The allelopathic effects of juglone and walnut leaf extracts on yield, growth, chemical and PNE compositions of strawberry cv. Fern

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ABSTRACT

Allelopathic effects of juglone and walnut leaf extracts from Persian walnut (*Juglans regia* L.) on yield, growth, chemical and plant nutrient element composition of the day-neutral strawberry (*Fragaria* × *ananassa* L.) cultivar Fern were investigated as part of an effort to identify tolerant species to plant adjacent to walnut. Strawberry plants were treated with juglone (5-hydroxy-1.4 naphthoquinone, 1mM) and walnut leaf extracts (undiluted, 1:2, 1:4, 1:8 diluted). Vegetative and reproductive plant growth was inhibited strongly by the treatment of both juglone and undiluted walnut leaf extracts. Fruit yield per plant, the number of fruit per plant, average fruit weight, crowns per plant, number of leaves, leaf area, fresh root weight, total soluble solid (TSS), vitamin C and acidity were reduced by juglone treatment compared to the control. All nutrient elements analyzed in the leaves generally were lower than the control for all treatments except 1:8 diluted.

Keywords: allelopathy; *Fragaria* × *ananassa* L.; *Juglans regia*; juglone; strawberry; walnut leaf extract

Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment (Rice 1984). One of the most famous allelopathic plants is Black Walnut (*Juglans nigra* L.) which produces a non-toxic colorless chemical called hydrojuglone. Hydrojuglone is found in leaves, stems, fruit hulls, inner bark and roots. When exposed to air or soil compounds, hydrojuglone is oxidized into the allelochemical juglone, which is highly toxic (Lee and Campbell 1969). Juglone has been isolated from many plants in the walnut family including *J. nigra*, *J. regia* and the others (Daglish 1950, Prataviera et al. 1983, Tekintaş et al. 1988a). Rain washes juglone from the leaves and carries it into the soil. Thus, neighboring 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). In particular, in Eastern part of Turkey, tree-based intercropping systems have been used and Persian walnut (*J. regia* L.) is one of the most common tree species used for this practice. Its high value, aesthetic qualities, capacity for nut production, rapid growth potential and adaptability to management makes the species very suitable to intercropping (Thevathasan et al. 1999).

Therefore, it is important to select tolerant species for planting in areas adjacent to walnut trees. To the best of our knowledge, no research has been reported on the effects of juglone and walnut leaf extracts on strawberry plants. It is hypothesized that, in a black walnut-based intercropping system, juglon released from walnut trees could be an inhibitor to the growth of strawberry plants close to the trees. Thus, the objective of this work was to test this hypothesis in a greenhouse experiment.

MATERIAL AND METHODS

Leaves of seven years old walnut trees were used in obtaining the extracts because walnut trees younger than seven years old do not contain sufficient juglone to cause toxicity (Prataviera et al. 1983, Piedrahita 1984). The leaves of *Juglans regia* L. cv. Sebin were collected in the first week of August and drying the leaves at 70°C in an oven, for 48 h. After drying 10 g of dried leaf was homogenized in 100 ml of distilled water with a Waring Blender for one hour and filtered with filter paper. The filtrate was centrifuged at 3000 rpm. The supernatant was decanted and used in experiments diluting 1:2, 1:4 and 1:8 ratios with distilled water or without diluting (Kocacaliskan and Terzi 2001). Juglone solution was prepared 10^{-3}M dissolving in distilled water by stirring at 40°C for 24 h. Juglone (1mM) was used, since it occurs in soil under field conditions at this concentration (Rietveld 1983).

The experiment was conducted with day-neutral strawberry plants cv. Fern as it is the most used strawberry cultivar in East Anatolia region of Turkey. Plants were grown in peat media in 20 cm diameter polyethylene pots. Cold-stored strawberry plants were planted at the end of November 2002. Plants were maintained in a heated greenhouse under natural light at a temperature between 10–21°C and a relative humidity of about 55–60%. The environmental parameters in the greenhouse, automatically controlled, were the same for all treatments during study. Until the end of study (June 2003), they were irrigated with 250 ml per plant once every 7 day with a nutrient solution (pH: 5.9) (Poly Plant, which obtained from AGREX-Agran, GmbH, Germany), with an electrical conductivity of 1.9 mS/cm. The composition of the nutrient solution was 213 ppm N, 77.5 ppm P, 251 ppm K, 72 ppm Mg and 221 ppm S.

The treatments were as follows: 1mM Juglone (Sigma, MO, USA), undiluted walnut leaf extract and walnut leaf extract dilutions of 1:2, 1:4 and 1: 8, distilled water was used as a control. Juglone, walnut leaf extracts and distilled water were applied on growing media 250 ml per plant once a month after planting date and continued one month interval until harvest. The experiment was a completely randomised design with three replications per treatment and ten plants each replicate.

Matured fruits were harvested twice a week during the experimental period and fruit yield per plant, average fruit weight and fruit number per plant were determined. In July 2003, plants were uprooted and crowns per plant, leaf number, leaf areas and fresh root weight were determined. Some chemical properties of fruits (Total Soluble Solids by refractometer, Vitamin C by titration with 2,6 Diclorophenolindophenol and Titratable Acidity by titration with 0.1N NaOH) and plant nutrient content of leaves (N, P, K, Ca, Mg, Fe, Mn and Zn) were also determined. Nitrogen was determined by Micro-Kjeldahl. Phosphorus was determined by spectrophotometer and K, Ca, Mg, Fe, Mn and Zn were determined by atomic absorption spectrometry after mineralization through wet combustion (AOAC 1970, Yildiz 1994).

Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan's multiple range test.

RESULTS

Our results showed plant growth of strawberry cv. Fern in terms of leaf areas, fruit yield per plant, number of fruit per plant and average fruit weight were significantly affected by both juglone and walnut leaf extracts (Figure 1 and Table 1).

The increase of crown per plant was significantly inhibited by juglone (2.6 per plant) and undiluted walnut leaf extract (2.8 per plant) treatments compared with the control (3.4 per plant) and the other treatments. Leaf number and leaf areas decreased from 16.22 to 15.30 and from 70.42 cm^2 to 60.04 cm^2 at the control and juglone treatments, respectively (Table 1).

Fruit yield per plant, number of fruit per plant and average fruit weight was also significantly decreased by juglone and undiluted walnut extract treatments (Figure 1). All juglone and walnut leaf extract treatments significantly decreased average fruit weight compared with the control ($P < 0.01$). The largest fruit (7.13 g) was obtained from the control treatments.

In comparing the effectiveness of treatments on the fresh root weights of strawberry plants, juglone and the undiluted extract were found to be the most inhibitive treatments (Table 1).

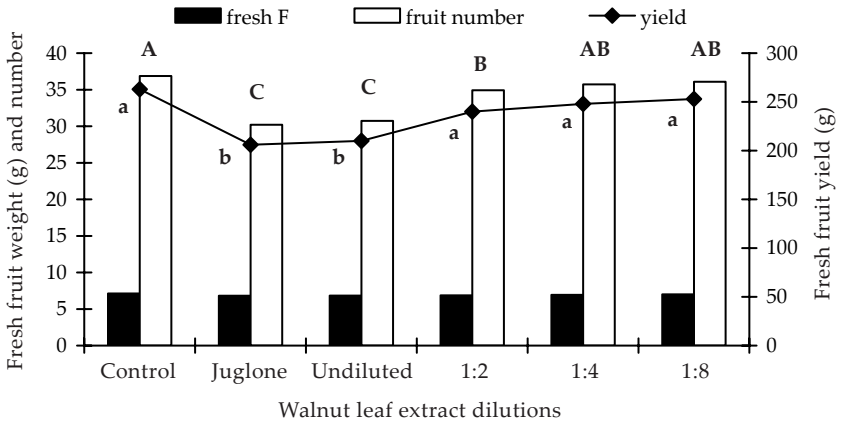

Figure 1. The effect of juglone and walnut leaf extracts on fruit weight, fruit number and yield of strawberry cv. Fern

Table 1. The effect of juglone and walnut leaf extracts on vegetative growth and PNE (plant nutrient elements) contents of strawberry cv. Fern

	Control	Juglone	Walnut leaf extracts				$LSD_{0.01}$
			undiluted	1:2	1:4	1:8	
Crown per plant	3.4	2.6	2.8	3.2	3.2	3.4	NS
Leaf area (cm^2)	70.42 a	60.04 b	60.42 b	65.35 ab	67.28 a	69.32 a	5.94
Leaf number	16.22	15.30	15.80	15.86	15.92	16.04	NS
Fresh root weight (g)	39.36	32.40	33.28	34.36	36.40	38.23	NS
N (%)	2.68 a	2.35 c	2.37 c	2.42 bc	2.43 bc	2.52 b	0.10
P (%)	0.35	0.31	0.31	0.32	0.33	0.34	NS
K (%)	2.02 a	1.66 c	1.68 c	1.82 bc	1.85 b	1.88 b	0.16
Ca (%)	2.51 a	1.80 d	1.88 d	2.10 c	2.11 c	2.34 b	0.12
Mg (%)	0.52	0.48	0.48	0.49	0.50	0.51	NS
Fe (ppm)	75 a	39 b	45 b	47 b	66 a	69 a	12.90
Mn (ppm)	77 a	48 d	57 cd	62 bc	65 bc	71 ab	9.98
Zn (ppm)	18	14	15	16	17	17	NS

TSS and vitamin C contents of fruits were highest (8.20% and 67 mg/100 ml) in the control treatments. However, the lowest TSS and vitamin C contents were observed from the juglone treatment as 7.40% and 54 mg/100 ml, respectively (Figure 2).

PNE (plant nutrient element) content of leaves was significantly decreased by the juglone and walnut leaf extracts treatments for N, K, Ca, Fe and Mn (Table 1). The N content decreased from 2.68% (at control) to 2.35% at juglone treatment. The P, K, Ca and Mg content increased from 0.31% to 0.35%; 1.66% to 2.02%; 1.80% to 2.51% and 0.48% to 0.52%, respectively, from to juglone to control treatments. However, there were no significant differences in the P, Mg and Zn contents among the juglone, walnut leaf extracts and the control.

DISCUSSION

In this research, fruit yield per plant; number of fruit per plant and the average fruit weight were greater at the control and the lowest at the juglone treatment. Moreover, root and vegetative plant growth were inhibited strongly by the treatment of both walnut leaf extracts and juglone in straw-

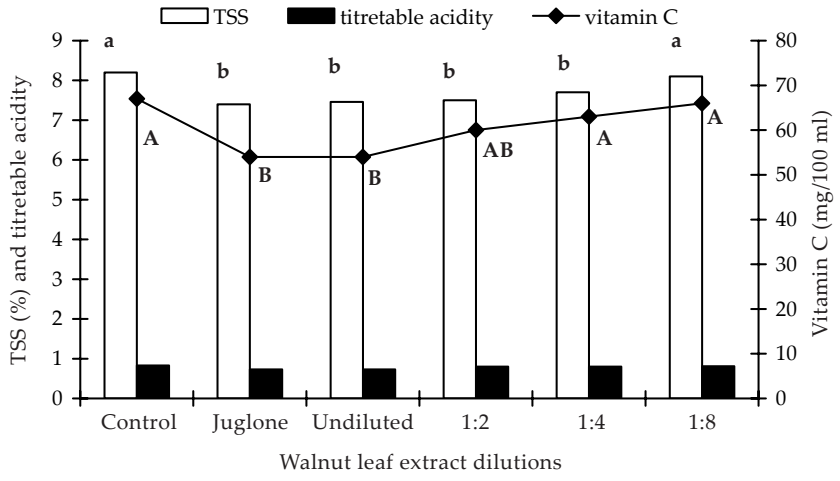

Figure 2. The effect of juglone and walnut leaf extracts on TSS, titretable acidity and vitamin C of strawberry cv. Fern

berry. Previous studies had shown that juglone had an inhibitory effect on plant growth of several plant species such as apple, blackberry, blueberry, grape, potato and tomato (Rietveld 1983, Coder 1999, Appleton et al. 2000). Some studies suggest that 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). Our results showed that juglone and walnut leaf extracts impairs both vegetative and reproductive growth in strawberries. Since we did not examine the effect of juglone and walnut leaf extract on plant metabolism such as photosynthesis, hormones and enzymes and evolves to endogenous solute like carbohydrates and plant growth regulators and enzymes in plant, further studies are underway to investigate the exact role of juglone and walnut leaf extract on metabolism in strawberry cultivars.

Kocacaliskan and Terzi (2001) demonstrated that both juglone and walnut leaf extracts inhibits germination and seedling growth of several plant species, such as watermelon, tomato, garden crest and alfalfa. Similar results were indicated with juglone in earlier studies in cucumber (Tekintaş et al. 1988b), tomato and bean (Neave and Dawson 1989).

In addition, juglone and walnut leaf extract has a negative effect on plant nutrient element uptake from growth media. Therefore, it can be concluded that juglone may play an important role in terms of decreasing cation uptake capacity and had positive effect on immobile plant nutrient uptake such as N, K, Ca, Fe and Mn. Besides, Hejl and Koster (2004) showed that juglone treatments significantly reduced H^+-ATPase activity which has important role in essential plant processes such as solute uptake and water uptake in soybean and corn.

As a conclusion, our results clearly revealed that juglone has inhibitory effects on strawberry plants. Therefore, juglone phytotoxicity cannot be ruled out when examining the causes for observed reductions in growth in strawberry in walnut intercropping. A future study is necessary to get more detail information about walnut-strawberry allelopathic relationships by using more strawberry cultivars.

REFERENCES

AOAC (1970): Official methods of analysis. Association of Official Agricultural Chemists, Benjamin Franklin St., Washington, USA.

Appleton B., Berrier R., Haris R., Alleman B., Swanson L. (2000): Trees for problem landscape sites-the walnut tree: Allelopathic effects and tolerant plants. Virginia State University, Virginia Cooperative Extension. Publication No. 430-021.

Coder K.D. (1999): Allelopathy in trees. Arborist News, *8*: 53–60.

Daglish C. (1950): The determination and occurrence of hydrojuglone glucoside in the walnut. Biochemical Journal, *47*: 458–462.

Funk D.T., Case P.J., Rietveld W.J., Phares R.E. (1979): Effects of juglone on the growth of coniferous seedlings. Forest Science, *25*: 452–454.

Hejl A.M., Einhellig F.A., Rasmussen J. (1993): Effects of juglone on growth, photosynthesis and respiration. Journal of Chemical Ecology, *19*: 559–568.

Hejl A.M., Koster K.L. (2004): Juglone disrupts root plasma membrane H^+-ATPase activity and impairs water uptake, root respiration, and growth in soybean (*Glycine max*) and corn (*Zea mays*). Journal of Chemical Ecology, *30*: 453–471.

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

Kocacaliskan I., Terzi I. (2001): Allelophatic effects of walnut leaf extracts and juglone on seed germination and seedling growth. Journal of Horticultural Science and Biotechnolgy, *76*: 436–440.

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

Neave I.A., Dawson J.O. (1989): Juglone reduces growth, nitrogenase activity and root respiration of actinorhizal black alder seedlings. Journal of Chemical Ecology, *15*: 1823–1826.

Piedrahita O. (1984): Black walnut toxicity. Ministry of Agriculture and Food, Ontario, Canada. Order No. 84-050.

Prataviera A.G., Kuniyuki A.H., Ryogo K. (1983): Growth inhibitors in xylem exudates of Persian walnut (*Juglans regia* L.) and their possible role in graft failure. Journal of the American Society of Horticultural Science, *108*: 1043–1050.

Rice E.L. (1984): Allelopathy. Academic Press, Orlando, Florida, USA.

Rietveld W.J. (1983): Allelopathic effects of juglone on germination and growth of several herbaceous and woody species. Journal of Chemical Ecology, *9*: 295–308.

Segura-Agular J., Hakman I., Rydstrom J. (1992): The effect of 5-OH-1,4 naphtoquinone on Norway spruce seed during germination. Plant Physiology, *100*: 1955–1961.

Tekintaş E., Tanrisever A., Mendilcioglu K. (1988a): Cevizlerde (*Juglans regia* L.) juglon izolasyonu ve juglon içeriginin yillik degisimi. Ege Universitesi, Ziraat Fakultesi, Dergisi, *25*: 214–225.

Tekintaş E., Tanrisever A., Mendilcioglu K. (1988b): Juglon'un tohum çimlenmesine etkileri. Ege Universitesi, Ziraat Fakultesi, Dergisi, *25*: 203–213.

Thevathasan N.V., Gordon A.M., Voroney R.P. (1999): Juglone (5-hydroxy-1,4 napthhoquinone) and soil

nitrogen transformation interaction under a wulnut plantation in southern Ontario, Canada. Agroforestry Systems, *44*: 151–162.

Yildiz N. (1994): Evaluation of chemical extraction methods used to determine plant available soil nitrogen of Pasinler plains soils. [Ph.D. Thesis.] Ataturk University.

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ABSTRAKT

Alelopatické účinky juglonu a extraktu z listů ořešáku královského na růst, chemické složení a obsah živin v jahodníku odrůdy Fern

Byly sledovány alelopatické účinky juglonu a extraktu z listů ořešáku královského (*Juglans regia* L.) na růst, chemické složení a obsah živin v rostlinách fotoperiodicky neutrální odrůdy jahodníku Fern (*Fragaria* × *ananassa* L.), a to za účelem nalezení druhů tolerantních na pěstování v blízkosti ořešáků. Na rostliny jahodníku byly aplikovány roztoky juglonu (5-hydroxy-1,4-naftochinon, 1 mM) a extraktu z listů ořešáku (neředěné nebo ředěné v poměru 1 : 2, 1 : 4 a 1 : 8). Vegetativní i reprodukční růst rostlin byl inhibován jak muflonem, tak i neředěným extraktem listů ořešáku. Ve srovnání s kontrolními rostlinami snižovala aplikace juglonu výnos a počet plodů na jedné rostlině, průměrnou hmotnost plodu, počet listů a listovou plochu, čerstvou hmotnost kořenů, celkový obsah rozpustných látek, obsah vitaminu C a kyselost. Aplikace juglonu i všech koncentrací extraktu listů s výjimkou ředění 1 : 8 snižovala obsah všech analyzovaných živin v listech.

Klíčová slova: alelopatie; *Fragaria* × *ananassa* L.; *Juglans regia*; juglon; jahodník; extrakt ořešáku královského

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