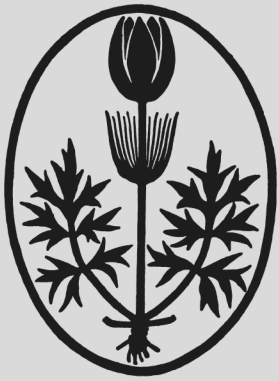
PHYTON

ANNALES REI BOTANICAE

FUNDATORES:
PROF. DR. F. WEBER † (GRAZ) et PROF. DR. F. J. WIDDER † (GRAZ)

EDITORES ET REDACTORES:
PROF. DR. H. GUTTENBERGER et PROF. DR. H. TEPPNER

VOL. 55, FASC. 2



HORN (AUSTRIA)
APUD FERDINANDUM BERGER & FIL.
2015

PHYTON 55 (2): 193–320

15. 12. 2015

ISSN 0079-2047

A PDF of the original article is send by the Publisher to the Corresponding Author. Corresponding Authors are allowed to pass electronic copies to all co-authors. All authors may distribute their original article as PDF or hardcopy to interested individuals. Any kind of public distribution, for example, on the internet, requires the permission of the copyright owner (Publisher: Verlag Ferdinand Berger & Söhne Ges.m.b.H., Wiener Straße 21–23, A-3580 Horn, Austria, www.verlag-berger.at E-mail: verlag@berger.at).

Author's personal copy

Phyton (Horn, Austria)	**Vol. 55**	Fasc. 2	271–277	15. 12. 2015

DOI: 10.12905/0380.phyton55(2)2015-0271

Effects of Walnut Leaf Extracts Prepared in Different Solvents on Seed Germination and Seedling Growth of Cress (*Lepidium sativum* cv. Zeybek)

By

İsmail Kocaçalışkan*), Berna Kiliç**) and İrfan Terzi***)

Received August 8, 2014

Accepted October 10, 2014

Key words: Cress, seed germination, seedling growth, solvents, walnut leaf extracts.

Summary

Kocaçalışkan I., Kiliç B. & Terzi I. 2015. Effects of walnut leaf extracts prepared in different solvents on seed germination and seedling growth of Cress (*Lepidium sativum* cv. Zeybek). – Phyton (Horn, Austria) 55 (2): 271–277, mit 2 Tabellen.

In this study, effects of walnut leaf extracts prepared in six different solvents "water, ethanol, methanol, aceton, tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO)" on seed germination and post-germinative seedling growth of cress (*Lepidium sativum* L. cv. Zeybek) have been investigated. All the treatments were seen to inhibit germination percentage of the seeds in second day, whereas only DMSO and methanol extracts inhibit it in fifth day. DMSO and methanol extracts decreased germination rate of the seeds significantly with respect to control, as well. Both root and shoot elongation were inhibited by the treatments of DMSO, water and methanol extracts in range of effectiveness grade from high to low. While shoot fresh weight was decreased by all the treatments, root fresh weight was decreased only by DMSO, water and methanol extracts. Among the treatments, the most

*) I. Kocaçalışkan (corresponding author), Molecular Biology and Genetics Department, Yıldız Technical University, İstanbul, Turkey. E-mail: ikocacaliskan@gmail.com

**) B. Kiliç, Science and Technology Institute, Dumlupınar University, Kütahya, Turkey

***) İ. Terzi, Biology Department, Education Faculty, Dumlupınar University, Kütahya, Turkey

Author's personal copy

strong inhibitory effect was found to have DMSO extract on both seed germination and seedling growth parameters. Thus, it was discussed in the manuscript that DMSO may be more efficient and proper solvent in preparation of walnut leaf extract.

Zusammenfassung

Kocaçalişkan I., Kiliç B. & Terzi I. 2015. Effects of walnut leaf extracts prepared in different solvents on seed germination and seedling growth of Cress (*Lepidium sativum* cv. Zeybek). [Effekte von Walnussblatt-Extrakt, hergestellt mit unterschiedlichen Lösungsmitteln, auf die Samenkeimung der Kresse (*Lepidium sativum* L. cv. Zeybek).]. – Phyton (Horn, Austria) 55 (2): 271–277, mit 2 Tabellen.

Der Einfluss von Walnussblätter-Extrakten, hergestellt mit sechs verschiedenen Lösungsmitteln (Wasser, Ethanol, Methanol, Aceton, Tetrahydrofuran (THF) und Dimethylsulfoxid (DMSO)), auf die Samenkeimung und das Wachstum nach der Keimung von Kresse (*Lepidium sativum* L.cv Zeybek) wurde in dieser Arbeit untersucht. Alle Behandlungen führten zu einer Verminderung der Keimrate am zweiten Tag, nur DMSO- und Methanol-Extrakte wirkten in fünf Tagen. Im Verhältnis zur Kontrolle senkten DMSO- und Methanol-Extrakte die Keimrate signifikant. Das Wurzel- und Sprosswachstum wurde durch den DMSO-Extrakt stark gehemmt, Wasser- und Methanol-Extrakte hemmten stark bis gering. Während das Frischgewicht des Sprosses durch alle Behandlungen geringer wurde, sank das Frischgewicht der Wurzeln nur bei DMSO-, Wasser- und Methanol-Extrakten. Bei der Keimrate und bei den Wachstumsparametern wirkte der DMSO-Extrakt am stärksten hemmend,. Daher wird in dieser Arbeit diskutiert, dass DMSO das am besten geeignete Lösungsmittel für Walnussblätterextrakte ist.

Introduction

Chemical interaction mainly between plants are called allelopathy, and the organic compounds involved in allelopathy are called allelochemicals. All parts of plants may contain allelochemicals but leaves and roots are the most important sources. The release of allelochemicals from plants occur by exudation from roots, leaching and volatilization from leaves and degradation of dead plant parts. Allelochemicals become stressful when they are toxic, and they are generally in toxic character. On the other hand, sometimes an allelochemical produced by a plant is harmful to another plant species but beneficial to a third one (Rice 1984, Rizvi & Rizvi 1992, Kocaçalişkan 2006).

The inhibitory effect of walnut on associated plants is one of the oldest examples of allelopathy. The chemical responsible for walnut allelopathy is juglone (5-hydroxy-1,4 naphthoquinone), and it has been isolated from many plant species in the walnut family including *Juglans nigra, Juglans regia* and the others (Davis 1928, Daglish 1950, Piedrahita 1984, Prataviera

Author's personal copy

& al. 1983). Juglone's reduced and nontoxic form hydrojuglone is abundant in root and leaves of walnut tree. When exposed to the air or to some oxidizing substance it is oxidized its toxic form, juglone. Rain washes juglone from the leaves and carries it into the soil. Thus, neighbour plants are affected by absorbing juglone through their roots (Lee & Campbell 1969, Segura-Aguilar & al. 1992). Walnut has been reported to be toxic to both herbaceous and woody plants (Funk & al. 1979, Rietveld 1983). Juglon's beneficial effect is rare that may be a few plant species. For example, muskmelon's growth has been increased by both juglone and walnut leaf extracts (Kocaçalişkan & Terzi 2001).

Walnut leaf extract are used in allelopathic researches because of its juglone content, and it has also been shown to have herbicidal, antimicrobial, and antioxidant activities (Rizvi & Rizvi 1992, Kocaçalişkan 2006, Altikat & al. 2013) . Several solvents such as distilled water, ethanol, methanol, aseton are being used in preparation of walnut leaf extract (Kocaçalişkan 2006). However, which solvent is more efficient and proper for the extraction is unclear. We could not encountered any study about this subject. Therefore, in this study, it has been aimed to compare the effects of walnut leaf extracts prepared with six different solvents and to find the efficient solvent.

Materials and Methods

Preparation of Walnut Leaf Extracts

Walnut leaf extracts were prepared from leaves of walnut (*Juglans regia* L. cv. Yalova-2). The leaves were picked in August and dryed at 70 °C in an oven for 48 h. Because juglone content in the leaves of walnut is in the highest level in August (Turan 2008). Later 10 g dried leaf was homogenized using a Waring Blender in 100 ml of distilled water, ethanol, mathanol, aseton, THF and DMSO solvents, separately. Then the homogenates were filtered and centrifuged at 3000 rpm for 10 min. The extraction procedure of distilled water solvent was stopped in this step and The supernatant sample was used as an extract source. The other supernatants were evaporated and the residue dissolved in distilled water of 50 ml. These samples were used as extract sources. Evaporation procedure was carried out by a rotary evaporatore, except DMSO whose evaporation was made in a heated oil bath. The obtained extracts were keeped in a refrigerator until to treatment.

Application of Treatments

Cress (*Lepidium sativum* L. cv. Zeybek) seeds surface sterilized with 1% sodium hypochloride and the seeds were placed in Petri dishes furnished with sheets of filter paper moistened with distilled water (control) or with the walnut leaf extracts as treatments. Then the dishes were left in an incu-

Author's personal copy

bator at 25 ˚C in continuous dark. During 5 days, germination percentages were recorded daily, and the end of 5th day postgerminative growth of the seeds were determined by measuring lengths and fresh weights of the seedlings.

The treatments were as follow:

1. Distilled water (control)
2. Walnut leaf extract prepared in distilled water
3. Walnut leaf extract prepared in ethanol
4. Walnut leaf extract prepared in methanol
5. Walnut leaf extract prepared in aseton
6. Walnut leaf extract prepared in THF
7. Walnut leaf extract prepared in DMSO

Results and Discussion

Walnut leaf extracts prepared in six different solvents "water, ethanol, methanol, aseton, tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO)" on seed germination and post-germinative seedling growth of cress have been found to have inhibitory effect at different levels, in this study. All the treatments were seen to inhibit germination percentage of the seeds in second day, whereas only DMSO and methanol extracts inhibit it in fifth day (Table 1). DMSO and methanol extracts decreased germination rate of the seeds significantly with respect to control, as well. Both root and shoot elongation were inhibited by the treatments of DMSO, water and methanol extracts in range of effectiveness grade from high to low. While shoot fresh weight was decreased by all the treatments, root fresh weight was decreased only by DMSO, water and methanol extracts (Table 2).

Table 1. Effects of walnut leaf extracts prepared in different solvents on germination percentages of cress seeds during five days.

Walnut leaf extracts	Days				
	1	2	3	4	5
Control (Dist. water)	0	73a	78a	78a	78a
Water extract	0	13b	48b	51ab	53ab
Ethanol extract	0	25b	64ab	65ab	65ab
Methanol extract	0	10b	43b	45b	46b
Aceton extract	0	11b	63ab	63ab	64ab
THF extract	0	23b	64ab	64ab	65ab
DMSO extract	0	0c	8c	10c	10c

Values with different letters in each coloumn are significantly different (Duncan, 5 %), n = 3.

Author's personal copy

Table 2. Effects of walnut leaf extracts prepared in differenet solvents on seed germination rate and seedling growth in cress.

Walnut leaf extracts	Germination rate	Root length (cm)	Shoot length (cm)	Root Fresh w. (mg)	Shoot Fresh w. (mg)
Control (Dist. water)	61a	2.5a	3.2a	80a	400a
Water extract	35b	1.5b	1.7b	30b	250b
Ethanol extract	43ab	2.4a	2.3ab	70a	400ab
Methanol extract	29b	1.7ab	1.6b	40b	340ab
Aceton extract	40ab	2.3a	2.2ab	70a	370ab
THF extract	43b	2.3a	2.2ab	60a	370ab
DMSO extract	5c	0.3c	0.3c	6c	46c

Values with different letters in each coloumn are significantly different (Duncan, 5 %), n = 3.

We preferred to use cress seed in this study. Because juglone and walnut leaf extracts were found to inhibit its germination and growth, and since cress was determined the most sensitive species, it has been suggested as a test plant in allelopathic studies by Kocaçalışkan & Terzi 2001. On the other hand, our study proves the previous study that walnut leaf extracts have showed more inhibitory effect on seedling gowth than on seed germination. This result is agree with other previous works that seedling growth is much more sensitive to the walnut extracts than seed germination (Rietveld 1983, Dornbos & Spencer 1990).

The main aim of this study was to establish the most efficient and proper solvent in preparation of walnut leaf extract. According to our results, this solvent may be DMSO. Because it was found the most effective solvent with respect to others as mentioned above and as seen in Table 1 and 2. It is difficult to explain exactly why DMSO is the most efficient solvent according to other solvents used in this study. In addition, there is no study to compare our results. We suppose that DMSO may solves Juglone exist in walnut leaf more efficiently than the other solvents used in the study. We don't know the mechanism of the effect of DMSO. But the efficient solubility of juglone in DMSO may be due to close chemical polarities depend on their hydroxy grops. On the other hand, DMSO penetrates all the cells and tissues passing cell memranes easly and carries dissolved compounds together. Due to this property of DMSO, it is being used to solve some dyes such as hematoxylin which used in dying cancer tissues and anti-tumor agent (Wood & Varella 1972, Guneral 2001). In this respect, juglone is a natural dye of walnut leaf in brownish colour, as well.

As seen in Table 2, shoot elongation was inhibited much more than root by DMSO extract. That is, although root elongation was decreased eight fold by DMSO extract according to control, the inhibition was more than ten fold in the case of shoot elongation. However, this situation was contrary in the

Author's personal copy

case of fresh weight. In former studies, it has been shown that inhibitory effects of allelochemicals are different on root and shoot growth. Because phytotoxicity is a function of both allelochemical and plant species studied (Rietveld 1983, Dornbos & Spencer 1990, Jose & Gillespie 1998).

In previous studies, Juglone has been shown to have herbicidal effect on several weeds (Topal & al. 2007), antimicrobial effects on bacteria and fungi (Clark & al. 1990, Dama & al. 1998, Tan 2012), anticancer effect on tumor cells (Sugie & al. 1998, Paulsen & Ljungman 2005, Kiran-Aithal & al. 2009, Montenegro & al. 2010, Xu & al. 2010, 2013), and antioxidant effect on related enzymes (Chobot & Hadeck 2009, Altikat & al. 2013). However, juglone use for these aims is not an economical approach due to it is rather expensive chemical. Since walnut leaf extract is rich in juglone, instead of pure juglone chemical walnut extract may be used for the practical aims such as herbicide, antimicrobial and antitumoral agent as mentioned above. In addition, as concluded in this study, DMSO may be used to prepare efficient walnut leaf extract.

References

Altikat S., Terzi İ., Kuru H. İ. & Kocaçalışkan İ. 2013. allelopathic effects of juglone on growth of cucumber and muskmelon seedlings with respect to antioxidant enzyme activities. – J. Environ. Protect. Ecol. 14 (3A): 1244–1253.

Clark A. M., Jurgens T. M. & Hufford C. D. 1990. Antimicrobial activity of juglone. – Phytotherapy Res. 4 (1): 11–14.

Chobot V. & Hadacek F. 2009. Milieu-dependent pro- and antioxidant activity of juglone may explain linear and nonlinear effects on seedling development. – J. Chem. Ecol. 35 (3): 383–390.

Daglish C. 1950. The ısolation and ıdentification of a hydrojuglone glycosyde occurring in the walnut. – Biochem. J. 47: 458–462.

Dama L., Poul B. & Jadhav B. 1998. Antimicrobial activity of naphtaquinonic compounds. – J. Ecotoxic. Environ. Monitor. 8 (3): 213–215.

Davis E. F. 1928. The toxic principle of juglans nigra as ıdentified with synthetic juglone and ıts toxic effects on tomato and alfalfa plants. – Am. J. Bot. 15: 620.

Dornbos D. L. & Spencer G. F. 1990. Natural products phytotoxicity. – J. Chem. Ecol. 16: 339-352.

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

Guneral İ. 2001. Kanserden korkma, p. 125, Remzi Kitabevi Press. – In Turkish, İstanbul, Turkey.

Jose S. & Gillespie A. R. 1998. 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.) growth and physiology. – Plant Soil 203: 199–205.

Kiran-Aithal K. B., Kumar M. R. S., Rao B. N., Udupa N. & Rao B. S. S. 2009. Juglone, a naphthoquinone from walnut, exerts cytotoxic and genotoxic effects against cultured melanoma tumor cells. – Int. Cell Biol. 33 (10): 1039–1049.

Author's personal copy

Kocaçalışkan İ. 2006. Allelopati, p. 132, Bizim Büro Press. - In Turkish, Ankara, Turkey.

Kocaçalışkan İ. & Terzi İ. 2001. Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. – J. Hortic. Sci. Biotechnol. 76: 436–440.

Lee K. C. & Campbell R. W. 1969. Nature and occurrence of juglone in *Juglans nigra* L. – Hortic. Sci. 4: 297–298.

Montenegro R. C., Araujoa A. J., Molina M. T., Filhoa J. D. B. M., Rochaa D. D., Montero E. L., Goulart M. O. F., Bentoc E. S., Alves A. P. N. N., Pessoaa, C., Moreas, M. O. & Costa-Lotufoa L. V. 2010. Cytotoxic activity of naphtoquinones with special emphasis on juglone and its 5-o-methyl derivative. – Chemico-Biological Interactions 184 (3): 439–448.

Paulsen M. T. & Ljungman M. 2005. The natural toxin juglone causes degradation of p53 and ınduces rapid H2Ax phosphorylation and cell death in human fibroblasts. – Toxic. Appl. Pharm. 209 (1): 1–9.

Piedrahita O. 1984. Black walnut toxicity. – Factsheet 11: 7–8.

Prataviera A. G., Kuniyuki A. H. & Ryugo K. 1983. Growth ınhibitors in xylem exudates of persian walnuts (*Juglans regia* L.) and their possible role in graft failure. – J. Am. Soc. Hortic. Sci. 108 (6): 1043–1045.

Rice E. L. 1984. Allelopathy, p. 422. – Academic Press, New York.

Rietveld W. J. 1983. Allelopathic effects of juglone on germination and growth of several herbaceous and woody species. – J. Chem. Ecol. 9: 295–308.

Rizvi S. J. H. & Rizvi V. 1992. Allelopathy, p. 480. – Chapman and Hall, New York, USA.

Segura J.-Aguilar, Hakman I. & Rydstrom J. 1992. The Effect of 5-OH-1,4 Naphtoquinone on Norway Spruce Seed During Germination. – Plant Physiol. 100: 1955–1961.

Sugie S., Okamotob K., Rahman K. M. W., Kawaid T. T. K. K. & Yamaharae J. 1998. Inhibitory Effect of Plumbagin and Juglone on Azoxymethane-induced Intestinal Carcinogenesis in Rats. – Cancer Letters 127 (1): 177–183.

Tan D. T. C. 2012. Synthesis and antibacterial activity of juglone derivatives. – J. Chemistry and Chem. Engin. 6 (1): 84–89.

Topal S., Kocaçalışkan İ. & Arslan O. 2007. Herbicidal Effect of Juglone as an Allelochemical. – Phyton 46 (2): 259–269.

Turan E. 2008. Determination of seasonal changes in juglone and phenolic content of walnut leaves. – PhD Thesis (In Turkish, Supervisor: İ. Kocaçalışkan), Dumlupınar University, Science and Technology Institute, Kütahya-Turkey.

Xu H., Yu X. F., Zhang R., Qu X. R., Chen Y. P., Ma X. Y. & Sui D. Y. 2010. Antiproliferative effect of juglone from juglans mandshurica maxim, on human leukemia cell HL-60 by ınducing apopitosis through the mitochondria-depenndent pathway. – Eur. J. Pharm. 645 (1): 14–22.

Xu H., Xiaofeng Y., Shaochun Q. & Dayun S. 2013. Juglone ısolated from juglans mandshurica maxim, ınduces apopitosis via down-regulation of ar expression in human prostate cancer LNCAP cells. Bioorg. – Med. Chem. Lett. 23: 3631–3634.

Wood D. C. & Varela V. L. 1972. Hematoxylin-DMSO mixture as an anti-tumor agent in mice, res. Commun. – Chem. Pathol. Pharmacol. 4 (3): 623–630.

Author's personal copy