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Herbicidal Effects of Juglone as an Allelochemical

By

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With 1 Figure

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Summary

TOPAL S.,.KOCACALISKAN I., ARSLAN 0. & TEL A. Z. 2007. Herbicidal effects of juglone as an allelochemical. - Phyton (Horn, Austria) 46 (2): 259-269, 1 figure. - English with German summary.

Juglone is a natural constituent of walnut with phytotoxic and allelopathic properties. Its herbicidal effects on the weed species wild mustard *(Sinapis arvensis* L.), creeping thistle *(Cirsium arvense L.),* field poppy *(Papaver rhoeas L.)* and henbit *(Lamium amplexicaule* L.) were investigated using wheat *(Triticum vulgare* Vill. cv. Gerek 79) and barley *(Hordeum vulgare L.* cv. KISLIK) varieties as control plants. A juglone concentration of 5.74 mM had a lethal effect on field poppy. Juglone concentrations of 1.15 and 2.30 mM decreased the elongation and fresh weight of the weeds, but not wheat and barley. The elongation of the shoot was more significantly decreased than that of the root in all weeds. Chlorophyll content of the weeds was also decreased by. juglone. The study reveals that juglone is a potent inhibitor of growth of the weeds and therefore it can be evaluated as a herbicide for future weed management strategies.

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Zusammenfassung

Topal S., Kocacaliskan I., Arslan 0. & TEL A. Z. 2007. Herbizide Effekte von Juglon als allelopathischer Wirkstoff. - Phyton (Horn, Austria) 46 (2): 259 - 269, 1 Abbildung. - Englisch mit deutscher Zusammenfassung .

Juglon ist ein naturlicher Bestandteil der Walnuss mit pflanzentoxischen and allelopathischen Eigenschaften. Die herbizide Wirkung wurde an den Wildkrautern Ackersenf *(Sinapis arvensis* L.), Ackerkratzdistel (Cirsium *arvense L.),* Klatschmohn *(Papaver rhoeas L.)* and Taubnessel *(Lamium amplexicaule* L.) mit Varietaten von Weizen *(Triticum vulgare* Vill. cv. Gerek 79) and Gerste *(Hordeum vulgare L.* cv. Kislik) als Kontrollen getestet. Eine Juglonkonzentration von 5,74 mM hatte einen letalen Effekt auf Klatschmohn. Juglonkonzentrationen von 1,15 and 2,30 mM hemmten das Langenwachstum and das Gewicht der Wildkrauter, nicht jedoch das von Weizen and Gerste. Das Langenwachstum der Sprosse war in alien Wildkrautern starker eingeschrankt als das der Wurzeln. Der Chlorophyllgehalt der Wildkrauter wurde Burch Juglon ebenfalls verringert . Die Studie zeigt, dass Juglon ein starker Wachstumshemmer fur Wildkrauter ist and deshalb als Herbizid fur zukunftige Unkrautbekampfungsmaßnahmen in Betracht gezogen werden sollte.

Introduction

The inhibitory effect of black walnut *(Juglans nigro)* on associated plant species is one of the oldest- examples of allelopathy. Juglone (5-hydroxy-1,4-naphthoquinone) is an allelochemical responsible for walnut allelopathy (Rice 1984). Juglone has been isolated from many plants of the walnut family *(Juglandaceae)* including J. *nigra* and. J. *regia* (DAGLISH 1950, Prataviera & al. 1983). A colourless, non-toxic reduced form called hydrojuglone is abundant, especially in leaves, fruit hulls, and roots of walnut trees. When exposed to air or to some oxidizing substance, hydrojuglone is oxidized to its toxic form, juglone (Lee & CAMPBELL 1969, Segura-Aguilar & 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 (Rietveld 1983). Juglone effects on plants are generally inhibitory but it has been found to be beneficial for the seedling growth of muskmelon *(Cucumis meld* cv. Kis kavunu) in pregermination treatment (Kocacaliskan & Terzi 2001, Terzi & al. 2003a).

Weed control research over the last 50 years has focused almost exclusively on synthetic herbicides. But widespread use of synthetic herbicides has resulted in herbicide-resistant weeds, has disturbed the ecological balance of the natural environment, and has sometimes affected human health. (HALL & al. 2000, Vyvyan 2002). Recently, there is an increasing interest in weed control strategies with natural compounds because they have short half-life in the environment and therefore they are considered environmentally and toxicologically safer than synthetic compounds. Compared with the long persistence, non-target toxicity, pollutant and mutagenic activity of synthetic herbicides, natural plant products are biodegradable and likely to be recycled through nature (Putnam & Duke 1974, Duke & Lydon 1987, Kumari & Waidyanath 1989, INDERJIT & al. 1999, DUKE & al. 2000, 2002, VYVYAN 2002, Boz & al. 2003, BHOWMIK & INDERJIT 2003).

Several allelochemicals have been studied for their herbicidal effects. For example, parthenin was found to have inhibitory effects on two weed species *Avena fatua* and *Bidens pilosa* (BATISH & al. 2002). Volatile 1,4 and 1,8-cineoles were toxic and injurious to bill goat weed *Ageratum conyzoides* (SINGH & al. 2002) and to. *Echinochloa crus-galli* and *Cassia obtusifolia* (ROMAGNI & al. 2000). Recently, a strong herbicidal effect of ailanthone on several weed species was found (HEISEY & HEISEY 2003). However, we have not encountered any reports about the herbicidal effect of juglone. Therefore, we studied the effect of juglone on four broadleaf weed species *(Sinapis arvensis L., Cirsium arvense L., Papaver rhoeas L.* and *Lamium amplexicaule L.)* to explore its herbicidal potential.

Material and Methods

Seeds of wheat *(Triticum vulgare* Vill. cv. Gerek 79) and barley *(Hord sum vulgare L.* cv. Kislik) were obtained from the Office of Agriculture in KUtahya (Turkey). These seeds were sown in plastic pots filled with sterilized turf. Wheat and barley were used to compare the effects of ju glone on weeds with that on crops and to determine juglone doses that are not harmful to these cultivated plants.

Four weed species, wild mustard *(Sinapis arvensis* L.), creeping thistle *(Cirsium arvense L.),* field poppy *(Pap aver rhoeas L.),* and henbit *(Lamium amplexicaule L.),* which were found to be the most common weeds in wheat and barley fields in Kutahya region, were used as test plants. These weed species are also common in most parts of the world (HOLM & al. 1997). Seedlings of the weeds, which had two or three leaves, were taken from the field in May and brought to the laboratory; then their roots were washed, and root and shoot lengths of the seedlings were measured and fresh weights of the seedlings were determined. These values were recorded as initial growth values. The seedlings were planted into plastic pots filled with sterilized turf. All the plants were maintained on laboratory benches. The temperature and relative humidity were about 20/15 °C (day/night) and 45/60 % (day/night), respectively. Under these conditions, all seedlings were left to grow for ten days. After that the juglone solutions were applied on the leaves of the plants by spraying at 0, 0.57, 1.15, 2.30 and 5.74 mM of juglone concentrations. Juglone (5-hydroxy-1,4-naphthoquinone) was purchased from Sigma, USA, as a 95 % pure compound. Juglone solutions were prepared freshly just prior to applying to the plants by continuously stirring in distilled water at 40 °C for 24 hours. Juglone is partly soluble in water, and its solubility can be increased by stirring at about 40 °C (KOCACALISKAN & *TERZI* 2001). In nature, juglone exuded from walnut will also dissolve in water. Therefore, this method was preferred since it represents the physiological conditions much better than the use of organic solvents.

Preliminary experiments were conducted to determine the maximum juglone concentration which would not harm ten-days grown wheat and barley plants. This concentration was 5.74 mM. Juglone solutions were directly sprayed to the foliar parts of the plants until the solutions started to drop from the leaves. Using this method of application, it was determined that about one ml of each solution was applied per plant. All solutions of juglone contained Tween-20 (polyoxyethylene sorbitan monolaurate), as a wetting agent, at 0.01 % (v/v) concentration. Control plants were sprayed only by 0.01 % solution of Tween-20 prepared in distilled water.

After 11 days of the treatments, all plants were taken out of the pots, and their roots were washed, then root, shoot lengths and seedling weights of the plants were measured. These values were recorded as the final growth values. Change in growth was determined by subtracting initial growth values from the final growth values for both elongation and fresh weight. The reason for using fresh weight instead of dry weight was that the difference between initial and final growth can only be determined on fresh weight, because the initial and final growth values were obtained from the same plant.

Chlorophyll contents of the leaves were determined by the method of ARNON 1949. Percentage inhibition in both growth and chlorophyll content was calculated using the following equation:

$$\text{Percentage inhibition (\%)} = \frac{\text{Control value} - \text{Treatment value}}{\text{Control value}} \times 100$$

The experiment was conducted using a completely randomized design with three replicates. Eight plants were used in each replicate per treatment. The data were analyzed by ANOVA, then the significant mean differences between the treatments of juglone and control were determined using Dunnet test (LITTLE and HILLS 1978).

Results and Discussion

In this study, foliar applications of juglone were carried out by mixing Tween-20, a non-ionic surfactant, in juglone solutions to increase its absorption by leaves. Several surfactants are generally used in foliar application of plant growth regulators (GULERYUZ 1982, NICKEL 1983) and agrochemicals such as herbicides (STOCK & HOLLOWAY 1993). Since wetting agents, especially non-ionic surfactants, reduce the surface tension of spray droplets, they improve retention of spray droplets on leaf surface, increase spray droplet contact with the leaf surface and reduce crystallization of the active ingredient on the leaf surface. In summary, they increase the rate of diffusion of agrochemicals across the cuticle of the leaves (STOCK & al. 1992, 1993). Further, foliar penetration of agrochemicals has also been shown to occur through stomata and the stomatal penetration of aqueous solutions into the leaves is improved by surfactants (GREENE & BUKOVAC 1974).

A typical property of an allelochemical is a growth inhibiting effect on some species, while it rarely has some growth stimulating effects on certain other species. Juglone has been shown to have such allelopathic effects (KOCACALISKAN & TERZI 2001). We have hypothesized that this property may be useful in weed management if juglone is harmful on weeds but not on cultivated plants. In agreement with this hypothesis, it was found that juglone was harmful for all the weeds studied here without causing any significant decrease in wheat and barley growth, except for fresh weight at 5.74 mM juglone (Table 1). Similarly, in a previous study, the allelochemical parthenin showed a significant herbicidal effect against *Ageratum conyzoides* weed with only marginal effects on wheat growth (BATISH & al. 1997). One fairly obvious factor might be that in the current study all the weeds are dicots and the crops (wheat and barley) are monocots. Although there is no corroborating evidence in the literature that juglone affects dicots and monocots differently, this study interestingly has showed such an effect of juglone. To date, the allelopathic effects of juglone were generally investigated by adding it into the growing medium of plant. The foliar application of juglone used in our study may have uncovered some differential effects on dicots and monocots. This phenomenon should be clarified in future studies.

Although the phytotoxic effect of juglone was demonstrated on several plant species (RIETVELD 1983, KOCACALISKAN & TERZI 2001), to date no information was available about its effect on any weed species. Among concentrations of juglone studied, 1.15 and 2.30 mM inhibited growth of the weeds significantly, and 5.74 mM had a strong growth suppressing effect on the weeds and even killed field poppy. 0.57 mM juglone was not effective on the weeds (data not shown).

As seen in Table 1, shoot elongation of all of the weeds was inhibited more severely than root elongation. This result is in agreement with the report of RIETVELD 1983 indicating that root elongation was less affected than the shoot elongation by juglone in 16 species. Significant decreases in fresh weight of the weeds were also recorded as compared to the control. Fresh weights of wheat and barley seedlings were also significantly decreased by juglone, but only at 5.74 mM concentration ($P < 0.05$), and this decrease was lower than that of the weeds. While percent inhibition in fresh weight of the weeds was more than 60%, it was just 14% and 13% in wheat and barley, respectively. On the other hand, creeping thistle was found to come second regarding the inhibitory effect of juglone. The percent inhibition of shoot elongation and seedling fresh weight were 83.6% and 98.6% in creeping thistle by 5.74 mM juglone, respectively, whereas in the wild mustard and henbit it was 49.2% and 81.6% of shoot elongation, and 60.7% and 65.1% of seedling fresh weight, respectively. As can be seen in Fig. 1 and Table 1, fresh weight of the wild mustard and creeping thistle was affected more than elongation.

Juglone activity against weeds was not investigated so far. However, the mode of action of juglone on the other plants is partially known. For example, HEJL & KOSTEL 2004 found that juglone disrupts root plasma membrane H+-ATPase activity and impairs water uptake, root respiration and growth in soybean *(Glycine max)* and corn *(Zea mays).* Juglone was found to decrease growth of cucumber seedlings by decreasing chlorophyll content and reducing some anatomical tissues (xylem vessel and bundle

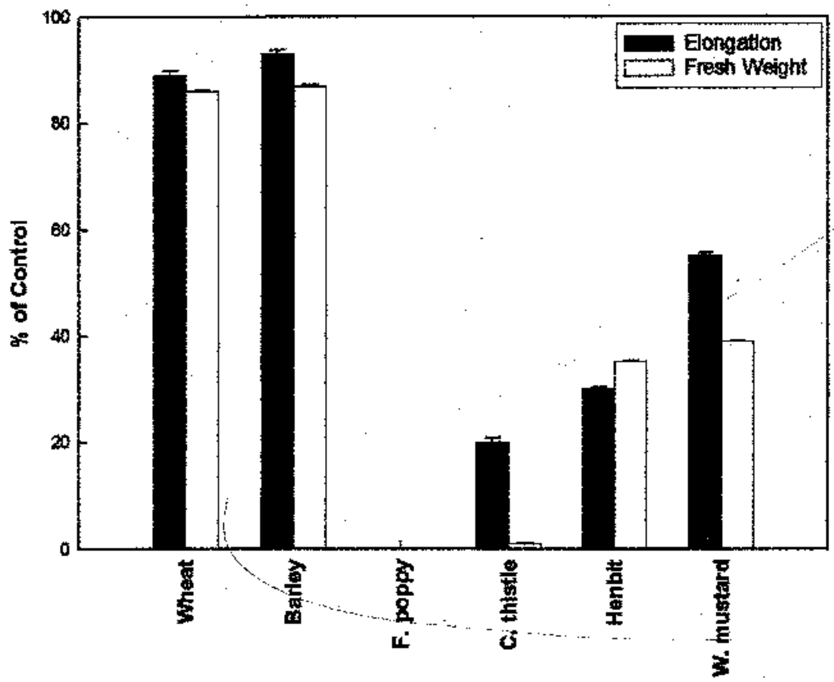

Fig. 1. Effect of 5.74 mM juglone on seedling elongation and fresh weight of the weed species. Vertical bars show standard error of the mean.

radius of stem, stomata length and stomata number of the cotyledons) (TERZI & al. 2003b).

Other natural compounds such as 1,3,7-trimethylxanthine, tentoxin, sorgoleone, artemisinin, hydantocidin, chaparrinone, pelargonic acid, parthenin, ailanthone and cineoles appear to inhibit weed growth (Rizvi & al. 1987, DUKE & al. 2001, BATISH & al. 2002, SINGH & al. 2002, HEISEY & HEISEY 2003), though the exact mechanism is not known. Possible mechanisms include disruption of mitotic activity in the growing cells (DAYAN & al. 2000), or reduction in photosynthesis due to loss of photosynthetic pigments (SINGH & al. 2002).

Both chlorophyll a and b contents of the weeds decreased significantly upon juglone application in this study. However, chlorophyll a was more affected than chlorophyll b. Among the weeds, it was determined that field poppy and henbit were the most affected species in chlorophyll content. Chlorophyll contents of wheat and barley were also decreased by juglone but not as much as in the case of the weeds. The reduction in both chlorophyll a and b content of wheat was less than that of barley (Table 2). The reduction in chlorophyll content in response to allelochemicals has been reported in a number of the plants (HEAL & al. 1993, ROMAGNI & al. 2000,

Table 1. Effect of juglone on growth parameters of wheat, barley and weeds. Each value in the table is the difference between initial and final growth and is mean of three replicates. Values in parentheses are percent inhibition.

	Juglone (mM)			
Species	0(Control)	1.15	2.30	5.74
Root length (cm)				
Wheat	22.6±0.5	21.9 (3)±1.1	20.8 (8)±0.7	19.8 (12)±0.8
Barley	27.4±0.5	27.9 (0)±0.6	26.3 (4)±0.8	25.8 (6)±0.7
Wild mustard	7.2±0.5	6.8 (5)±0.6	5.5 (23)±0.4**	4.2 (42)±0.5**
Creeping thistle	16.0±1.1	12.9 (20)±0.4**	10.6 (34)±0.4**	3.9 (76)±0.7**
Field poppy	4.1±0.2	3.0 (30)±0.3**	1.2 (71)±0.2**	0.0 (100)**
Henbit	2.4±0.1	2.5 (0)±0.1	2.4 (3)±0.1	1.1 (57)±0.2**
Shoot length (cm)				
Wheat	28.8±0.8	27.1 (6)±0.5	26.7 (7)±0.9	26.0 (10)±0.7
Barley	27.3±0.6	27.6 (0)±1.3	26.4 (3)±0.6	25.2 (8)±0.3
Wild mustard	41.5±2.3	37.4 (10)±1.6*	33.3 (20)±1.8**	21.1 (49)±2.3**
Creeping thistle	11.4±1.0	9.0 (21)±0.5**	4.1 (64)±0.3**	1.9 (84)±0.1**
Field poppy	11.0±0.5	10.4 (7)±0.4**	6.3 (44)±0.2**	0.0 (100)**
Henbit	6.4±0.3	5.8 (15)±0.2**	4.8 (29)±0.2**	1.2 (82)±0.1**
Seedling fresh weight (mg)				
Wheat	0.22±0.01	0.21 (5)±0.01	0.21 (7)±0.01	0.19 (14)±0.01*
Barley	0.36±0.01	0.35 (3)±0.01	0.34 (4)±0.01	0.31 (13)±0.01*
Wild mustard	1.86±0.12	1.16 (38)±0.12**	0.97 (48)±0.07**	0.73 (61)±0.03**
Creeping thistle	1.31±0.12	0.96 (27)±0.08**	0.30 (77)±0.14**	0.02 (99)±0.01**
Field poppy	0.42±0.02	0.16 (63)±0.03**	0.11 (74)±0.02**	0.00 (100)**
Henbit	0.29±0.02	0.22 (0)±0.03	0.19 (12)±0.01	0.08 (65)±0.01**

** ($P < 0.01$), * ($P < 0.05$). Treatments differ significantly from the control (Dunnet). ±SE.

BATISH & al. 2002). Generally, the first obvious symptom of the herbicide effect on weeds is that the affected plants present a bleached appearance because of the loss of chlorophyll. The effect modes of some herbicides on weeds are mainly by inhibiting photosynthesis. But the nature of this effect is different depending on the herbicide (CAMPBELL 1991). In *Zea mays and Glycine max,* juglone inhibits plant growth especially by reducing photosynthesis (JOSE & GILLESPIE 1998). Juglone has also been shown to inhibit growth by reducing chlorophyll content and inhibiting photosynthesis in *Lemna minor.* In that case, the inhibition in photosynthesis was attributed to the decrease in photosynthetic electron transport activity rather than chlorophyll loss (Ha & al. 1993).

To our knowledge, no study has examined the effect of juglone on chlorophyll content of weeds. However, some other allelochemicals have

Table 2. Effect of juglone on chlorophyll a and b content (mg g' leaf) of wheat, barley and weeds. Values in parentheses are percent inhibition.

Species	Juglone (mM) 0(Control)	1.15	2.30	5.74
Chlorophyll a				
Wheat	1.249±0.015	0.988 (21)±0.054*	0.927 (26)±0.027**	0.914 (27)±0.037**
Barley	1.093±0.051	0.950 (13)±0.036	0.704 (36)±0.047**	0.700 (36)±0.045**
Wild mustard	0.797±0.071	0.635 (20)±0.104	0.608 (24)±0.073	0.424 (47)±0.067**
Creeping thistle	0.944±0.064	0.687 (27)±0.057**	0.645 (32)±0.012**	0.484 (49)±0.013**
Field poppy	0.745±0.062	0.667 (11)±0.058	0.447 (40)±0.021**	0.000 (100)**
Henbit	0.829±0.139	0.469 (43)±0.016*	0.427 (49)±0.037**	0.378 (54)±0.009**
Chlorophyll b				
Wheat	0.775±0.006	0.685 (12)±0.028	0.701 (10)±0.062	0.694 (10)±0.012
Barley	0.900±0.074	0.701 (22)±0.099	0.627 (30)±0.028*	0.607 (33)±0.047*
Wild mustard	0.655±0.086	0.527 (20)±0.071	0.480 (27)±0.035	0.495 (24)±0.068
Creeping thistle	0.854±0.044	0.722 (16)±0.055	0.647 (24)±0.049*	0.518 (39)±0.059**
Field poppy	0.790±0.011	0.547 (31)±0.104*	0.369 (53)±0.036**	0.000 (100)**
Henbit	0.781±0.116	0.554 (29)±0.030	0.438 (44)±0.039**	0.403 (48)±0.013**

** ($P < 0.01$), * ($P < 0.05$). Treatments differ significantly from the control (Dunnet). ± SE.

been shown to decrease chlorophyll content in weeds. For example, cineole and citronellol have been recorded to decrease chlorophyll content of bill goat weed (SINGH & al. 2002). The loss of chlorophyll is likely to reduce the photosynthetic ability and thereby the growth of the plants. Significant decreases in growth of the weeds were accompanied by significant chlorophyll losses in the present study. This result suggests that the observed growth inhibition of the weeds may be due to chlorophyll loss. However, in addition to the chlorophyll loss, some other mechanisms may play roles in plant growth inhibition depending on species investigated. Thus, in our study, although chlorophyll contents of wheat and barley were decreased significantly, this decrease was not reflected in the growth of the plants to the same degree. PANDEY 1996 reported that growth inhibition of hyacinth by parthenin was due to various physiological changes such as damage to the cell membranes, loss of dehydrogenase activity in roots and also loss of chlorophyll in leaves.

In eukaryotic cells, juglone induces cell death in human fibroblasts (PAULSEN & LJUNGMAN 2005) and blocks transcription by inhibiting RNA polymerases (CHAO & al. 2001). In addition, juglone has-been found to have mutagenic effects in *Salmonella* bacterium (TIKKANEN & al. 1983). In contrast, in rat models, it has been reported that juglone has inhibitory effects on intestinal carcinogenesis (SUGIE & al. 1998). Although juglone may have some adverse effects in some experimental models, it may not be as harmful to human health as synthetic herbicides. Since juglone was applied during the seedling stage of the plants (wheat and barley), it would be almost completely degraded (as it is a natural chemical) until the plant matures and produces grains. Therefore, it is highly unlikely that a harmful amount of juglone will remain in the mature plant. Obviously, that has to be investigated in future studies.

In conclusion, our study indicates that juglone is a potent inhibitor of the investigated weeds, especially field poppy, without affecting wheat and barley growth. This ability of juglone can be utilized for future weed management strategies. As synthetic herbicides disturb the .ecological balance of the natural environment, juglone may be safer than synthetic herbicides used in weed management, because it is a natural and biodegradable compound.

Acknowledgements

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