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Short Communication

Effects of juglone on seedling growth in intact and coatless seeds of cucumber (*Cucumis sativus* cv. Beith Alpha)

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The main aim of this study was to test if the seed coat is a barrier to restrict juglone's entrance into the seed using intact and coatless seeds of cucumber (*Cucumis sativus* cv. Beith Alpha). Elongation, fresh and dry weights of cucumber seedlings were decreased significantly by juglone in both intact and coatless seeds. Protein content in cotyledons of cucumber seedling was also decreased by juglone. The result do not prove the seed coat barrier hypothesis Because juglone's inhibitory effect on seedling growth and protein content was not so much different in coatless seeds than was in intact seeds. However, in the present study, catechol and dopa oxidizing activities of polyphenol oxidase enzyme were increased by juglone in both seedlings. The increase in the activities of this enzyme was attributed to a reaction against to allelochemical stress produced by juglone.

Key words: Cucumber, juglone, polyphenol oxidase activity, protein content, seedling growth.

INTRODUCTION

The chemical interactions that occur among living organisms including plants, insects and microorganisms are called allelopathy, and the organic compounds involved in allelopathy are called allelochemicals. The release of allelochemicals from plants occur by volatilization, leaching from leaves, exudation from roots and degradation of dead plant parts. All parts have been shown to contain allelochemicals but leaves and roots are the most important sources. Allelochemicals become stressful when they are toxic. Sometimes a single chemical produced by one organism is harmful to another but benefical to a third organism (Whittaker and Feeny, 1971; Rice, 1979; Hale and Orcutt, 1987; Rizvi and Rizvi, 1992). In addition temperature was the most effective agent in enhancing synthesis and exudation of allelochemicals (Pramanik et al., 2000). Thus, allelopathic interactions in nature has been indicated to have ecological importance (Inderjit, 1999).

The inhibitory effect of black walnut (*Juglans nigra* L.) 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 *J. nigra*, *Juglans regia* and the others (Daglish, 1950; Prataviera et al., 1983). A colourless, nontoxic reduced form called hydrojuglone is abundant, especially in leaves, fruit hulls and roots of walnut. When exposed to the 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). Change in juglone content of walnut (*J. regia* L.) has been researched and its maximum level has been detected in July and August (Tekintaş et al., 1988).

Walnut has been reported to be toxic to both herbaceous and woody plants (Funk et al., 1979; Rietveld, 1983). Sed germination and seedling growth of cucumber

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Table 1. Effect of juglone on growth of root and shoot, protein content and PPO activities of cotyledons in intact and coatless seeds of cucumber.

	Control (distilled water)	Juglone (1 mM)
Intact seeds **Root**		
Elongation (cm)	16.2 ± 0.3	3.1 ± 0.1*
Fresh weight (mg)	132.0 ± 2.3	56.0 ± 1.7*
Dry weight (mg)	5.8 ± 0.3	4.3 ± 0.2*
Shoot		
Elongation (cm)	3.1 ± 0.2	2.2 ± 0.1*
Fresh weight (mg)	102.8 ± 2.8	37.9 ± 1.9*
Dry weight (mg)	2.2 ± 0.1	2.1 ± 0.1
Cotyledon		
Protein (mg/g fr. wt.)	1.9 ± 0.3	1.1 ± 0.1*
Catecholase (A_{430} / g. fr. wt.)	10.5 ± 0.9	24.5 ± 1.3*
Dopa oxidase (A_{490} / g. fr. wt.)	11.0 ± 0.7	17.8 ± 0.8*
Coatless seeds		
Root		
Elongation (cm)	19.5 ± 0.6	4.0 ± 0.1*
Fresh weight (mg)	118.9 ± 2.5	45.2 ± 1.2*
Dry weight (mg)	6.7 ± 0.8	3.6 ± 0.2*
Shoot		
Elongation (cm)	2.7 ± 0.2	2.0 ± 0.1*
Fresh weight (mg)	77.2 ± 2.3	40.8 ± 1.4*
Dry weight (mg)	2.6 ± 0.2	2.1 ± 0.1*
Cotyledon		
Protein (mg/g fr. wt.)	1.2 ± 0.1	1.8 ± 0.2
Catecholase (A_{430}/g fr. wt.)	10.1 ± 0.4	12.7 ± 0.5
Dopa oxidase (A_{490}/g fr. wt)	12.7 ± 0.5	14.9 ± 0.6

*(P< 0.05) “t” test, ± SD, n = 4.

was effected by juglone lesser than cress and tomato (Kocaçalışkan and Terzi, 2001; Terzi, 2008). Probably because of thicker seed coat in cucumber than in cress and tomato this reasult was obtained. Seed coat of some seeds are thin and some are thick. This may be the reason for different allelopathic effect of juglone on seed germination. Thus, seed coat has been suggested to be a barrier to restrict juglon’s entrance into the seed (Segura – Aguilar et al., 1992). Therefore, the aim of this study was to test this hypothesis in cucumber seed by comparing intact and coatless seeds.

EXPERIMENTAL

Juglone application and experimentation

Seeds of Cucumber (*Cucumis sativus* cv. Beith Alpha) was obtained from AGROMAR Company, Bursa, Turkey and were surface sterilized with 1% sodium hypochloride. Then, they were left in distilled water for 2 h. Next, they were dried on filter papers at room temperature until they reached their previous weight. The chubby, strong and similar sized seeds were selected from these seeds. At least 10 seeds were placed in a Petri dish furnished with sheets of filter paper moistened with distilled water (control) or with juglone solution.

The seeds were divided in two groups. One group intact seeds and the other coatless seeds whose coat was removed after 2 h of water imbibition as mentioned above. Two groups of the seeds were treated with both distilled water (control) and juglone. Then the seeds in dishes were left in a growth chamber for 20 days. Growth conditions of the chamber were “14 h light (20 000 lux) / 24℃ / 70% humidity and 10 h dark / 18℃ / 80% humidity” (Şeniz, 1993). The experiment was replicated four times.

1 mM juglone solution was prepared by dissolving in distilled water by stirring at 40℃ for 24 h (Kocaçalışkan and Terzi, 2001). This concentration of juglone was used since in the soil of walnut plantation, it is generally found in less than 1 mM concentration, depending on walnut species, season and distance from trunk of walnut (Rietveld, 1983; Jose and Gillespie, 1998). Therefore, a maximum possible concentration of juglone (1 mM) in the soil was selected. Juglone chemical (5-hydroxy-1,4-naphthoquinone) was purchased from SIGMA.

Determinations

After 20 days of growth in the chamber, seed germination percentage was recorded. Then the seedlings were separated two parts as root and shoot by cutting from the connection point. Seedling growth was determined by measuring root and shoot lenght and fresh and dry weights. For measuring protein content and polyphenol oxidase activities 0.5 g of cotyledons was homogenized in 5 ml 0.1 M phosphate buffer of pH 6.5 and centrifuged at 3 500 g for 10 min. The supernatant was used in determinations of protein content and enzyme activity. Protein content was determined spectrophotometric method of Bradford (1976). Catecholase and dopa oxidase activities were determined spectrophotometrically by measuring absorbances of reaction products at 430 and 490 nm, respectively (Kabar and Kocaçalışkan, 1990).

RESULTS AND DISCUSSION

Seed germination of cucumber was not changed by juglone. That is, it was 100% in both control and juglone treated seeds. The result therefore was not shown on the Table. However, the growth parameters as elongation, fresh and dry weights of the seedlings were decreased significantly by juglone in both intact and coatless seeds. Root elongation was inhibited by juglone more than shoot in both intact and coatless seeds (Table 1). In the previous study (Kocaçalışkan and Terzi, 2001), juglone was found to decrease both seed germination and seedling growth, whereas in the present study seed germination was not changed by juglone. This different results may be stem from the used seeds belong to different cucumber varieties.

Protein content was significantly decreased by juglone in intact seeds but it did not change significantly in coatless seeds. It has generally been assumed that protein content may be a criterion of plant growth, and leaves are the most metabolically active organ and rich in protein. Therefore, in this study, protein content was measured in cotyledonary leaves of cucumber seedlings. Changes in protein content of the cotyledons by juglone was almost parallel to growth of the seedlings. That is, juglone decreased elongation and fresh and dry weights of root and shoot with decreased protein content of cotyledons with respect to control.

Polyphenol oxidase activities were enhanced by juglone, in contrast to protein content and seedling growth (Table 1). Polyphenol oxidase oxidizes mainly diphenolic subtrates. The main substrates of the enzyme are catechol and dopa in plants, and the enzyme oxidizing these subtrates are called catecholase and dopa oxidase, respectively (Mayer, 1987). Polyphenol oxidase activity has also been shown to increase as a result of juglone treatment in cucumber (Terzi et al., 2003). 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).

In conclusion, the present study did not prove the seed coat barrier hypothesis that seed coat may restrict juglone entrance into the seed suggested by Segura - Aguilar et al. (1992). Because, juglone's inhibitory effect on seedling growth and protein content was not more in coatless seeds than was in intact seeds.

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