorbance values were expressed as the enzyme activity units per g cotyledon tissue of fresh weight (A_{430} / g fr.wt) Duplicate measurements of three replicates were made.

Anatomical examinations

Some seedlings were fixed in 70 % ethanol for anatomical examination. This process provides rigidity for the tissues, so that sections can be taken more easly and properly. Later, cross sections from the cotyledons and stems, and superficial sections from subepidermal tissue of the cotyledons were taken. Then the fixed preparations were obtained from the sections in glycerine – gelatine medium, and the sections were examined under light microscope at proper magnifications. Xylem vessel and vascular bundle radius of stem and stomata length and mesophyll thickness of the cotyledons were measured using ocular micrometer. Stomata number and palisade layering were determined by microscope examination.

Results and Discussion

In this study, the mode of juglone action on cucumber seedling was examined by comparing growth parameters with physiological and anatomical ones. All the growth parameters such as seedling elongation, fresh and dry weights were reduced significantly by juglone. The fresh and dry weights of the seedlings were more affected than was seedling elongation (Table).

Juglone was found to reduce growth and both chlorophyll a and chlorophyll b contents of the seed-

Table. Effect of juglone on growth and physiological and anatomical parametres of cucumber seedlings

Growth parametres of whole seedlings	Control (dist. water)	Juglone (1 mM)
Seedling elongation (cm)	12.9±0.40	7.4*±0.07
Seedling fresh weight (mg)	330±2.00	120*±2.12
Seedling dry weight (mg)	13±0.57	5*±0.57
Pigment and protein contents of the cotyledons		
Chlorophyll a (mg/g fr.wt)	1.24±0.016	0.73*±0.006
Chlorophyll b (mg/g fr.wt)	1.22±0.002	0.64*±0.001
Carotenoid (mg/g fr.wt)	0.16±0.010	0.19±0.010
Protein (mg/g fr.wt)	8.3±0.070	16*±0.570
Enzyme activities of the cotyledons		
Catecholase (A_{430} / g fr.wt)	8.8±0.100	48.5*±0.590
Tyrosinase (A_{430} / g fr.wt)	8.6±0.029	19*±0.290
Anatomical parameters		
Xylem vessel radius of stem(μm)	26.7±2.06	20.4*±1.67
Bundle radius of stem(μm)	311±7.31	297*±8.93
Stomata length of the cotyledon(μm)	18±1.41	14*±0.89
Stomata number of the cotyledon(per mm^2)	564±1.14	505±2.00
Mesopyll thickness(μm)	367±2.07	466*±2.00
Palisade layering(cell line)	2±0.00	3±0.00

* ($P < 0.05$) " t " test, ± SD for means of triplicate samples.

lings without significantly changing carotenoid content. In a report, juglone was found to inhibit growth by reducing photosynthesis and chlorophyll content in *Lemma minor*. But this inhibition was found to be correlated with photosynthetic electron transport functions of chloroplast rather than chlorophyll loss (Hejl *et al.* 1993). In the present study, although chlorophyll contents were significantly reduced by juglone, it could not be related to growth inhibition of the seedlings by juglone because juglone was also found to inhibit cucumber seedling growth in darkness (Kocaçalişkan and Terzi 2001).

In the present study, protein content was enhanced by juglone, in contrast to seedling growth. As contrary to the general assumption that increase in total protein content is a criterion of plant growth, the present study has shown that it might not always be the case. Increase in protein content may stem from increases in some enzymatic proteins such as catecholase and tyrosinase, because these enzymes, which belong to the group of phenol oxidases, were found to significantly increase their activities with juglone (Table). This activity increase may be the result of oxidative defence mechanism produced by allelochemical stress of juglone. Thus, juglone was found to induce oxidative stress during germination (Segura-Aguilar *et al.* 1992).

However, from anatomical parameters xylem vessel and bundle radius of stem and stomata length and stomata numbers of the cotyledons were found to show parallelism with seedling growth. That is, seedling growth was decreased by juglone as anatomical paramaters were reduced (Table). Since juglone is translocated through xylem vessels from roots to leaves (Daglish 1950), this narrowing in the vessels may cause decrease in water supply for the plant. On the other hand, decreasing of stomata length and stomata number by the effect of juglone may cause growth inhibition as well, because decreasing of stomata length may reduce photosynthesis by limiting CO_2 entrance into the cotyledon.

The findings in the present study seem to indicate that while there are two palisade layers in control cotyledon, the number of palisade layers in a cotyledon treated with juglone may be up to three (Table). Also, mesophyll tissue of the cotyledons was much thicker in juglone treatment than control. It can be said that this alteration may be a result of stress created by juglone. Thus, it has been indicated that leaf thickness increases under several stresses (Hale and Orcutt 1987).

As a conclusion, reduction in anatomic tissues such as xylem vessel and bundle radius of stem, and

stomata length and stomata number of the cotyledons may be the causes of plant growth inhibition by juglone. But increases in mesophyll thickness, palisade layering and catecholase and tyrosinase activities may be the result of a reaction to allelochemical stress produced by juglone.

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